Evaluation of cardiotoxicity of rupatadine, an antihistamine, as recommended by the ICH E14

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A todos los que me han dado la fuerza y la ilusión para llegar aquí

A mis padres, hermanas y Ricard,
por su cariño, sus cuidados y su ejemplo,
sin vosotros no hubiese sido posible.

A Iñaki,

porque cuando te fuiste,
te llevaste parte de mi vida,
pero lo vivido me acompañará siempre,
y tu recuerdo me ayuda a ser mejor.

EVALUATION OF CARDIOTOXICITY OF RUPATADINE, AN ANTIHISTAMINE, AS RECOMMENDED BY THE ICH E14

AIMS:
To evaluate the effects of therapeutic and supratherapeutic doses of rupatadine on cardiac repolarization in line with a 'thorough QT/QTc study' protocol performed according to International Conference on Harmonization guidelines.

METHODS:
This was a randomized (gender-balanced), parallel-group study involving 160 healthy volunteers. Rupatadine, 10 and 100 mg od, and placebo were administered single-blind for 5 days, whilst moxifloxacin 400 mg/day was given on days 1 and 5 in open-label fashion. ECGs were recorded over a 23-h period by continuous Holter monitoring at baseline and on treatment days 1 and 5. Three 10-s ECG samples were downloaded at regular intervals and were analysed independently. The primary analysis of QTc was based on individually corrected QT (QTcI). Treatment effects on QTcI were assessed using the largest time-matched mean difference between the drug and placebo (baseline-subtracted) for the QTcI interval. A negative 'thorough QT/QTc study' is one where the main variable is around < or =5 ms, with a one-sided 95% confidence interval that excludes an effect >10 ms.

RESULTS:
The validity of the trial was confirmed by the fact that the moxifloxacin-positive control group produced the expected change in QTcI duration (around 5 ms). The ECG data for rupatadine at both 10 and 100 mg showed no signal effects on the ECG, after neither single nor repeated administration. Furthermore, no pharmacokinetic/pharmacodynamic relationship, gender effects or clinically relevant changes in ECG waveform outliers were observed. No deaths or serious or unexpected adverse events were reported.

CONCLUSIONS:
This 'thorough QT/QTc study' confirmed previous experience with rupatadine and demonstrated that it had no proarrhythmic potential and raised no concerns regarding its cardiac safety.

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LIST OF THE MOST COMMON ABBREVIATIONS

AE:	*Adverse event*
AEM:	*Spanish Drug Agency*
Alpha:	*Level of significance*
a.m.:	*Ante meridien*
$AUC^{\infty}_{0:}$	*Area under the concentration-time curve from zero to infinity*
$AUC^{\tau}_{0:}$	*Area under the concentration-time curve from zero to τ*
BMI:	*Body Mass Index*
β-HCG:	*Beta Chorionic Gonadotrophine*
ºC:	*Centigrade*
$C_{avg:}$	*Mean concentration at steady-state*
$C_{max:}$	*Maximum observed plasma level reached after administration*
$C_{min:}$	*Minimum observed plasma level achieved just before the administration*
CI:	*Confidence interval*
CLss/F:	*Apparent total plasma clearance based on the terminal phase*
CRF:	*Case report form*
CV:	*Curriculum Vitae*
d:	*Day*
EC:	*Ethical Committee for Clinical Research*
ECG:	*Electrocardiogram*
FDA:	*Food and Drug Administration*
g:	*Gram*
GCP:	*Good Clinical Practice*
h:	*Hours*
HIV:	*Human Immunodeficiency Virus*
IEC:	*Independent Ethics Committee*
IRB:	*Institutional Review Board*
lsmean:	*Least square mean (from ANCOVA model)*
ITT:	*Intent to treat analysis population*
Kg:	*Kilograms*
LC/MS/MS:	*Liquid chromatography tandem mass spectrometry*
Log:	*Logarithm*
LOCB:	*Last observation carried forward Limit of Quantification*
LOCF:	*Last observation carried backward*
LOQ:	*Lower limit of confidence*
Max:	*Maximum value*
mg:	*Milligrams*
Min:	*Minimum value*
ml:	*Millilitre*
mmHg:	*Millimetres of Mercury*
MRT:	*Mean Residence Time*
ms:	*Millisecond*
n:	*Number of subjects*
N.A.:	*No available*
Nmiss:	*Number of missing values*
od:	*Once daily*
p.m.:	*Post meridien*
PP:	*Per protocol analysis population*
PTF:	*Peak through fluctuation*
P25:	*Percentil 25*
P50:	*Percentil 50*
P75:	*Percentil 75*
Rtheor:	*Theoretical accumulation ratio*
Rx:	*Medication*

SOP:	*Standard Operating Procedure*
s.d:	*Standard deviation*
Treat:	*Treatment*
$t_{1/2}$:	*Terminal half-life*
Tmin:	*Time of minimum concentration*
µg:	*Microgram*
Vd/F:	*Upper limit of confidence interval Apparent Volume of Distribution*

1. INTRODUCTION

1.1 An overview of the drug discovery and development process

Although human civilization has been experimenting and consuming drugs for many centuries, it is only in the past hundred years that the foundation was laid for the systematic research and development, from scientists, clinicians, and medical practitioners to statisticians. Even people from seemingly disparate occupations, such as economists, lawyers, and regulatory staff, play a vital role as well. This is due to the pharmaceutical industry is perhaps one of the most regulated industries in the world (1).

Unfortunate events have catalyzed the development of medicines regulation more than the evolution of a knowledge base. In 1937 over 100 people in the United States died of diethylene glycol poisoning following the use of a sulfanilamide elixir, which used the chemical as a solvent without any safety testing (2). This facilitated introduction of The Federal Food, Drug and Cosmetic Act with the premarket notification requirement for new drugs in 1938. The second catastrophe that influenced the development of medicines regulation far more than any event in history was the thalidomide disaster. Thalidomide was a sedative and hypnotic drug that first went on sale in Western Germany in 1956. Between 1958 and 1960 it was introduced in 46 different countries worldwide resulting in an estimated 10.000 babies being born with phocomelia and other deformities (3). As a result, the whole regulatory system was reshaped in the UK where a Committee on the Safety of Drugs (CSD) was started in 1963 followed by a voluntary adverse drug reaction reporting system (Yellow Card Scheme) in 1964. In the United States, The Drug Amendments Act of 1962 was passed by Congress requiring, the now called, Food and Drug Administration (FDA) to approve all new drug applications (NDA) and, for the first time, demanded that a new drug should be proven to be effective and safe. Of equal importance, the FDA was also given the authority to require compliance with current Good Manufacturing Practices (GMP), to officially register drug establishments and implement other requirements. The EEC Directive 65/65/EEC (4) on the approximation of provisions lay down by law, regulation and administrative action relating to medicinal products was also induced by the thalidomide disaster. The Council Regulation EEC/2309/93 (5) established the European Medicines Evaluation Agency (EMEA), now European Medicines Agency (EMA), in 1993 and the Committee on

Proprietary Medicinal Products (CPMP) was created as an advisory committee to formulate the opinion of the Agency on questions relating to the submission of applications and granting marketing authorizations in accordance with the centralized procedure (6).

Somewhat parallel with the ongoing harmonization and movement towards creating a common market for medicines inside the EU, the need for wider harmonization was, after preliminary contacts between officials from Japan, EU and US, discussed during the International Conference of Drug Regulatory Authorities (ICDRA – organized by WHO every second year) in Paris in 1989. The preliminary informal discussions had revealed a need for the harmonization of requirements relating to the new innovative drugs and the green light given in Paris led to the establishment in 1990 of the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), a collaborative initiative between the EU, Japan and the United States with observers from WHO, EFTA and Canada. ICH harmonization focuses primarily on technical requirements for new, innovative medicines and it composed a mutually accepted body of regulations concerning the safety, quality and efficacy requirements that new medicines have to meet in order to receive market approval. The ICH technical Topics are divided into four major categories and specific ICH Topic Codes are assigned according to these categories. "Q" means 'Quality' Topics i.e., those relating to chemical and pharmaceutical Quality Assurance, "S" mean 'Safety' Topics, i.e., those relating to *in vitro* and *in vivo* preclinical studies and "E" means 'Efficacy' (7).

The regulations are designed to protect the public welfare and ensure that new therapies reach the market safely. From discovering a new drug to registering it for marketing and commercialization, pharmaceutical organizations have to negotiate through very complex and lengthy process. It is estimated that, on average, a drug takes 10-12 years from initial research to reach the commercialization stage. It is not surprising that from conception to market most compounds face an uphill battle to become an approved drug. For approximately every 5.000 to 10.000 compounds that enter preclinical testing, only one is approved for marketing. The cost of this process is estimated to be more than US$800 million in 2000 dollars. Pharmaceutical development is a high-risk undertaking, in which many promising leads prove disappointing, often after millions of dollars are invested in them. Then, the industry reflects a complex, and sometimes controversial, amalgam of science and business (8).

1.1.1 Drug development process

From discovery to marketing approval of a drug, the stages involved are the following (1):

- **Drug discovery**: This process involves finding the target that causes or leads to the disease. Before any potential new medicine can be discovered, scientists work to understand the disease to be treated as well as possible, and to unravel the underlying cause of the condition. They try to understand how the genes are altered, how that affects the proteins they encode and how those proteins interact with each other in living cells, how those affected cells change the specific tissue they are in and finally how the disease affects the entire patient. This knowledge is the basis for treating the problem. Once they have enough understanding of the underlying cause of a disease, pharmaceutical researchers select a "target" for a potential new medicine. Next, chemical or biological compounds are screened using specific assays and are tested against these targets to find leading drug candidates for further development.
- **Preclinical trials**: With one or more optimized compounds in hand, researchers turn their attention to testing them extensively to determine if they should move on to testing in humans. Scientists carry out *in vitro* and *in vivo* tests. *In vitro* tests are experiments conducted in the lab, usually carried out in test tubes and beakers and *in vivo* studies are those in living cell cultures and animal models. Scientists try to understand how the drug works and what its safety profile looks like. The tests, often called preclinical research activities include toxicology, pharmacodynamics, and pharmacokinetics, as well as optimization of drug delivery systems.
- **Clinical trials**: These are studies conducted on human subjects. The pertinent parameters for clinical trials and protocols include safety and respect for human subjects, responsibilities of the investigator, institutional review board, informed consent, trial monitoring and adverse event reporting. Clinical trials must follow guidelines from the International Conference of Harmonization and, in our case, regulations from the European Medicines Agency (EMA) to be registered and commercialized.
- **Manufacturing**: The drug designated for clinical trials and large-scale production has to be manufactured in compliance with current Good Manufacturing Practice (GMP), following also the EU regulations or directives. The emphasis is that drugs

should be safe, pure, effective, and of consistent quality to ensure that they are fit to be used for their intended functions.

• **Marketing Application**: A drug is not permitted for sale until the marketing application for the new drug has been reviewed and approved by regulatory authorities. Medicines regulation incorporates several mutually reinforcing activities all aimed at promoting and protecting public health. Extensive dossier and samples are provided to the authorities to demonstrate the safety, potency, efficacy and purity of the drug. After the drug has been approved and marketed, there is a continuous monitoring of safety and performance of the drug to ensure that it's prescribed correctly and adverse effects are reported and investigated.

We are going to focuse attention in the main stages for the research and approval of a new drug with verified data on safety: preclinical trials, clinical trials and marketing approval.

1.1.2 Preclinical trials

Preclinical development encompasses the activities that link drug discovery in the laboratory to initiation of human clinical trials. Preclinical studies can be designed to identify a lead candidate from several hits; develop the best procedure for new drug scale-up; select the best formulation; determine the route, frequency, and duration of exposure; and ultimately support the intended clinical trial design. The details of each preclinical development package can vary, but all have some common features. The development process includes pharmacological studies of the lead compound and its effects on toxicity, carcinogenicity, mutagenicity, and reproductive development. These data are important to determining the safety and effectiveness of the lead compound as a potential drug. The aim of these studies is obtain data on the safety and effectiveness of the lead compound, but may iterations of optimization of the lead compound may be necessary to yield a potential drug candidate for clinical trial. The potency, efficacy and safety of a drug depend on the chemical and structural specificity of drug-target interaction. In pharmacology, the concerns are the evaluation of pharmacodynamics, pharmacokinetics and toxicity.

Pharmacodynamics is the study of interactions between drugs and the body, while pharmacokinetics describes the absorption, distribution, metabolism and excretion (ADME) of drugs by the body. Pharmacodynamic studies allow us to understand the potency, effectiveness, therapeutic index and safety margins of drugs.

Pharmacokinetic information on ADME provides us with an understanding of how drugs are transported, diffused into the bloodstream and become available to the cells and act on the target sites (1)(9). In addition to the preclinical research of pharmacodynamics and pharmacokinetics, study of the toxicology of a potential drug is critical to demonstrate that it is safe before it is given to human in clinical trials. Toxicological studies show the functional and morphological effects of the drug. They are performed by determining the mode, site, and degree of action, dose relationship, sex differences, latency and progression and reversibility of these effects.

The batteries of studies that must to be conducted in order to obtain the marketing authorization approval are described by the ICH guidelines on safety studies (10):

Table 1: Battery of ICH preclinical studies

S1	*Carnicogenicity studies*
S1A	Need for carcinogenicity studies of pharmaceuticals
S1B	Testing for carcinogenicity studies of pharmaceuticals
S1C	Dose selection for carcinogenicity studies of pharmaceuticals and limit dose
S2	*Genotoxicity studies*
S2A	Specific aspects of regulatory genotoxicity tests for pharmaceuticals
S2B	Genotoxicity: a standard battery
S3	*Toxicokinetics and pharmacokinetics*
S3A	Assessment of systemic exposure in toxicity studies
S3B	Pharmacokinetics: Guidance for repeated dose tissue distribution studies
S4	*Toxicity testing* *Duration of chronic toxicity testing in animals*
S5	*Reproductive toxicology* *Toxicity to reproduction for medicinal products and toxicity to male fertility*
S6	*Biotechnological products* *Preclinical safety evaluation of biotechnology-derived pharmaceuticals*
S7	*Pharmacology studies*
S7A	Safety pharmacology studies for human pharmaceuticals
S7B	The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals
S8	*Immunotoxicology studies* *Immunotoxicity studies for human pharmaceuticals*
S9	*Non clinical evaluation for anticancer pharmaceuticals*
S10	*Photosafety evaluations of pharmaceuticals*

1.1.3 Clinical trials

After the lead compound has been optimized and tested in the laboratory, and pharmacological studies have been conducted to show that the lead compound has the potential to become a drug, it is ready for clinical trial in humans (1). A clinical trial is a research study to answer specific questions about new therapies or vaccines, or new ways of using known treatments. Clinical trials are used to determine whether new drugs or treatments are both safe and effective. Carefully conducted clinical trials are the fastest and safest way to find treatments that work in people. Trials are in four phases: Phase I, tests a new drug or treatment in a small group; Phase II, expands the study to a larger group of people; Phase III, expands the study to an even larger group of people; and Phase IV, takes place after the drug or treatment has been licensed and marketed (1)(11).

The phase I clinical trial is the first experiment in which a drug is tested on the human body. The primary aim of the trial is to assess the safety of the new drug. Other areas of study include pharmacokinetics and pharmacodynamics in humans. Normally, healthy volunteers are recruited for phase I trial. In many cases, volunteers are compensated financially for participation in this trial. However, in some situations, patients who are critically ill or have terminal disease are presented with the option to be included in the trial after due consideration of the risk-benefit ratio. Generally, 20 to 100 volunteers are enrolled in a phase I trial. These studies usually start with very low doses, which are gradually increased. On average, about two thirds of phase I compounds will be found safe enough to progress to phase II.

The phase II clinical trials are controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks. A series of doses of varying strengths may be used. It is now common to conduct phase II trials with a control group in conjunction with the test group given the drug. The control group is given either the current standard treatment or placebo. Again, the risk-benefit profile has to be assessed as to whether the trial should use placebo or standard treatment to ensure the subjects' well being is not compromised during the trial. The result of the phase II trial is information needed to determine the effective dose and the dosing regimen of frequency and duration. Typically, phase II studies involve 100 to 300 patients who suffer from the condition the new drug is intended to treat. The success rate of phase I and II studies is estimated around 30%.

After the successful completion of the phase II trial, the objective of phase III is to confirm the efficacy of the drug in a large patient group. Phase III trials are the final step before seeking marketing approval. During phase III, researchers try to confirm previous findings in a larger population. These studies usually last from 2 to 10 years and involve thousands of patients across multiple sites. Because, the results are crucial to the determination of the drug's effectiveness, the phase III trial is referred to as the pivotal trial, as it can make or break the success of a drug. The methodology of the trial has to be carefully prepared so that meaningful results can be gathered at the conclusion of the trial. Extensive statistical analyses are performed to evaluate the data. The study results provide comprehensive data for understanding the critical parameters of safety and effectiveness of the drug. These results enable the pharmaceutical company to set the dosage, treatment frequency, duration and target patient groups for the drug. Despite the intense scrutiny a product receives before undergoing expensive and extensive phase III testing, approximately 10% of medications fail in phase III trials.

Phase IV clinical trials are post-marketing approval trials to monitor the efficacy and side effects of the drug in an uncontrolled real-life situation. This is also known as a post-market surveillance trial. Post marketing surveillance is important, because even the most well designed phase III studies might not uncover every problem that could become apparent once a product is widely used. Furthermore, the new product might be more widely used by groups that might not have been well studied in the clinical trials, such as elderly patients. Information about the effectiveness of the drug compared with established treatment, side effects, patient's quality of life, and cost effectiveness is collated. Any adverse events are reported and acted on to ensure patients' welfare is not compromised by the drug (1)(8)(9)(12).

The main documents that most clinical trials for a drug development plan are based on, are the documents from the ICH. These relevant documents are listed below (13).

Table 2: Battery of ICH clinical studies

E1-E2	*Clinical safety*
E1	The extent of population exposure to assess clinical safety for drugs intended for long-term treatment of non-life threatening conditions
E2A	Clinical safety data management: Definitions and standards for expedited reporting
E2B	Maintenance of the clinical safety data management including data elements for transmission of individual case safety reports
E2C	Clinical safety data management: Periodic safety update reports for marketed drugs
E2D	Post-approval safety data management: Definitions and standards for expedited reporting
E2E	Pharmacovigilance planning
E2F	Development safety update report
E3	*Structure and content of clinical study reports*
E4	*Dose-response information to support drug registration*
E5	*Ethnic factors in the acceptability of foreign clinical data*
E6	*Good clinical practice*
E7-E11	*Clinical trials*
E7	Studies in support of special populations: Geriatrics
E8	General considerations for clinical trials
E9	Statistical principles for clinical trials
E10	Choice of control group and related issues in clinical trials
E11	Clinical investigation of medicinal products in the pediatric population
E12	*Clinical evaluation by therapeutic category*
E14	*Clinical evaluation* *The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non antiarrhytmic drugs*
E15-E16	*Pharmacogenomics*
E15	Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories
E16	Biomarkers Related to Drug or Biotechnology Product Development Context, Structure and Qualification Submissions

Requirements for the conduct of clinical trials in the EU are provided for "Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use" ("the Clinical Trials Directive") (14). The Clinical Trials Directive is concretized further by "Commission Directive 2005/28/EC of 8 April 2005" (15) laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of

such products" (Good Clinical Practice - "the GCP Directive"). Clinical trials performed in the European Union are required to be conducted in accordance with the Clinical Trials Directive.

The Clinical Trials Directive harmonizes the rules in the EU for the approval of a clinical trial conducted in a Member State. As regards national competent authorities, the details are set out in the "Commission Detailed guidance on the request to the competent authorities for authorization of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1)" published in EudraLex Volume 10 (16).

A normal course of event in initiating a clinical trial is for the sponsor (a organization or individual that initiates the clinical trial and finances the study) to select an investigator, an expert in the field of the disease to be investigated that, on the basis of the investigator's brochure information, supports the clinical trial design and who conducts the trial. The investigator responsibility is to ensure that GCP is being implemented during the course of the trial and the subjects' rights and welfare are respected. Another important point is that the investigator has to maintain impartiality. Investigator is not an employee of the company, to show that there is transparency and no conflict of interest, no is the financial gain if the drug is successful.

Once the sponsor and the investigator have prepared the trial protocol, all required documentation (clinical trial application) is submitted to the Independent Ethics Committee for approval in the hospitals and to the competent authority in each member state where the trial is to be conducted.

According Spanish legislation, in the time that this study was conducted, the following documents must to be included (17):

- **For authorization of Ethical Committee:**

Cover letter: It's a official letter where it's requested the clinical trial approval and where it's included all the hospitals where the clinical trial will be conducted and Ethical Committes involved.

Index of documentation

Official Request Form (according the European model and also in XML format)

Protocol: Its content and structure is on the basis of Directiva 2001/20/CE(14) and the ICH guideline of Good Clinical Practice (CPMP/ICH/135/95 Topic E6 step 5 Note of guidance on Good Clinical Practice)(18). This document sets out how a trial is to be

conducted. It contains the rationale for the clinical trial, the methodology on how the trial is designed, the number of subjects to be recruited, the endpoints, the statistical methods to be used to analyze the data, how the subjects are protected in the trial, informed consent and confidentiality, as well as welfare and frequency of monitoring.

Patient information sheet and Informed consent: Subjects are to be informed about the aims, methods, risks and benefits of the trial. The availability of alternatives should be explained to the subjects. Subjects should not be pressured into enrolling in the trial, but rather should voluntarily join in and should be able to leave the trial at any time without duress of penalty. For young and incapacitated people who are not able to understand the requirements and implications of the trial, proxy decision from their representatives must be obtained.

Investigator's brochure or summary of product characteristics: The investigator's brochure is a collection of information prepared and updated by the sponsor for the investigator and the regulatory authorities. The information consists of all the data relevant to the drug under investigation, including properties of the drug, the pharmacokinetic, pharmacodynamic and toxicity results on animals.

Documentation about the adequacy of facilities and researchers

Compensation for the investigators included in the study

Insurance policy

Subjects enrollment plan

Investigator's agreement form

- **For the authorization of Health Authorities:**

Cover letter

Index of documentation

Official Request Form (according the European model and also in XML format)

Protocol

Patient information sheet and Informed consent

Investigator's brochure or summary of product characteristics

Certification on Good Manufacturing Practices for the study drug

Ethical Committee approval

Hospital agreement from all centers involved in the study

Once the trial is completed as if it is terminated prematurely, the sponsor should ensure that the clinical trial reports are prepared and are provided to regulatory

agencies as specified by the relevant legal requirements. The developer must also ensure that reports of clinical trials included in marketing applications meet the standards of the ICH Guideline for Structure and Content of Clinical Study Reports.

1.1.4 Role of regulatory authorities: European Medicines Agency

Following successful clinical trials, the sponsor has to apply for authorization to market the drug in Europe. It is the role of public regulatory authorities to ensure that pharmaceutical companies comply with regulations. Regulatory authorities perform the watchdog role to ensure that animal studies comply with Good Laboratory Practice (GLP), clinical trials are performed in accordance with Good Clinical Practice (GCP), and drugs are manufactured under current Good Manufactured Practice (cGMP) conditions. A large body of legislation has developed around this principle, with the progressive harmonization of requirements for the granting of marketing authorizations since the 1960s, implemented across the European Community. The requirements and procedures for the marketing authorization for medicinal products for human use, as well as the rules for the constant supervision of products after they have been authorized, are primarily laid down in Directive 2001/83/EC (19) and in Regulation (EC) 726/2004 (20).

Community authorization procedures (centralized, mutual recognition) are in place since the mid-90s and in addition the system is supported by a Community regulatory agency in charge of providing the EU institutions with scientific advice on medicinal products: the European Medicines Agency (19).

Centralized procedure: Under the European community Regulation 726/2004 (20) and Directive 2004/27/EC (21), the Centralized procedure is a single authorization procedure that is mandatory for medicinal products of the following categories: derived from biotechnology processes, intended for the treatment of HIV/AIDS, cancer, diabetes or neurodegenerative disorders, orphan medicines.

Mutual recognition procedure: It's stated in Council Directive 93/39/EEC (22). A medicine is first authorized by one member state, according to the member state's own national procedure. The applicant can seek further authorizations through a mutual recognition procedure. When there is a dispute between member states on the issue of mutual recognition, the EMA is called upon to arbitrate, and its decision is binding on the member states.

Descentralized procedure: This is applicable where authorization has not yet been approved in any member state. The applicant may apply for simultaneous

authorization in more than one EU member state for medicines that do not fall within the mandatory scope of the centralized procedure (23).

According the ICH recommendations, sponsor must to submit the application following the common technical document (CTD) structure. The CTDs were implemented in July 2003. They are format-based documents for submission to the regulatory authorities; the country-specific process of review is not affected. The harmonized CTDs help to reduce cost and accelerate approval time. The CTD is structured in five modules: module 1 (regional administrative information specific to each country); module 2 (summary of quality, non clinical and clinical); module 3 (quality); module 4 (non clinical study reports) and module 5 (clinical study reports) (24).

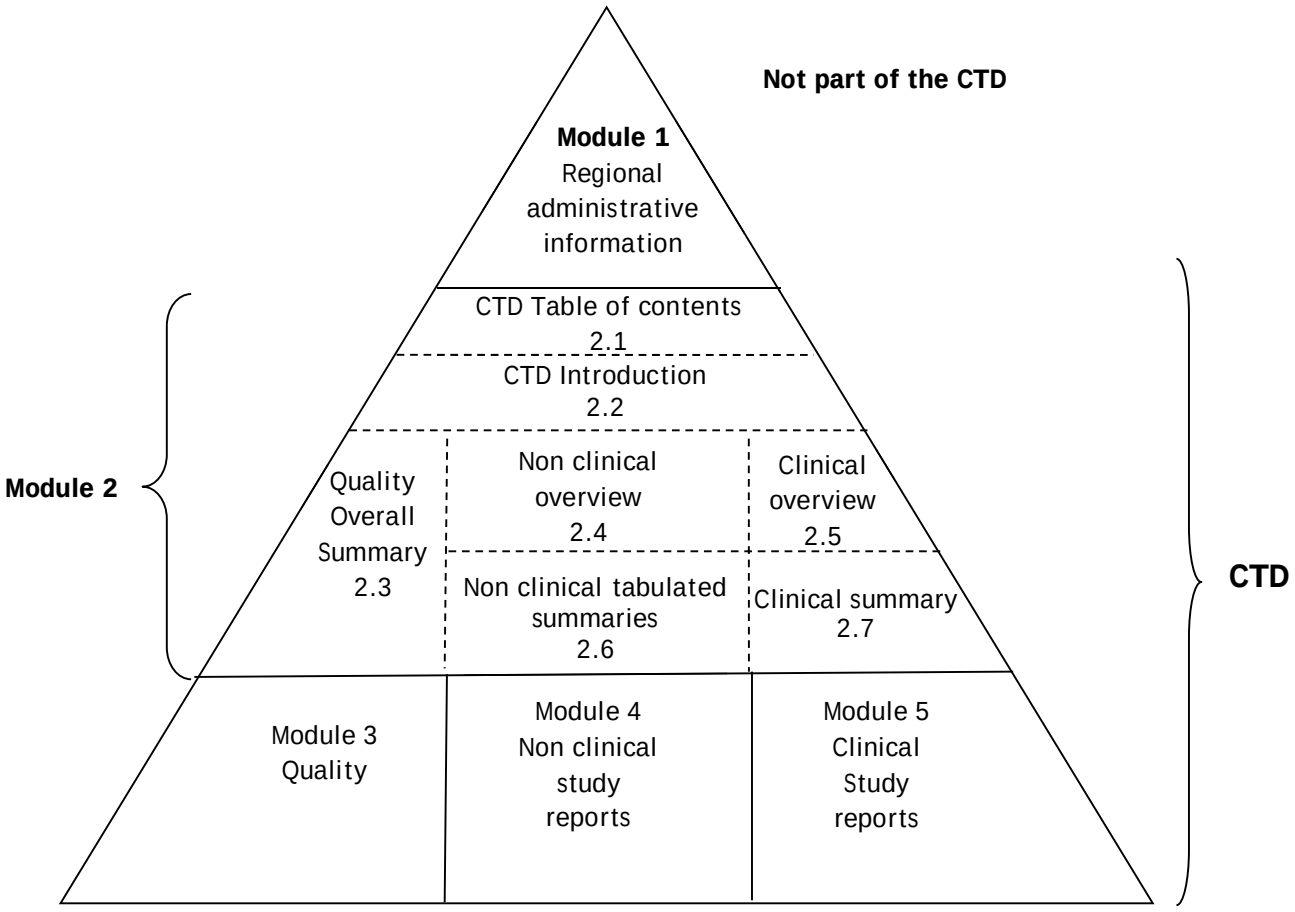

Figure 1: CTD structure

Assessment of the application by the Committee for Medicinal Products for Human Use (CHMP) is published initially as a Summary of Opinion-positive or negative. After the granting of a Marketing Authorization by the European Commission, a more detailed report is published as the European Public assessment Report (EPAR). The

EPAR shows the scientific conclusion reached by CHMP at the end of the centralized evaluation process. It is available to the public, with commercial confidential information deleted. The EPAR gives a summary of the reasons for the CHMP opinion in favor of granting a marketing authorization for a specific medicinal product. It results from the Committee's review of the documentation submitted by the applicant and from subsequent discussions held during CHMP meetings. The EPAR is updated throughout the authorization period as changes to the original terms and conditions of the authorization are made (1)(24).

1.2 Cardiac safety in drug development

It is generally easy to define the efficacy of a new therapeutic agent. However, what is even more difficult and more challenging, yet more important is to define its safety when administered to millions of patients with multi-faceted diseases, co-morbidities, sensitivities and concomitant medications. One of the most common cause of new drug discontinuations, cause for disapproval from marketing and removal from the market after approval, is a drug's effect on cardiac repolarization which is essentially identified by increasing the duration of the QT interval duration on the standard 12-lead electrocardiogram (ECG). A prolonged QT interval predisposes to the development of ventricular tachyarrhythmias such as torsades de pointes (TdP) and ventricular fibrillation, which could cause syncope, cardiac arrest or sudden cardiac death. Drug-induced QT prolongation and TdP has been recognized as a side effect of many commonly used medications. An up-date information can be found at the website www.qtdrugs.org supported by The Critical Path Institute and University of Arizona. The increasing incidence of polypharmacy in current clinical practice causes a need for further attention to side effects of drugs and their interactions, possibly leading to adverse fatal events (25)(26)(27).

1.2.1 History of drug induced-QT syndrome

Cases of life-threatening ventricular tachyarrhythmias and sudden death, associated with prescription drugs, have been reported since the 1960's, however this association was broadly recognized in the 1980's (28)(29)(30)(31)(32).

The first drug to be clearly associated with QT interval prolongation and TdP was quinidine, an extract of the cinchona bark. The drug was originally developed as an antimalarial (33), a purpose for which it continues to be used. Indeed, even in the initial use of the drug for a range of infectious diseases (malaria, typhoid fever, scarlet fever) in the 19th century, sudden deaths were reported (34). The drug began to be used for conversion of atrial fibrilation to normal rhythm in the early 20th century by the Dutch cardiologists (35). In a summary of the first 460 cases reported in the literature, Levy (34) identified five cases of abrupt syncope or sudden death, just over 1%. He presented a case of a woman with atrial fibrilation treated with quinidine for 6 days, after which normal rhythm was observed; shortly thereafter, the patient developed abrupt loss of consciousness and seizure-like activity; there seems little doubt that this represents a case of TdP occurring after conversion from atrial

fibrilation to sinus rhythm. The arrhythmia responsible for quinidine syncope was not documented until the advent of online monitoring in the 1960s. In 1964, Selzer and Wray (28) identified “paroxysmal ventricular fibrillation” in eight patients with quinidine syncope; a review of the published tracings shows typical episodes of what we would now call quinidine-associated marked QT prolongation and TdP, although no specific mention of the QT interval was made at the time.

A variety of drugs prolong the QT interval, although the major example is the so-called class III antiarrhytmics. These drugs generally exert therapeutic effect by affecting potassium ion channels, thereby, reducing the outward repolarising current, and prolonging the action potential duration and the QT interval in the electrocardiogram (ECG). Many of these drugs have been developed for conversion of atrial fibrillation and/or mainteinance of sinus rhythm in patients with recurrent atrial fibrillation. However, these anti-arrhytmic drugs, which prolong cardiac repolarization, are not harmless, as they may induce the potentially fatal arrithmia, torsade de pointes. Antiarrhythmic drugs with QT interval prolonging potential carry a 1 to 3% risk of TdP over 1 to 2 years of exposure (36)(37)(38), with the exception of amiodarone (39).

Drug induced long QT syndrome also occurs with drugs not prescribed for cardiovascular indications, and the incidence seems orders of magnitude lower. The first such “noncardiovascular” drugs to be implicated in QT prolongation and TdP were antipsychotic agents, specifically thioridazine (40)(41). Since the 1960s, drug induced long QT syndrome has been reported with dozens of marketed noncardiovascular drugs in many therapeutic categories. Although the idea that noncardiovascular drugs could also prolong the QT interval and trigger TdP or even sudden death was recognized during the 1970s and 1980s, it remained an electrocardiographic curiosity until the initial report of terfenadine-associated TdP in the late 1980s (42)(43). Terfenadine was so widely used that it was being considered for over-the-counter status at the time. After further QT studies with terfenadine, a prolongation on QT interval was demonstrated. Although QT prolongation was known and production of TdP was uncontested, terfenadine remained on the market (“appropriately” labeled) until another nonsedating antihistamine that did not have the QTc effects became available. Finally, in January (1997), FDA proposed removing all terfenadine products from the marketplace because of the approval of a safer alternative drug: fexofenadine. Terfenadine was withdrawn after reported 350 deaths attributed to the drug, so the recognition of this rare serious adverse event was an

initiator for the regulatory and drug development issues discussed below (39). The medical community and regulatory agencies became more sensitive to the possibility that drugs causing QT prolongation might increase the risk of sudden death (32).

Nine drugs have been withdrawn from the market worldwide over the past years because of this problem: terolidine, lidoflazine, terfenadine, astemizole, grepafloxacin, droperidol, sertindole, levomethadyl and cisapride (25).

Yet, other drugs such as beripril, moxifloxacin, and newer antiarrhytmic drugs have been approved for marketing despite known effects on increasing QTc interval duration with some having known TdP associated with their use before approval. These decisions were primarily based on an analysis of the risk versus benefit of a drug. Such assessment is a difficult task and subject always to increasing knowledge and experience (25)(32)(39).

1.2.2 Electrophysiology of cardiac activity

pacemaker cells of sino-atrial node. An efficient propagation of the electrical waveform initiated by this spontaneous electrical activity (action potentials) to the atria and, via the cardiac conduction system, atrioventricular node, Bundle of His, Bundle branches, and Purkinje fibre network, to the rest of the heart brings about a highly coordinated rhythmic mechanical activity characterised by synchronised contraction and relaxation of different regions of heart. The classical work of Carl Wiggers (44) on cardiac cycle identified the correlation between the electrical activity and different parameters of mechanical activity, making it evident that the electrical cycle initiates the mechanical cycle and its importance in the heart diseases (45).

In 1887, the first recording of electrical activity of the human heart was done using Waller's capillary meter. The recorded signal included four waves called A, B, C and D. Einthoven mathematically modified this signal to correct the inertia associated with the movement of the mercury column in the capillary electrometer. To avoid confusion with Waller's recording, Einthoven named the five identified deflections P, Q, R, S and T, having used the O point as the origin of the time scale (by mathematical convention O is used for the origin of the Cartesian coordinates) (46)(47).

The electrical cycle, recorded with an ECG, begins with the P wave, reflecting the atrial depolarization. The PR interval, the time taken for atrial depolarisation to reach the atrioventricular node and beyond, is characteristically well preserved in

different mammals. The QRS complex represents entry of the wave of excitation via the cardiac conduction system into the ventricles and depolarisation of ventricular myocytes. Subsequent repolarisation of the ventricles is reflected in the T wave, which is often followed by U wave, the cause of which is still unclear (45). The QT interval is a measure of the time between the start of the Q wave and the end of the T wave on the ECG. The duration of the QT interval is a representation of the ventricular action potential duration, the time during which the ventricles depolarize and repolarise. The ventricular activation, determined by cardiac action potential duration, is a complex physiological process. The mammalian heart operates as an electromechanical pump, the proper functioning of which depends critically on the sequential activation of cells throughout the myocardium and the coordinated activation of the ventricles. It is delay in cardiac repolarization, which creates an electrophysiological environment, which favours the development of cardiac arrhythmias. Since, the measured QT interval is inversely related to heart rate, it is by convention corrected for heart rate. Various formulae are used for measuring the corrected QT (QTc) (25)(45)(48).

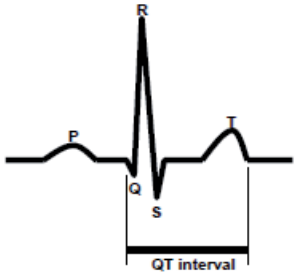

Figure 2. Representation of a normal ECG

To understand how these action potentials are generated, it's necessary to know the electrochemical processes that happen in heart cells. Electrical signaling in the heart is mediated through regenerative action potentials that reflect the synchronized activity of multiple ion channels that open, close, and inactive in response to changes in membrane potential. The cell membrane is made of lipids and as such is a perfectly hydrophobic milieu, which hydrophilic ions cannot directly cross. To penetrate the cell membrane, ions need to find hydrophilic pathways, which are formed by specialized proteins (the ion channels). It happens that the ion channel pathway is not permanently available, but inversely flips between an open and a closed state. Once a hydrophilic pathway is available (the channel is open), ions move passively across the cell membrane depending on their respective electrochemical gradient. If the

gradient for a given ion species is directed inward then ions enter the cell. If the gradient is outward then ions leave the cell. "Electrochemical" means that two independent forces can move ions across the membrane: the electrical gradient and the chemical gradient.

The chemical gradient makes ions move from a compartment of higher concentration to a compartment of lower concentration (according to their chemical gradient K+ ions are keen to move from the intracellular to the extracellular compartment; inversely Na+ ions are keen to move from the extracellular to the intracellular compartment).

The electrical gradient makes ions move in the direction of their inverse sign (negative for a cation and positive for an anion). A negatively charged compartment will attract cations but reject anions.

In some instances, the electrical gradient and the chemical gradient can oppose each other and eventually be equal; in this situation the force promoting the move of an ion in one direction equals that promoting its move in the reverse direction. Equilibrium is so reached. Therefore, there is a transmembrane potential value for which the electrical gradient perfectly opposes the chemical gradient and permits the equilibrium of an ion species (49).

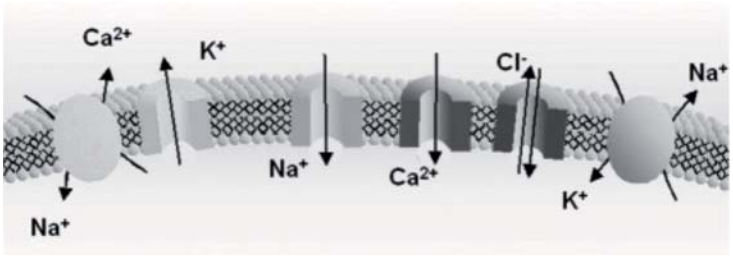

Figure 3. Representation of the ion and electrical transmembrane gradient of the cardiomyocyte (49)

Electrophysiological studies have detailed the properties of the major voltage-gated inward (Na+ and Ca+) and outward (K+) currents, which determine the heights and the duration of cardiac action potentials. In contrast to the Na+ y Ca2+ currents, there are multiple types of myocardial K+ currents, particularly K voltage (Kv) currents. A rapidly activating and inactivating transient outward current (Ito1) and several components of delayed rectification, ultrarapid (IKur), rapid (IKr) and slow (IKs) have been distinguished. In addition to Kv currents, the inwardly rectifying K+ (Kir) current (IK1) plays a role in myocardial action potential repolarization. The fact that channel conductance is high at negative membrane potentials underlies the

contribution of IK1 to resting membrane potentials. Because the driving force on K+ is high at depolarized potentials, IK1 channels do contribute outward K+ current during the plateau phase of the action potential, as well as during phase 3 repolarization. There are other known K+ currents, the ligand gated channels, that include those activated by a decrease in the intracellular concentration of adenosine triphosphate (KATP) or activated by acetylcholine (KAch) (50)(51)(52).

The rapid upstroke of the action potential (phase 0) in ventricular and atrial cells is attributed to inward currents through voltage-gated Na+ (Nav) channels. Phase 0 is followed by a rapid phase of repolarization (phase 1), reflecting Nav channel inactivation and the activation of voltage-gated outward K+ (Kv) currents: Ito1 and IKur. During phase 2 inward depolarizing currents through Na+ (slowly inactivated) and L-type Ca2+ channels (ICaL) are balanced by the different components of the delayed rectifier K+ current IKur, IKr and IKs. The terminal phase 3 of repolarization is due to the increasing conductance of the IKr, IKs components of the delayed rectifier and the inward rectifier (IK1). IK1 is also responsible for the maintenance of the resting potential.

The height and duration of the plateau, as well as the time-and voltage-dependent properties of the underlying Na+, Ca2+ and K+ channels determine action potential durations in individual cardiac cells. Changes in the properties of these channels owing to underlying cardiac result or as a result of the actions of cardiac and noncardiac drugs, therefore, is expected to have dramatic effects on action potential waveforms, refractory periods and cardiac rhythms. There are marked regional differences in action potential waveforms in the myocardium, and these contribute to the normal propagation of activity through the heart and the generation of normal cardiac rhythms (25)(50).

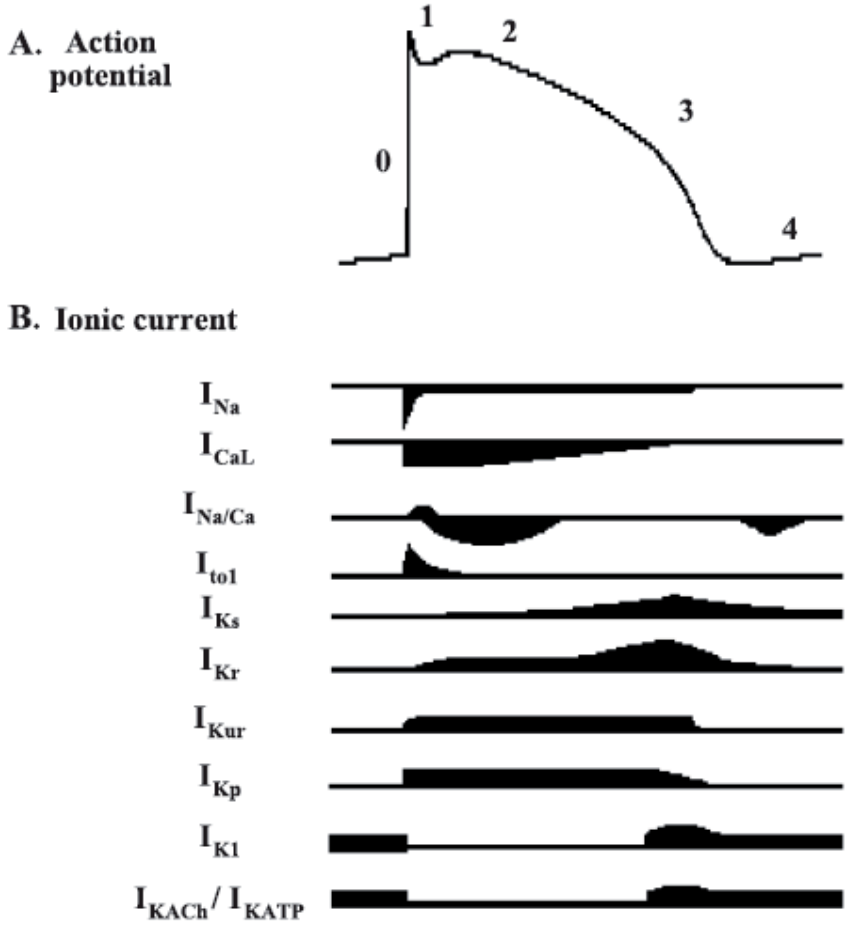

Figure 4. Action potentials and underlying ionic currents in atrial and ventricular myocytes (50)

Therefore, multiple types of voltage-gated inward and outward currents contribute to action potential repolarization in the mammalian myocardium. Cardiac myocytes express a repertoire of Kv channels/currents that contribute importantly to shaping the waveforms of action potentials, as well as influencing automaticity and refractoriness. Because of this, changes in the properties of cardiac Kv channels have rather dramatic effects on myocardial action potential waveforms and the generation of normal cardiac rhythms. In addition to the demonstrated importance of repolarizing Kv channels, however is also quite clear that Cav channel currents and Nav channel "window" current also contribute importantly to action potential repolarization. This has been demonstrated for Nav channels with the characterization of inherited mutations in the cardiac Nav SCN5A gene, mutations that underlie Long QT3, Brugada syndrome and conduction defects. Functional characterization of these mutants and computer simulations of cellular electrical activity together have provided new insights into the effects of altered channel functioning on action potential waveforms and rhythmicity. These studies

demonstrate that small changes in Nav channel currents can have profound effects on repolarization because the plateau phase of the action potential is maintained by the delicate balance of small (inward and outward) currents. It is very clear therefore, drugs that affect Nav channel currents will influence action potential durations. Because cardiac Cav channels also control the plateau phase of cardiac action potentials and action potential repolarization, changes in Cav channel currents will also have functional consequences. Indeed, it seems reasonable to suggest that drugs that affect the functional expression and/or the properties of any of the (inward or outward current) channels that contribute to shaping action potentials would have been expected to impact the propagation of activity and the generation of cardiac rhythms. When considering screening of noncardiac (as well as cardiac) drugs, therefore, effects on all of the various cardiac ion channels that contribute to repolarization should be considered.

In addition to the diversity of voltage-gated ion channel, molecular and biochemical studies have now demonstrated that there are multiple accessory subunits that contribute to the formation of the various cardiac inward and outward current channels. Although it seems quite clear that the relationship between channel subunits and regulatory molecules are important in determining channel expression, very little is presently known about their molecular interactions and the role of these interactions in determining the functioning of the various cardiac ion channels involved in mediating repolarization. Nevertheless, these channel subunit-subunit and channel subunit-regulatory protein interactions are also potential sites of action of cardiac and noncardiac drugs. Probing these molecular mechanisms in detail is requisite to understanding the factors controlling channel expression. Clearly a major focus of future research will be on defining the molecular mechanisms controlling the properties and the functioning of myocardial ion channels in great detail (25).

1.2.3 Role of electrical activity in long QT interval and TdP

The configuration and duration of the cardiac action potentials vary considerably among species and different cardiac regions (atria versus ventricle) and specific areas within those regions (epicardium, midmyocardium (M) and endocardium).

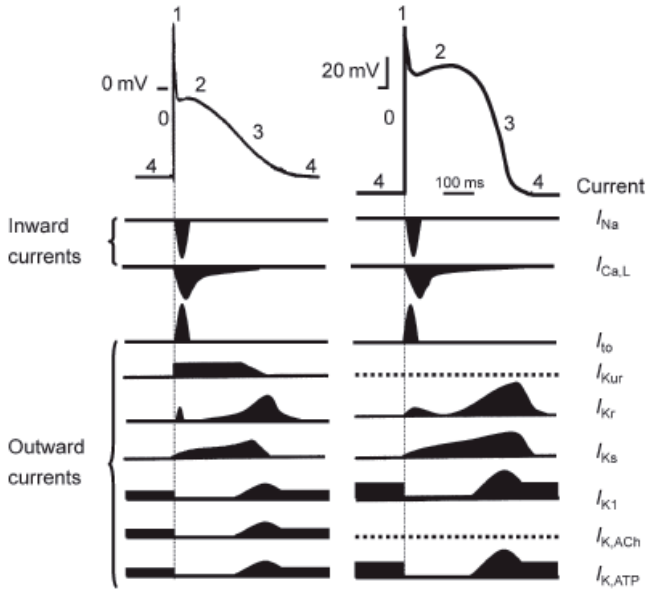

Figure 5. Inward, depolarizing and outward, repolarizing currents that underlie the atrial and ventricular action potential (53)

One of the major discoveries in basic electrophysiology was the identification of the different types of myocardial cells across the ventricular wall, as well as the description of their electrical characteristics: the epicardial, midmyocardium and endocardial cells. The differences between the shapes of the action potentials of these three types of cells reside in the differences in phase 1 and phase 3. Epicardial and M cells have a prominent dome during phase 1, explained by more pronounced outward Ito currents. M cells have action potential prolonging more than epicardial and endocardial cells when the heart rate slows down or in reaction to agent that prolong action potential duration. This heterogeneity mainly reflects differences in the type and/or expression patterns of the K+ channels that participate in the genesis of the cardiac action potential, mainly IKr and IKs (53)(54)(55)(56)(57).

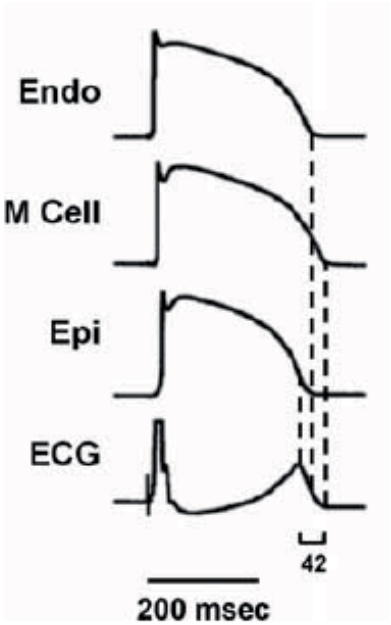

Figure 6. Transmembrane action potentials in the different types of cells and transmural ECG (57)

An alteration in these channels may prolong the action potential duration and alter the cardiac repolarization. The best studied of the channelopathies are the long QT syndromes (LQTS). The congenital long QT syndromes are familiar disorders characterized by QT prolongation and a propensity to ventricular tachyarrhythmias (usually torsades de pointes) frequently leading to sudden death at a young age. Identification of specific ion channel abnormalities causing QT prolongation in LQTS has increased our understanding of mechanisms related to myocardial electrophysiology and cardiac arrhythmias. The congenital syndromes of QT prolongation (cLQTS) associated with a high risk of sudden death were first described in the 1950s and 1960s (Jervell and Lange-Nielsen, 1957 (58); Romano, 1963 (59); Ward, 1964 (60)). Mutations in 13 genes are now recognized as causes of the cLQTS (39). Six of these encode a voltage-gated ion channel, including KCNQ1 (KvLQT1) and KCNE1 (minK) KCNH2 and KCNE2, the genes encoding α and ß subunit of the delayed rectifier potassium channels that conduct IKr and IKs, and the remaining seven encode proteins that modulate ion channel function. It is clear that mutation induced reduction in these key currents underlies the disease phenotype; however, the prevalence of these inherited disorders is rare (54)(55)(61)(62).

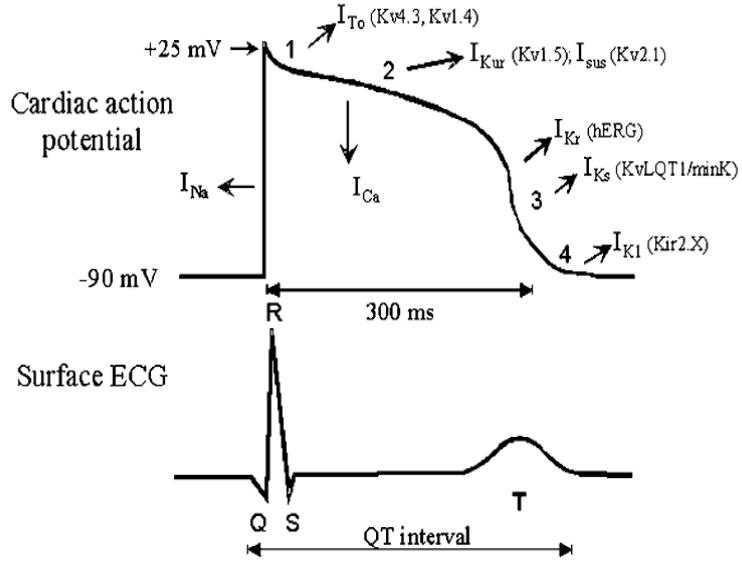

Figure 7. Relationship among cardiac membrane currents, action potential duration and the QT interval of the ECG. The commonly used gene names are in parentheses (25)

A more common and related disorder is excessive delay in repolarization caused inadvertently by drug-induced reduction in the repolarizarizing cardiac membrane current IKr. IKr is the product of the human ether-a-go-go gene (hERG) and, also, mutations in hERG have been linked to a form of hereditary long QT syndrome. The logical expansion was for the hERG product to be a molecular target for drug-induced acquired long QT syndrome (63). The study of these disorders has led, in turn, to a vastly improved understanding of the role of individual ion currents in control of the cardiac action potential and thus the QT interval on the surface electrocardiogram and it has had important implications for drug development and approval (64)(65)(66)(67).

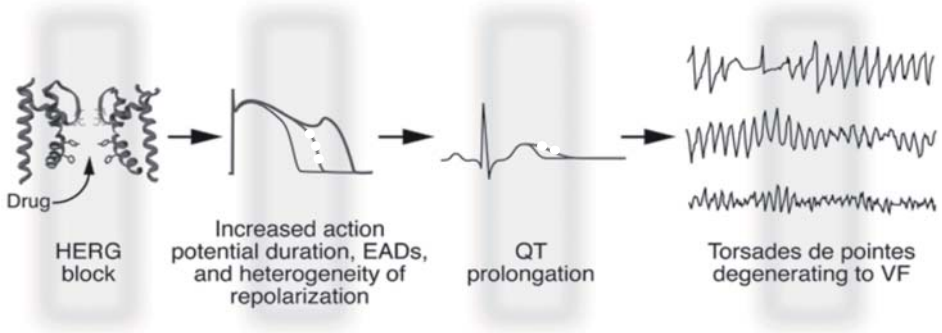

Figure 8: Mechanisms of sudden death with HERG blockade. Drug blockade of the HERG channel (left) produces prolongation (discontinued line) and an EAD (continued line) in the cardiac action potential. With permission (68).

Ikr block and QT prolongation have attracted considerable attention in recent years as a result of their association with life-threatening cardiac arrhythmias such as the torsades de pointes (TdP). QT interval prolongation is used as a surrogate biomarker for the assessment of the risk of TdP in drug-safety studies. The TdP describes ventricular tachycardia with the cardiac axis rotating, changing from one direction to another and back again. It's an uncommon polymorphic ventricular tachycardia that by definition is associated with baseline QT prolongation. First reported in 1966 by Dessertenne in an 80-yr-old woman. The specific morphology of the malignant arrhythmias was suspected to be generated by two competing foci leading to presentation with changing QRS morphology (29). This hypothesis was confirmed 15 yr later in a canine model where the TdP could be recorded from both the left and right ventricular sites at a similar, but periodically changing rate (69).

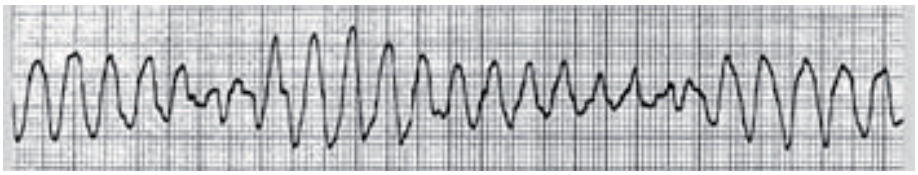

Figure 9. Torsade de pointes

Since the original work by Dessertenne(29), it has been well recognised that many conditions may cause prolonged or abnormal repolarisation (that is, QT interval prolongation and/or abnormal T or T/U wave morphology), which is associated with TdP. If TdP is rapid or prolonged, it can lead to ventricular fibrillation and sudden cardiac death.

Most known cases of TdP have occurred when the QT interval exceeded 500 ms. Although QT prolongation by itself is not clearly associated with increased risk of TdP, a 1997 report by the Center for Propietary Medicinal Products (CPMP) stated that QTc is a significant risk factor for TdP when it is rises above 500 ms or when an increase of 60 ms or more is seen. A 60 ms change more reflects that the drug in question is responsible for the observed change rather than spontaneous variability because no data on a change by 60 ms and incidence of TdP are available (25).

In summary, the most likely primary event underlying the arrhythmia called *Torsade de Pointes*, is inhibition of the potassium current known as IKr. Because IKr plays a key role in repolarization of the cardiac action potential, selective inhibition of this current prolongs the action potential, and this is manifested as a prolongation of the QT interval on the electrocardiogram (70). Although drug-induced QT interval

prolongation is not a safety concern per se, in a small percentage of people it has been associated with TdP, which either spontaneously terminates or degenerates into ventricular fibrillation. It is also true to say that QT interval prolongation is associated with an increased death rate (71) whether there is a proven link to TdP or not (72).

However, not all medications that block Ikr are associated with TdP, which means that IKr blockade and QT prolongation might not be sufficient to trigger TdP e.g., if it is offset by block of sodium and/or sodium and/or calcium currents. So, the effect of a drug on action potential duration (APD) is determined by the balance between its action to alter inward and outward currents. A prolonged APD can be caused by drug effects on a single, or on many ion channels, pumps or exchangers (55).

Furthermore, QT prolongation must be accompanied by a significant increase in spatial dispersion of repolarization to create conditions suitable for the development of TdP. TdP most commonly develops in patients receiving an IKr blocker, that induces early after depolarizations (EAD) and triggered activity in M cells. An EAD-induced extrasystole is believed to be responsible for the premature beat that initiates TdP, but the maintenance of the arrhythmia is generally thought to be a result of circus movement re-entry (32). The role of the transmural dispersion of repolarization in the mechanism of acquired TdP arrhytmias is crucial, as Weissenburger et al documented in anesthetized dogs receiving sotalol. TdP was observed only in animals anesthesized using halothane but not pentobarbital, due to the fact that halothane was associated with significant increased transmural dispersion of repolarization in comparison to pentobarbital (73).

There are significant differences in repolarization in the various layers of the myocardium, as previously we comment, with the epicardial cells having the shortest action potential duration, endocardial cells having an intermediate duration, and M cells having the longest action potential duration (74)(75). QT duration on ECG represents the longest repolarization in the M cell zone. This physiologic transmural dispersion of repolarization usually does not lead to TdP. However, proarrhythmic states cause, as a result of specific gene mutations or actions of medications, selective action potential prolongation in certain areas of the heart (usually M cells) that lead to increased transmural repolarization gradients (55). This increased transmural gradient may contribute to reentrant arrhythmias leading to TdP. For example, amiodarone is known to prolong QT duration, but since this drug is not increasing transmural heterogeneity of repolarization (or it might decrease it), TdP is not observed in patients taking this drug. Similarly, novel compound ranolazine may

increase QT duration but simultaneously decreases heterogeneity of repolarization. Interestingly both of these drugs are mild calcium and sodium channel blockers, which might contribute to a decreased propensity to proarrhythmias (32)(76).

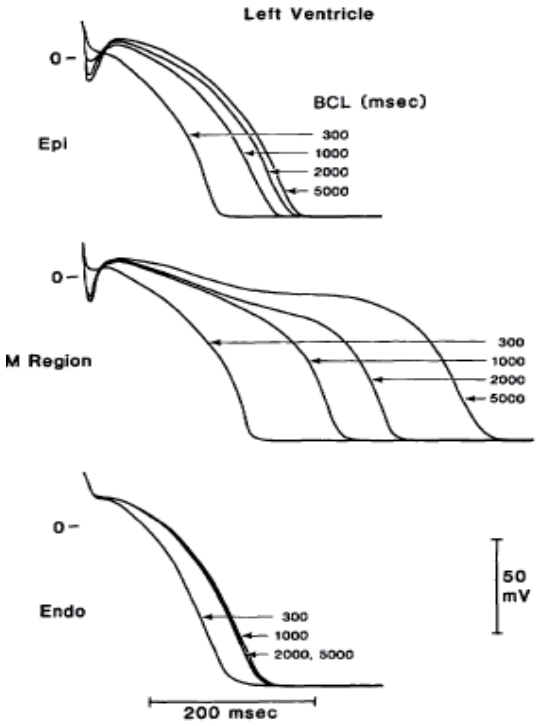

Figure 10. Transmembrane activity recorded from epicardial, endocardial, and M cells at basic cycle lengths of 300, 1,000, 2,000, and 5,000 msec (steady-state conditions). With permission (77)

Another important myocardial dysfunction that is likely to play a role in the triggering of TdP is the inter-ventricle dispersion, as it was demonstrated by experiments involving drugs such as sotalol and almokalant. Some studies revealed that the occurrence of spontaneous TdP is lower with sotalol; and sotalol was associated with significantly lower inter-ventricle dispersion than almokalant. Studies conducted in heart of canine models with chronic atrioventricular (AV) blocks and after almokalant revealed spontanous triggering of TdP following a consistent mechanism: increase of dispersion of action potential duration (APD) between the left and right ventricles, occurrence of EADs increasing further endocardial inter-ventricle dispersion, and finally genesis of ventricular ectopic beats initiation TdP (78). Furthermore, transmembrane APDs recorded from the right ventricle are usually longer than those from the left, and APDs from the apical regions are generally longer than those recorded near the base. Such apico-basal repolarization gradients have been proposed to contribute to the electrocardiographic T wave, although the magnitude and

direction of ventricular gradients within the hearts remain poorly defined. Lacking are data relative to transmural gradients across the ventricular wall. As a consequence, the mechanisms responsible for the T wave under normal as well as pathophysiological conditions are still not well understood. In order to evaluate how do these distinctions in repolarization time contribute to the inscription of the T wave, the arterially-perfused canine left ventricular (LV) wedge preparation was developed to address these issues, due to that permits direct temporal correlation of cellular transmembrane and ECG events. Floating microelectrodes were used to record transmembrane APs simultaneously from epicardial, M-region, and endocardial sites or subendocardial Purkinje fibers. A transmural ECG was recorded concurrently (79). Studies involving the arterially perfused wedge have provided significant insight into the cellular basis of the T wave showing that currents flowing down voltage gradients on either side of the M region are in large part responsible for the T wave. The interplay between these opposing forces establishes the height and width of the T wave and the degree to which either the ascending or descending limb of the T wave is interrupted, leading to a bifurcated appearance of the T wave (25)(79).

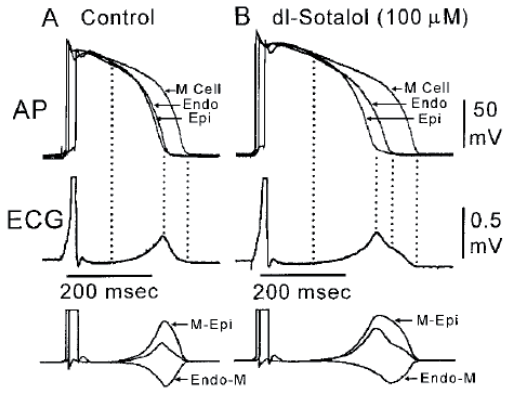

Figure 11. Interruption of the descending limb of the T wave. Each panel shows APs recorded from epicardial (Epi), M-region (M), and endocardial (Endo) sites (top), a transmural ECG (middle), and the calculated voltage differences between epicardial and M-cell APs (M-Epi) and between the M-cell and endocardial responses (Endo-M) (bottom). With permission (79)

Recent findings clearly indicate that what many clinicians refer to as an accentuated or inverted U wave is not a U wave, but rather a component of the T wave whose descending or ascending limb (especially during hypokalemia) is interrupted. A transient reversal in current flow across the ventricular wall caused by shifthing voltage gradients between epicardium and the M region and endocardium and the M region underlies this phenomenon. The data suggest that the pathophysiologic U wave that develops under conditions of acquired or congenital long QT syndrome is part of the T wave and that the various hump morphologies represent different levels of interruption of the ascending limb of the T wave, arguing for use of the term T2 in place of U to describe these events. The distinction between a T2 and U wave is critically important from the standpoint of identifying potential risk of agents that prolong the QT interval. QT may be grossly underestimated if a T2 is judged to be a U wave and excluded from the measurement of the QT interval.

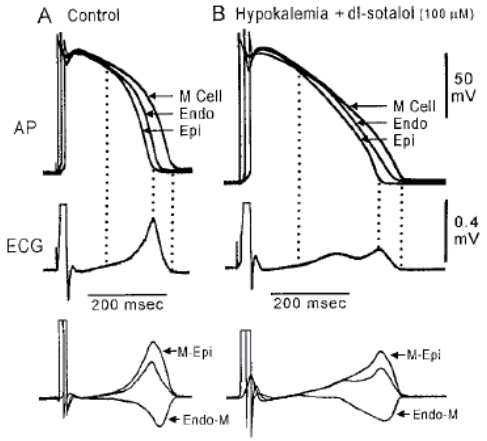

Figure 12. Interruption of the ascending limb of the T wave following exposure to dl-sotalol during the transition from normokalemia to severe hypokalemia (4.0 to 1.5 mmol/L). Each panel shows superimposed APs recorded from epicardial (Epi), M-region (M), and endocardial (Endo) sites and a transmural ECG. With permission (79)

In conclusion, prolongation of the QT interval in the ECG occurs when the action potential of a significant proportion of cells in the ventricular myocardium are prolonged, as a result of a reduction in one or more repolarizing currents and/or an augmentation of inward currents due to an alteration in the function of the ion channels (80)(81). The development of TdP is thought to arise as a consequence of this QT prolongation that favours an increase in dispersion of repolarization secondary to amplification of electrical heterogeneities intrinsic to ventricular myocardium.

1.2.4 Drug induced QT prolongation

Many of the drugs that were initially known to prolong the QT interval were antiarrhythmics, and quinidine was the most commonly implicated agent. Surprisingly, many non-cardiac drugs have also been reported to cause QT prolongation and/or TdP recently. In a survey in both the UK and Italy, non-cardiac drugs that have pro-arrhythmic potential (that is, have an official warning on QT prolongation or TdP, or with published data on QT prolongation, ventricular tachycardia, or class III effect) alone represented 3% and 2% of total prescriptions in both countries, respectively (82). The danger of drug induced pro-arrhythmia is therefore serious. This issue has been identified as a considerable public health problem and has attracted attention from the drug regulatory authorities. The exact incidence of drug induced TdP in the general population is largely unknown. Most of our understandings of the incidence, risk factors, and drug interaction of pro-arrhythmic drugs are derived form epidemiological studies, anecdotal case reports, clinical studies during drug development, and post-marketing surveillance. The awareness of drug induced TdP in the last few years has resulted in an increase in the number of spontaneous reports. Nevertheless, the absolute total number remains very low, although it has been suggested that the system of spontaneous reporting under-reports the true incidence of serious adverse reactions by a factor of at least 10 (83). Between 1983 and December 1999, 761 cases of TdP, of which 34 were fatal, were reported to the World Health Organization Drug Monitoring Centre by the member states (84). The WHO data provide an insight into the incidence of TdP on the most commonly reported pro-arrhythmic drugs. However, such a reporting system is undermined by the widely variable content and clinical information between different countries and sources. In all, 225 pharmaceutical compounds have been associated with torsade de pointes in spontaneous ADR reports collected by the WHO Drugs Monitoring Centre. Of the 20 most commonly reported drugs, 10 were cardiovascular agents and these appeared in 348 of the reports (46%). This presentation will mainly deal with non-cardiovascular drugs. The information on drug-associated TdP is constantly growing in line with the increasing awareness and concern (85). (available at https://crediblemeds.org/index.php/?cID=328).

Table 3: Drugs that can prolong QT interval and torsades de pointes (not comprehensive) (85)

Antiarrhytmic drugs Type 1[a] (TdP reported in all)	Quinidine (TdP reported) Procainamide (TdP reported) Disopyramide (TdP reported) Ajmaline (TdP reported)
Antiarrhythmic drugs Type 1C (increase QT by prolonging QRS interval)	Encainide Flecainide
Antiarrthmic drugs Type 3 (TdP reported in all)	Amiodarone Sotalol d-Sotalol Bretylium Ibutilide Dofetilide Amakalant Semantilide
Calcium channel blockers	Prenylamine (TdP reported, withdrawn) Bepridil (TdP reported, withdrawn) Terodiline (TdP reported, withdrawn)
Psychiatric drugs	Thioridazine (TdP reported) Chlorpromazine (TdP reported) Haloperidol (TdP reported) Droperidol (TdP reported) Amitriptyline Nortriptyline Imipramine (TdP reported) Desipramine (TdP reported) Clomipramine Maprotiline (TdP reported) Doxepin (TdP reported) Lithium (TdP reported) Chloral hydrate Sertindole (TdP reported, withdrawn in UK) Pimozide (TdP reported) Ziprasidone
Antihistamines	Terfenadine (TdP reported, withdrawn in USA) Astemizole (TdP reported) Diphenhydramine (TdP reported) Hydroxyzine Ebastine Loratadine Mizolastine
Antimicrobial and antimalarial drugs	Erythromycin (TdP reported) Clarithromycin (TdP reported) Ketoconazole Pentamidine (TdP reported) Quinine Chloroquine (TdP reported) Halofantrine (TdP reported) Amantadine (TdP reported) Sparfloxacin Grepafloxacin (TdP reported, withdrawn in UK and USA) Pentavalent antimonial meglumine
Serotonin agonists/antagonists	Ketanserin (TdP reported) Cisapride (TdP reported, withdrawn in UK and USA)
Immuosuppressant	Tacrolmus (TdP reported)
Antidiuretic hormone	Vasopessin (TdP reported)
Other gents	Adenosne Organophosphates Probucol (TdP reported) Papaverine (TdP reported) Cocaine

1.2.4.1 Antiarrhythmic drugs

All class I antiarrhythmic drugs have the potential to cause life-threatening ventricular proarrhythmias. The early landmark report by Selzer and Wray (28) observed that quinidine use was associated with syncope and ventricular fibrillation or flutter. It has been estimated that 1–8% of patients treated with quinidine will develop TdP (32). In their report, the risk of TdP with quinidine was not necessarily a consequence of excessive doses of the drug. Others have confirmed that TdP with class Ia drugs can occur at low therapeutic or subtherapeutic concentrations (86). Indeed most of the class Ia drugs, including quinidine, disopyramide, and procainamide, are similar in this regard. On the other hand, antiarrhythmic agents such as sotalol are associated with a greater incidence of TdP as the dose increases (85). One possible explanation for such discrepancy is that the blockade of sodium channels by class Ia drugs suppresses the QT prolonging effect at higher concentrations. Pure IKr potassium blocking antiarrhythmic drugs such as d-sotalol prolong the QT interval and induces TdP at an incidence directly proportionate to their concentration until the potassium currents are completely blocked.

It is noteworthy that while class Ia drugs are strongly concordant in their production of TdP, concordance with class III antiarrhythmic agents is less clear. For example, while both sotalol and amiodarone have the same potent effects on QT prolongation, the incidence of TdP is very low with amiodarone compared with sotalol. A literature review revealed that the incidence of TdP with amiodarone was only 0.7% in 17 uncontrolled studies (2878 patients) between 1982 and 1993, and that no pro-arrhythmia was reported in seven controlled studies (1464 patients) between 1987 and 1992. The risk of TdP with amiodarone mainly occurs in patients with other co-existing risk factors such as hypokalaemia or bradycardia. In contrast, d-sotalol has a 0.3% incidence rate of TdP for a daily dose of 80 mg, which rises to 3.8% for a daily dose of >680 mg. The risk is greater in female patients, and patients with reduced creatinine clearance, congestive heart failure, or sustained ventricular tachycardia. Similar to d,-sotalol, dofetilide also exhibits a dose dependent effect on QTc prolongation and TdP. The incidence of TdP ranges from 0-10,5% at doses <250 µg to >500 µg. Other new class III intravenous antiarrhythmics, such as ibutilide, are equally toxic in inducing TdP (85).

1.2.4.2 Antihistamines

Since 1986, certain non-sedating antihistamines, the so-called second generation antihistamines (mainly terfenadine and astemizole), have been reported to cause QT prolongation and, in some cases, TdP (87). The first 25 reported cases with terfenadine-associated TdP indicated that the parent substance, but not its main metabolite, was the problem (87), and the importance of pharmacokinetic interaction with ketaconazole was identified (88). These incidents have occurred when the recommended dose has been exceeded, at normal doses with concurrent use of drugs that inhibit hepatic cytochrome P450 enzymes (for example, imidazole, antifungals, and macrolide antibiotics), impaired liver function, or in patients with congenital long QT syndrome. Like class III antiarrhythmics, terfenadine and astemizole were found to prolong the monophasic action potential and QT interval, which led to the development of early after-depolarisation and TdP through inhibition of the IKr channel. As almost all of the non-sedating antihistamines were metabolised via the hepatic cytochrome P450 CYP3A4 system, concomitant administration of drugs or food (grapefruit juice) that inhibit the hepatic cytochrome P450 or severely compromise liver function may result in the accumulation of the parent drug and cardiotoxicity. Furthermore, co-administration of non-sedating antihistamines with other drugs that prolong the QT interval by the same or other mechanism (for example, antiarrhythmics, antipsychotics, tricyclic antidepressants) also increases their adverse effect on cardiac repolarisation. Other drug related factors such as the physicochemical properties of the antihistamines, their metabolic profile, and tissue distribution may also contribute to the cardiac response of antihistamines. Newer non-sedating antihistamines (loratadine, cetirizine, acrivastine, mizolastine, ebastine, and fexofenadine) continue to be introduced into the market. The cardiac safety profile of these newer non-sedating antihistamines requires confirmation. Antihistamines with low or no potential to block the K+ rectification channel (for example, IKr) channels are likely to possess cardiac safety advantages. The overall evidence so far indicates that the potential to cause TdP is not a class effect of non-sedating antihistamines; certain non-sedating antihistamines such as terfenadine and astemizole have potent pro-arrhythmic risk, whereas others have low risk (for example, azelastine, mizolastine) or are probably not associated with (for example, loratadine, cetirizine, ebastine and fexofenadine) QT prolongation, TdP or other ventricular arrhythmias. It should be emphasized that apart from the specific contraindications described, the incidence of cardiotoxicity with antihistamines is

very low in view of the widespread use of the drugs (89). Nevertheless, as they are widely prescribed for a self-limiting, non-fatal disease, the risk attributable must be assessed very carefully.

1.2.4.3 Antimicrobials

Macrolides (erythromycin, clarithromycin), fluoroquinolones, antifungals, and antimalarials have been implicated in predisposing to TdP as a result of QT prolongation (85)(32)(84)(90). Similar to class III antiarrhythmics and antihistamines, macrolides prolong the QT interval and cause dispersion of repolarisation across the ventricular wall, resulting in the induction of TdP.

In the case of fluoroquinolones, sparfloxacin lengthened the duration of the action potential in a concentration dependent manner (91), whereas ofloxacin and levofloxacin did not alter the action potential duration at a variety of concentrations (1–100 mM). Thus, sparfloxacin exerts a pure class III electrophysiological effect whereas levofloxacin and ofloxacin do not. Clinically, it is not yet known whether sparfloxacin will cause any spontaneous TdP, particularly in low risk patients (91). Grepafloxacin, a fluoroquinolone available in the UK since 1998, has recently been withdrawn voluntarily by its distributor because of its effect on QT prolongation and some reported cases of TdP. It is well established that moxifloxacin produces a predictable small prolongation of the corrected QT (QTc) interval by reversible and dose-dependent but weak blockage of the rapidly activating delayed rectifier potassium channel, IKr or human cloned counterpart, the hERG potassium channels. However, despite the observed QTc prolongation with moxifloxacin, there is no evidence that its use is associated with an increase in the overall number of cardiac events. It is interesting to note that moxifloxacin has been widely used in clinical studies as a positive control without any reports of TdP. Even in patients, no obvious association has been found between this drug and TdP in the absence of other risk factors. Probably, the lack of TdP reports for moxifloxacin is attributable to its predictable PK profile and other dose-limiting effects. Moxifloxacin, unlike many other fluoquinolones, lacks PK interactions with food or a number of drugs and therefore large variations in plasma exposure are not expected. In addition, it is possible that other side effects (e.g. dizziness, headache, diarrhea, and vomiting, etc.) would limit the possibility of higher drug exposures and, therefore, the risk of TdP. Therefore, the available evidence from preclinical and clinical studies suggested

that there are significant differences in the potency to prolong QT interval among the fluoroquinolones, and the risk of arrhythmias varies between drugs (92). Sporadic cases of TdP have been reported in association with most, but not all, fluoroquinolones. As a whole, apart from grepafloxacin and possibly sparfloxacin, the fluoroquinolones that are currently on the market or soon to be launched are safe from the point of view of QT prolongation and TdP, with a frequency of this adverse event generally occurring at a rate of about one per million prescriptions.

Antimalarials deserve some attention, as they are commonly prescribed worldwide. Quinine, quinidine, and halofantrine are capable of prolonging the QT interval. Quinine prolongs the QT interval at standard doses, as does halofantrine (60%). Halofantrine induces a dose related prolongation of the QT interval whereas mefloquine has no effect on QT interval (93).

The antifungal agents ketoconazole and itraconazole prolong the QT interval by blocking the IKr channels (94)(88)(94). Similar to macrolide antibiotics, ketoconazole and itraconazole also inhibit the hepatic cytochrome P450 CYP3A4 isoenzyme. Therefore, coadministration of ketoconazole or itraconazole with another QT prolonging drug that is metabolised by the cytochrome P450 CYP3A4 isoenzyme, such as terfenadine, will result in a notably prolonged QT interval and increase the risk of TdP (85).

1.2.4.4 Tricyclic antidepressants

The use of tricyclic antidepressants (TCAs) has raised some concern about their cardiotoxicity. The effect of TCAs on the QT interval have been investigated, but with mixed results (95)(96)(97). Amitriptyline, doxepin, desipramine, imipramine, and clomipramine have been associated with a prolonged QT interval, whereas dothiepin has no effect on QT interval. In children, concerns about possible TCA associated adverse effects were raised after a few cases of sudden death in children treated with TCAs (98)(99). The TCAs implicated were desipramine, clomipramine, and imipramine. These cases of sudden death occurred without acute overdose. A possible mechanism is the ‘‘fast’’ or ‘‘slow’’ metabolism of TCA by hepatic cytochromes (100). For example, impaired metabolism caused by a genetically determined ‘‘slow metaboliser’’ phenotype of cytochrome CYP2D6 is suggested as a possible mechanism for the apparent toxicity of these tricyclic antidepressants. Co-administration of drugs that can alter the concentrations of both parent drug and metabolites will therefore

affect the QTc interval (85). Evidence from cellular studies suggest that, similar to class Ic drugs, amitriptyline, phenothiazine, and fluoxetine induce cardiac sodium channel blockade and reduce Ito activation, which may shorten the action potential durations and induce an intramyocardial electrical gradient that produces the typical ECG changes described above. However, such ECG changes will probably only occur upon massive overdose of these drugs. Furthermore, it is also possible that some patients may have subclinical dysfunctional sodium channels that would be unmasked by these drugs. Nevertheless, further studies are required to investigate this phenomenon (85).

1.2.4.5 Neuroleptics

Neuroleptics have long been associated with sudden death and are reported to cause QT prolongation and TdP at therapeutic doses or in overdose (phenothiazines, thioridazine, haloperidol, chlorpromazine, trifluoperazine, pericycline, prochlorperazine, and fluphenazine) (101). Among them, thioridazine was the most potent in causing QT prolongation and arrhythmia. At both therapeutic and toxic doses, thioridazine can induce TdP (102). In the presence of hypokalaemia, TdP can develop even with a low dose (50 mg daily) of thioridazine (103). Sertindole is a relatively new atypical antipsychotic for the treatment of schizophrenia (104). Slight QT prolongation was seen with sertindole in early clinical trials although TdP was not reported in pivotal studies. However, 12 unexplained sudden deaths and 23 cases of syncope occurred among 1446 patients during the pre-marketing trials of sertindole. A total of 27 deaths associated with its use had been reported to the US Food and Drug Administration (FDA) by 1996. Although an independent review panel then did not find a causal relationship between sertindole and these deaths, in 1996 the drug was not approved for marketing in the USA. Nevertheless, sertindole was marketed in Europe. However, in the UK the Committee of Safety of Medicines was notified of 36 suspected adverse drug reactions with a fatal outcome by the end of November 1998. Not all of these reports were related to sudden cardiac death. In addition, 13 reports of serious but non-fatal cardiac arrhythmia were also reported in the UK during the same period. Because of the number of adverse drug events, fatal and non-fatal, reported since the marketing of sertindole in the UK, it was considered that the risks of treatment with this drug outweighed its benefits. The manufacturers of sertindole voluntarily suspended its marketing and use from December 1998 in the UK, pending further safety evaluations (85). It is now known that sertindole is a high affinity

antagonist of the human cardiac IKr potassium channel, and this blockade underlies, at least in part, the prolongation of the QT interval observed with this drug (105). Pimozide is a diphenylpiperidine neuroleptic agent with known cardiovascular side effects including QT prolongation. TdP has been described after acute poisoning. The risk of pimozide cardiotoxicity may be increased with the concomitant use of drugs that inhibit the cytochrome P450 CYP3A4 isoenzyme, for example, clarithromycin, ketoconazole, etc. Forty reports (16 fatal) of serious cardiac reactions (predominantly arrhythmias) with pimozide use were reported to Committee of Safety of Medicines between 1971 and 1995, and restricted labelling has now been introduced for pimozide in the UK (85).

1.2.4.6 Prokinetics

Cisapride is gastrointestinal prokinetic agent used to treat gastro-oesophageal reflux, gastroparesis, and childhood chronic intestinal pseudo-obstruction; it is structurally similar to procainamide. In the USA, while cisapride was being marketed from 1993 to 1999, the FDA received reports on a total of 341 individual patients who had serious adverse cardiac effects following the use of cisapride: 117 developed QT prolongation; 107 TdP; 16 polymorphic ventricular tachycardia; 27 ventricular tachycardia; 18 ventricular fibrillation; 25 cardiac arrest; 16 serious (unspecified) arrhythmia; and 15 sudden death (106). Eighty (23%) of the 341 patients died. Deaths were directly or indirectly associated with an arrhythmic event. Many of the patients (56%) were also taking an imidazole compound or a macrolide antibiotic, which could inhibit the P450 CYP3A4 isoenzyme that metabolises cisapride and results in increased serum concentrations. In the UK, since cisapride was first marketed in 1988 the Medicines Control Agency has received reports of 60 serious cardiac adverse drug reactions, five of which were fatal. These included 24 reactions comprising ventricular arrhythmias, sudden unexplained death, cardiac arrest, and QT prolongation. Worldwide, there have been 386 reports of serious ventricular arrhythmias associated with cisapride treatment, 125 of which were fatal, and 50 reports of sudden unexplained death. In the UK, several relabellings of cisapride were required following these incidences, which had a limited effect in reducing co-prescription of cisapride with contraindicated medication. Serious cardiovascular reactions, including fatalities, continued to be reported. As a result, the Medicines Control Agency suspended the product licences for cisapride in the UK in July 2000 as

the risks versus benefits balance was no longer considered favourable. Similarly, in the USA, the risk of fatal arrhythmia with cisapride was believed to outweigh the benefit for the approved indication, leading to the drug's discontinuation there (85). Cisapride inhibited the IKr current in isolated guinea pig ventricular myocytes in a concentration dependent manner with an IC50 of 15 nmol/l (therapeutic levels, 50–200 nmol/l). This explained the lengthening of cardiac repolarisation observed in patients receiving clinical doses of cisapride (107).

1.2.4.7 Other QT prolonging drugs

Early reports of TdP associated with cardiac drugs incriminated not only antiarrhythmics, but also antianginal agents such as bepridil and prenylamine, both of which have been well documented to cause TdP (85). These antianginal agents have now been withdrawn from the market in most regulatory jurisdictions.

Terodiline, an antispasmodic agent used to treat urinary incontinence, was withdrawn in the UK following 69 reported cases of serious arrhythmias. Fourteen of these patients had sudden death and the remaining 55 patients had non-fatal arrhythmias, including 37 with ventricular tachyarrhythmia of which 24 were caused by TdP. It is now clear that the pro-arrhythmic effect of terodiline is a consequence of the blockade of IKr current, which occurs in a concentration dependent manner (108).

With regards to antimigraine drugs, naratriptan, sumatriptan and zolmitriptan have all been shown to prolong the QT interval, but no cases of TdP are reported in the literature (84).

Tamoxifen, an anti-oestrogen drug commonly used to treat breast cancer, prolongs the QT interval at high doses (109) and has been demonstrated to block the IKr and calcium currents in rabbit myocytes (110), but has not been shown to induce TdP.

QT prolongation has been described with probucol, a cholesterol-lowering drug, since the early 1980s (111).

Tacrolimus, a macrolide used for prevention of hepatic allograft rejection, has also been described as the cause of TdP in a case report (112), and animal studies have shown a sustained QT prolongation after intravenous administration (113).

Certain inhalation anaesthetics, such as sevoflurane and isoflurane, prolong the QT interval (114)(115).

Also worth mentioning is that a Chinese herbal remedy that contains extract from the same root as is used in liquorice (116), as well as liquorice itself, maycause TdP, presumably through hypokalaemia (117).

1.2.5 Other risk factors to trigger Torsade de pointes

As we have just commented, some drugs affect IKr kinetics and prolongs the QT interval, but they rarely cause life-threatening arrhythmias. Susceptibility to drug induced QT prolongation and TdP is multifactorial and a combination of several factors is needed for arrhythmia to occur.

1.2.5.1 Genetic variations

Most likely genetic variations (not obligatory mutations causing LQTS) in genes encoding the function of ion channels operate as a key factor underlying susceptibility to drug-induced QT prolongation and TdP (68)(118). Virtually, all QT-prolonging drugs block the hERG potassium-ion channel. Variations (polymorphisms) in genes encoding ion channels may cause an increased sensitivity of these channels to drugs blocking IKr. Recent studies focused on the associations between genetic polymorphisms and QT duration provided proof of the concept of potential importance of genetic predisposition to QT prolongation in the general population (119)(120). Polymorphisms in genes encoding enzymes metabolizing drugs may increase serum levels of drugs to excessive levels blocking the channel (68). Borderline prolonged QT duration might be the phenotypic expression of such polymorphisms and therefore it is worth paying attention to a baseline ECG when prescribing drugs that could block IKr or could interfere with metabolism of drugs blocking potassium currents. The addition of a hERG blocker can be a second hit that will decrease the conductivity of their IKr channel, prolonging the cardiac action potential to the point that significant QT prolongation is seen, which in turn increases the risk of TdP. The field of pharmacogenomics is still in its infancy but there is growing evidence that genetic make-up might be critical for drug-induced QT prolongation (67)(84).

1.2.5.2 Women

Women account for 70% of cases of drug-induce QT prolongation and TdP indicating that sex-related differences in repolarization duration might predispose women to proarrhythmias (75). A summary of published data led Makkar et al. (121) to identify a 2:1 to 3:1 female predominance in drug induced LQTS. An increased risk of cardiac

events is also observed in women with some forms of the congenital LQTS but only after childhood (122). These clinical observations, coupled with the finding that the QT shortens after puberty in men but not women (123), suggest that sex hormones modulate repolarization. Testosterone, by increasing IKr and IKur, shortens QTc and has been implicated as the major factor lowering risk of TdP in men (124). Although sex hormones play a role in QTc differences between men and women, they explain only part of the observed differences: androgens are protective against drug-induced prolongation of repolarization, whereas estrogens seem to be proarrhythmic. Although sex differences in the density of ionic channels do exist (125), they seem to only partially explain women's increased risk of drug induced LQTS and TdP. This suggests that heretofore unrecognized mechanisms, such as modulation of the pharmacokinetics of IKr blockers, may be important in determining sex related differences in risk of developing drug-induced prolongation of the QT interval (126).

1.2.5.3 Bradycardia

Bradycardia is a commonly observed risk factor for drug induced LQTS and TdP. Although this association was first described over 40 years ago (29), remarkably little is known about the ECG predictors of this potentially lethal complication of bradyarrhythmias (127)(128). Indeed, the "short-long-short" series of cycles before TdP is so characteristic of drug induced LQTS that lack of a "pause" before onset calls into question the diagnosis. Bradycardia is largely caused by a slow sinus rhythm, hypothermia, or hypothyroidism, all of which can physiologically prolong ventricular repolarization and lead to a higher risk of TdP. However, a pause provoked by extrasystoles may be more important than bradycardia per se at the initiation of TdP (129). The association between this stereotypical series of cycle-length changes and the initiation of TdP (The "Short-Long-Short" Series of Cycling Changes before the Initiation of an Event) is probably a clue to underlying mechanisms (30) (31). These cycle-length changes have two major effects that could, in theory, promote TdP: 1) there is striking deformity of the pause after the QT interval, often with the development of a large U-wave (130)(131)(132), suggesting a role for after depolarizations and 2) the short-long sequence maximizes the heterogeneity of repolarization times of the last sinus beat, thereby increasing the likelihood of reentrant excitation (129).

1.2.5.4 Hypokalemia

Lowering of extracellular potassium decreases IKr, an effect that is likely to contribute to QT interval prolongation in hypokalemic patients (133). However, this effect on IKr is unexpected because simple electrochemical considerations predict an increase in outward potassium current with lowering of extracellular potassium. Two possible mechanisms have been advanced to explain this paradoxical behavior: one is that sodium and potassium ions compete for access to extracellular binding sites on the channel, and sodium is a potent blocker of the current (134). Consequently, when extracellular potassium is lowered, the inhibitory effect of sodium on IKr becomes more apparent. The second explanation involves the very rapid inactivation that IKr undergoes after opening during depolarizing pulses (135). Lowering of extracellular potassium enhances this fast inactivation, so with hypokalemia, more channels are in the inactivated state and fewer in the open configuration during depolarizing pulses. This very rapid inactivation also explains why KCNH2 channel, which generates IKr, plays such a key role in repolarization. Another twist on hypokalemia as a risk factor for TdP has been the observation that drug blockade is actually enhanced at low levels of extracellular potassium (133)(136). Thus, hypokalemia enhances TdP risk through at least two mechanisms: 1) decrease in the repolarizing current itself and 2) potentiation of drug blockade of residual current. Hypomagnesemia increases TdP risk, possibly by modulating the L-type calcium channel function that contributes to EADs. Recognition of hypomagnesemia as a contributor to acquired QT prolongation and TdP (31)(137) led to empiric testing of intravenous magnesium as a therapy (138). Although no randomized prospective trial has been conducted, intravenous magnesium has become a first-line therapy for TdP due to drug induced LQTS.

1.2.5.5 Atrial fibrillation conversion

The most common arrhythmia requiring drug therapy is atrial fibrillation (AF) (139)(140). However, many antiarrhythmic drugs block IKr as a major mechanism of action, and marked QT prolongation and TdP are the major class toxicities. Multiple lines of clinical evidence suggest that AF itself protects against TdP and that after conversion, risk is increased. One common clinical observation is that TdP often [but not always (141)] occurs in patients with AF after conversion to sinus rhythm (34)(142). This may reflect the decrease in heart rate that often accompanies such conversion, but studies conducted in the late 1990s indicate that the mechanisms

must be more complicated. In a small study, it was examined the extent of QT prolongation by intravenous dofetilide during AF and shortly after conversion to sinus rhythm. Despite the fact that dofetilide did not change heart rate, the extent of QT prolongation was much greater in sinus rhythm than in AF (143). Indeed, more recently, it was showed that QT-RR slopes are extraordinarily flat during AF (i.e., even with long pauses, the QT interval does not prolong) and steepen very sharply, to greater than normal values, shortly after conversion to sinus rhythm (144). These authors infer that AF itself may exert a heretofore poorly understood influence on the QT interval both during arrhythmia and shortly after its conversion to normal rhythm. AF is associated with significant atrial remodeling that includes alterations in L-type calcium current, inward rectifier current, transient outward current, and ultrarapid delayed rectifier current (among other changes) (145). The magnitude of these changes is sufficient to alter responses to antiarrhythmic drugs. However, molecular changes in the left ventricle secondary to AF are less well established. Nonetheless, there are echocardiographic data that links AF, atrial remodeling, reverse remodeling, and changes in left ventricular systolic function (146). Although the precise mechanism by which AF reduces susceptibility to TdP is unknown, AF-associated electrophysiological and cellular remodeling may be associated with reduced susceptibility to the development of TdP among patients with drug induced LQTS (32).

1.2.5.6 Drug Metabolism

Another factor to be taking account is the role of variable drug concentrations in TdP risk. Initial reports with quinidine noted that the adverse effect often occurred within 24 h of starting the drug, at a time when excessive accumulation of drug (or potentially active metabolites) would not be expected. Indeed, with routine plasma concentration monitoring came the frequent observations of “subtherapeutic” quinidine concentrations in patients developing TdP (34)(147)(30). Studies as early as the 1940s (148)(149) identified multiple quinidine metabolites, raising the possibility that variability in response to the drug might reflect variable activity or accumulation of metabolite(s). However, subsequent studies established that the multiple metabolites demonstrate less in vitro electrophysiologic activity than the parent drug (150) and that plasma concentrations at the time of TdP were generally lower for the metabolite compared with the parent drug (151). The lack of a relationship between

plasma quinidine concentrations and TdP risk probably reflects the drug's inhibition of multiple ion currents with a range of potencies: block of IKr at low concentrations (133) to prolong action potentials, block of other potassium currents at higher concentrations to prolong action potentials (152)(153), and block of sodium current (in a frequency-dependent fashion) to shorten action potentials (154)(155). By contrast, TdP developing during therapy with most other antiarrhythmic agents (sotalol, dofetilide) and noncardiovascular therapies (thioridazine, methadone) seems to be dose- or concentration-related (156)(157). Thus, conditions leading to accumulation of QT-prolonging agents in plasma are, in general, risk factors for TdP. Sotalol and dofetilide undergo renal excretion and therefore require dose reductions in patients with reduced renal function to avoid TdP (158)(156). This concept extends to drug metabolism: thioridazine is a CYP2D6 substrate, and some data suggest that the drug accumulates in plasma in poor metabolizers with more marked QT prolongation (159). Likewise, the QT prolonging S-enantiomer of methadone is eliminated by CYP2B6-mediated metabolism, and persons with reduction-of-function alleles in this gene may therefore be at increased risk for methadone- induced TdP (160).

CYP2D6 offers the prototypical example of a pharmacokinetic/pharmacogenomic link (25). The protein expressed by this gene metabolizes a wide variety of pharmaceutical compounds, among which are several drugs associated with QT prolongation and risk of TdP. An individual can have zero, one or two copies of the gene, so that the metabolic activity can nominally be in one of these three levels. There is still considerable spread in *in vivo* activity, however, because the level of protein expression varies among individuals and because there is typically a backup pathway that can metabolize drugs even in the absence of any CYP2D6 activity. Because the link between genotype and activity is so clear for CYP2D6, it is possible to carry out a genotyping assay and use the results to tailor the dosage of CYP2D6 metabolized drugs to the particular patient. This is especially valuable in situations where the patient is on multiple drugs that are substrates for this gene.

A more complex example is provided by CYP3A4 (25)(161). This gene is responsible for metabolizing even more compounds that CYP2D6 –perhaps 60% of all prescription drugs. It also shows even variable metabolic efficiency, but the specific alleles responsible for this variability have not been identified. CYP3A4 has several variant forms, but none of these is associated with differential *in vivo* activity. Instead, this

functional variability is driven by changes in expression that can be induced or by activity changes caused by inhibition.

Both CYP2D6 and CYP3A4 can be inhibited by a variety of other drugs as well as by common foods. For instance, grapefruit juice is a CYP3A4 inhibitor and should not be consumed while taking certain drugs. Although this mechanism of drug-drug interaction is well understood, and should be well known among physicians, there are many reports of incidents of TdP where a patient was simultaneously taking a hERG-blocking drug and a corresponding enzyme inhibitor.

Pharmacokinetics cover not only drug metabolism, but also the entire ADME process. There are likely person-to-person differences in how these drugs are processed in the other ADME steps, and candidate gene pharmacogenomic studies often will include polymorphisms in these pathways when looking at a variable drug action. However, little is known currently about how the other ADME pathways affect the potential for TdP among QT-prolonging drugs (25).

In summary, pharmacogenomic factors can affect the risk of TdP. These risk factors are broken down into pharmacodynamic factors, mainly involving cardiac ion channel genes and pharmacokinetic factors, which centered on drug metabolizing enzymes. Most individuals have sufficient repolarization reserve to be able to tolerate decreased potassium current caused by a hERG-blocking drug at normal doses. However, if the patient has inborn defects in their potassium channels or has a decreased expression of the channels, then the effect of the drug can be significantly enhanced. Likewise, if the effective concentration of the drug is greatly increased because the normal routes of metabolism are ineffective, then the patient is again at increased risk. The main metabolic enzymes (CYP2D6 and CYP3A4) have inborn variation in activity, and both can be inhibited by certain other medications and foods. Furthermore, several phenotypic factors are well documented. These included female gender, advanced age, renal or hepatic impairment, hypokalemia, hypomagnesia, use of diuretics, certain cardiac disorders (congestive heart failure, cardiac hypertrophy, bradycardia), recent conversion from atrial fibrillation, drug overdose, and baseline ECG abnormalities such as a long QT interval or T wave liability. It can be that a mild, and usually benign pharmacogenomic factor when combined with one of these phenotypic factors, can finally lead to an arrhythmia.

1.2.6 Preclinical cardiac safety evaluation

General pharmacology studies have been considered an important component in drug safety assessment. In order to help protect clinical trial participants and patients receiving marketed products from potential adverse effects of pharmaceuticals, while avoiding unnecessary use of animals and other resources. On the other side, as a practical matter, drug developers cannot wait until phase I clinical trials to determine drug safety. Rather, this determination should be made long before an investigational new drug submission, preferably during lead development. A 10% improvement in predicting QT liability might save as much as $100 million per drug in drug development costs (25)(72).

In line with these assumptions, ICH develops the Safety Pharmacology Studies for Human Pharmaceuticals (S7A). This guideline provides a definition, general principles and recommendations for safety pharmacology studies. All three regions have accepted data from general pharmacology studies (Japan and EC) or safety pharmacology studies (USA) in the assessment of a marketing application (162).

These studies were originally referred to as those designed to examine effects other than the primary therapeutic effect of a drug candidate and they were focused on identifying adverse effects on physiological functions. The term "safety pharmacology studies" first appeared in the ICH topics, "Timing of Non- Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (M3)" and "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (S6)", as studies that should be conducted to support use of therapeutics in humans (163)(164). The S7A guideline generally applies to new chemical entities and biotechnology-derived products for human use although it can also be applied to marketed pharmaceuticals when appropriate.

It is important to adopt a rational approach when selecting and conducting safety pharmacology studies. The specific studies that should be conducted and their design will vary based on the individual properties and intended uses of the pharmaceuticals. Some safety pharmacology endpoints can be incorporated in the design of toxicology, kinetic, clinical studies, etc., while in other cases these endpoints should be evaluated in specific safety pharmacology studies.

Since pharmacological effects vary depending on the specific properties of each test substance, the studies should be selected and designed accordingly. The following factors should be considered: effects related to the therapeutic class of the test substance, since the mechanism of action may suggest specific adverse effects;

adverse effects associated with members of the chemical or therapeutic class, but independent of the primary pharmacodynamic effects; ligand binding or enzyme assay data suggesting a potential for adverse effects; results from previous safety pharmacology studies, from secondary pharmacodynamic studies, from toxicology studies, or from human use that warrant further investigation to establish and characterize the relevance of these findings to potential adverse effects in humans.

The purpose of the safety pharmacology core battery is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory and central nervous systems are usually considered the vital organ systems that should be studied in the core battery. In some instances, based on scientific rationale, the core battery should be supplemented or need not be implemented (162).

Central Nervous System: Motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature should be evaluated.

Cardiovascular System: Blood pressure, heart rate, and the electrocardiogram should be evaluated. In vivo, in vitro and/or ex vivo evaluations, including methods for repolarization and conductance abnormalities should also be considered.

Respiratory System: Respiratory rate and other measures of respiratory function should be evaluated. Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies.

Follow-up and Supplemental Safety Pharmacology Studies: Adverse effects may be suspected based on the pharmacological properties or chemical class of the test substance. Additionally, concerns may arise from the safety pharmacology core battery, clinical trials, pharmacovigilance, experimental in vitro or in vivo studies, or from literature reports.

According to the recommendations of the cardiovascular safety pharmacology studies (S7A) for human pharmaceuticals, a guideline (S7B) was prepared to present some currently available methods and discuss their advantages and disadvantages (165). This guideline describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization and it includes information concerning non-clinical assays and integrated risk assessments (165).

The objectives of studies recommended to be conducted are to: identify the potential of a test substance and its metabolites to delay ventricular repolarization, and relate

the extent of delayed ventricular repolarization to the concentrations of a test substance and its metabolites. The study results can be used to elucidate the mechanism of action and, when considered with other information, estimate risk for delayed ventricular repolarization and QT interval prolongation in humans.

Non-clinical methodologies can address the following:

- Ionic currents measured in isolated animal or human cardiac myocytes, cultured cardiac cell lines, or heterologous expression systems for cloned human ion channels;
- Action potential parameters in isolated cardiac preparations or specific electrophysiology parameters indicative of action potential duration in anesthetized animals;
- ECG parameters measured in conscious or anesthetized animals;
- Proarrhythmic effects measured in isolated cardiac preparations or animals.

These four functional levels can be investigated by *in vitro* and/or *in vivo* methods.

In vitro electrophysiology studies can explore potential cellular mechanisms that might not be evident from *in vivo* data. Changes in other cardiovascular parameters or effects on multiple ion channels can complicate interpretation of data. Although delay of repolarization can occur through modulation of several types of ion channels, inhibition of IKr is the most common mechanism responsible for pharmaceutical-induced prolongation of QT interval in humans. Due to this, a hERG assay is pivotal to the lead optimization phase. To be of maximum value it is essential that the assay would be positioned very early in the optimization phase so that any hERG liability in the chemical area of interest is identified as soon as possible, thus giving time for the activity to be designed out (166).

In vivo models that possess the full complement of molecular, biochemical, and physiological systems can also be informative with regard to the response in humans to the test substance. Carefully designed and conducted *in vivo* studies allow evaluation of parent substance and metabolites, and can enable estimation of safety margins.

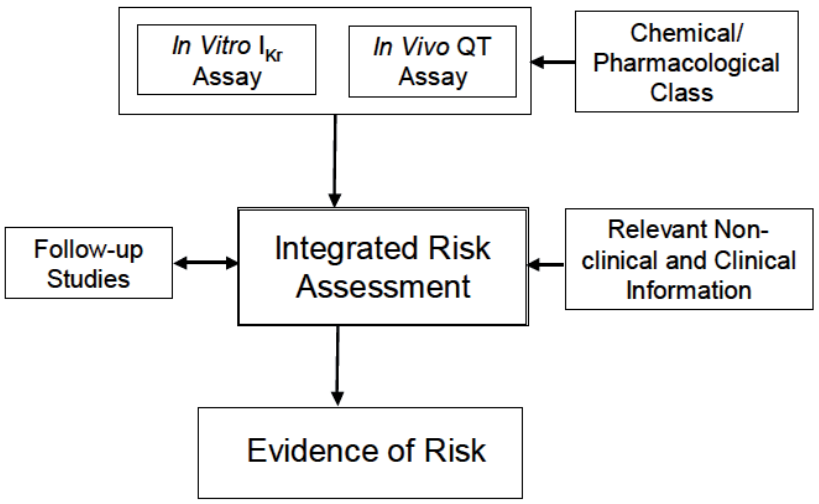

Figure 13: Non-clinical testing strategy (165)

The following paragraphs describe the general non-clinical testing strategy for assessing risk for delayed ventricular repolarization and QT interval prolongation that is pragmatic and based on currently available information.

1.2.6.1 *In Vitro* IKr Assay

An *in vitro* IKr assay evaluates the effects on the ionic current through a native or expressed IKr channel protein, such as that encoded by hERG. As noted, hERG is the only proven molecular target for noncardiac drugs that carry a defective repolarization liability, and drug block of the hERG current expressed heterologously in cell lines is the most direct test of this propensity. The ideal scenario with respect to hERG is that the chemical leads are not active, even at high concentrations. However, given the promiscuity of hERG this may only be the case in certain chemical areas (e.g. acids). Hence in many drug discovery projects there will be multiple iterations of the screening process, attempting to lower hERG potency while retaining desirable properties. Therefore, it's necessary to establish the potency that needs to be achieved before a compound would be considered to carry a low enough QT prolongation risk to be developed to the later stages of the discovery process (166). Although the hERG safety margin concept does not appear in the final non-clinical regulatory guidance, for internal decision making, companies have necessarily required an estimated target for the extent to which hERG potency must be reduced and used this criterion as the basis for whether to progress a given compound for further evaluation (167) (168).

A number of factors have combined to allow companies to make some progress in terms of screening out hERG activity. A variety of assay types have evolved that indirectly aim to assess the potency of test compounds in a time frame short enough to influence chemical design. Advances in technology have also facilitated the generation of electrophysiology-based assays that directly assess effects on hERG channel function with significantly higher throughput than via conventional means. Tangible evidence of this progress is publications showing chemical strategies for how hERG liability can be reduced while retaining desirable properties. The requirement to fit into the synthesis-screening cycle initially limited hERG assays to those indirectly assessing potency (e.g. binding and rubidium efflux assays), but the 'gold standard' assay – which is based on voltage clamp electrophysiology – gradually has become a viable alternative owing to the development of high-throughput electrophysiology devices (169). In addition, in silico methods are evolving that may add to the armoury of the medicinal chemist (72).

A number of retrospective, literature based exercises have provided some indication of the required margin between the maximum free plasma exposure required for clinical efficacy and hERG potency (usually IC50: the concentration which inhibits 50% of the potassium channels) (166)(168)(170) Furthermore, some authors have tried to quantify a suitable safety margin in order to provide a target value for those aiming to screen out hERG activity. This can be achieved using the ratio of the IC50 for drug block of hERG (numerator) to the IC50 for the drug at its primary targe, the effective therapeutic plasma concentration of the drug (denominator). The ratio is referred to as the safety margin (SM). Overall, these reports suggest that a minimum safety margin of 30-fold is required. Obviously margins should reflect disease severity and medical need. For example, one could envisage that a 10-fold margin might be acceptable for drugs used in diseases which are lethal if untreated (e.g. cancer, AIDS, some other infections, etc.), a 30-fold margin may be acceptable for drug treatments for serious debilitating diseases (e.g. stroke, Parkinson's disease, schizophrenia, epilepsy, asthma, arthritis, etc.), but a margin of 100-fold or even higher might be required in the case of less serious diseases (e.g. Raynaud's, seasonal rhinitis, eczema, etc.) (171). However, the safety margins need to be applied with care for because they are based on measured Cmax (free) values for efficacy, whereas drug discovery projects usually only have predicted estimates of this figure that are notoriously difficult to define with any certainty.

Before the central role of IKr/hERG *in vitro* assay was widely appreciated, the mainstay of the first regulatory documents was considered the *in vitro* assay in action potential duration (APD) recording from single cardiac cells. The most commonly used preparation was the Purkinje fibre, owing to the relative technical ease of making stable intracellular recordings in these large, non-contractile fibres responsible for rapid action potential conduction to the ventricular muscle mass. The assay is to determine the concentration drug values for eliciting a 10–20% increase in the duration of the cardiac action potential at 90% repolarisation (APD90) in *in vitro* preparations. A 10% increase in APD90 is typically the minimum increase that reaches statistical significance in such studies, and it is also the degree of change considered physiologically significant by the majority of pharmaceutical companies (171).

APD assays were also prominent in the draft versions of the latest non-clinical regulatory guidance, where they appeared as a so-called 'core assay' before being relegated to a 'follow-up' study. Nevertheless, it would be a logical step to insert a Purkinje fibre assay before the *in vivo* assessment, as the data are rich in useful information. (166).

Other point to consider is that tissue and cell preparations for *in vitro* assays are obtained from different laboratory animal species including rabbit, ferret, guinea pig, dog, swine, and occasionally from humans. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans; therefore, use of tissues from these species is not considered appropriate.

Test substance concentrations for *in vitro* studies should span a broad range, covering and exceeding the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration limiting. Ideally, the duration of exposure should be sufficient to obtain steady-state electrophysiological effects, unless precluded by the viability of the cell or tissue preparation. The duration of exposure should be indicated. Appropriate positive control substances should be used to establish the sensitivity of the *in vitro* assay system.

Factors that can confound or limit the interpretation of *in vitro* electrophysiology studies include the following:

- The testing of high concentrations of the test substance can be precluded by limited solubility in aqueous physiological salt solutions;

- Adsorption to glass or plastic or non-specific binding to the test matrix can reduce the concentration of the test substance in the incubation or perfusion medium;
- Test substance concentrations can be limited by cytotoxic or physicochemical attributes of the test substance that disrupt cell membrane integrity so that electrophysiological endpoints cannot be obtained;

Cardiac cells and tissues have limited capacity for drug metabolism; therefore, in vitro studies using the parent substance do not provide information on the effects of metabolites. When *in vivo* non-clinical or clinical studies reveal QT interval prolongation that is not consistent with data from *in vitro* studies using the parent substance, testing metabolites in the *in vitro* test systems should be considered (165).

1.2.6.2 *In Vivo* QT Assay

An *in vivo* QT assay measures indices of ventricular repolarization such as QT interval. This assay can be designed to meet the objective of both ICH S7A and S7B (162)(165).
The QT interval of the ECG is the most commonly used endpoint to gauge effects of a test substance on ventricular repolarization. Additional safety parameters of interest, including blood pressure, heart rate, PR interval, QRS duration, and arrhythmias, can be assessed simultaneously.
The QT interval and heart rate have an inverse, non-linear relationship, which varies among species and between animals within a species. Thus, a change in heart rate exerts an effect on QT interval, which can confound the assessment of the effect of the test substance on ventricular repolarization and the QT interval. There are two important situations where there is variability in heart rate among animals: one is due to difference in autonomic tone, and the other is due to effects of test substances on heart rate. Therefore, the interpretation of data from *in vivo* test systems should take into account the effect of coincident changes in heart rate. Ideally, QT interval data obtained after administration of a test substance should be compared with control and baseline data at similar heart rates. When the heart rate variability is not due to the test substance, it can be reduced by acclimatization, or the use of anesthetized animal models. When the effects are due to a test substance, the most common approach is to correct the QT interval for heart rate (QTc) using formulae such as Bazett or Fridericia. When differences in heart rate between treatment and control are large, the correction formulae may not be effective for assessing risk of QT

interval prolongation. An alternative approach is to maintain a constant heart rate using cardiac pacing. An analysis of QT/RR relationship, including correction of the QT interval using formulae for individual animals, may be more appropriate.

Laboratory animal species used for in vivo electrophysiology studies include dog, monkey, swine, rabbit, ferret, and guinea pig. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans; therefore, use of these species is not considered appropriate.

The dose range should include and exceed the anticipated human exposure. The dose range can be limited by animal intolerance to the test substance. A sub-maximally effective concentration of a positive control substance should be used to demonstrate the responsiveness of *in vitro* preparations for ion channel and action potential duration assays and should be included in every study. In the case of *in vivo* studies, positive control substances should be used to validate and define the sensitivity of the test system, but need not be included in every study.

For test substances belonging to a chemical/pharmacological class that is associated with QT interval prolongation in humans, the use of concurrent reference compound(s) (member(s) of the same class) in *in vitro* and *in vivo* studies should be considered to facilitate ranking the potency of the test substance in relation to its comparators.

The precise relationship between test substance-induced delay of ventricular repolarization and risk of proarrhythmia is not known. Directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking. Indices of proarrhythmic activity (e.g., electrical instability, temporal and/or spatial dispersion of refractoriness, reverse use-dependency, changes in action potential configuration) and animal models might have utility in assessing proarrhythmia.

1.2.6.3 Integrated risk assessment

Factors that should be considered in conducting studies and interpreting the results include the following (165):

- Data acquisition and analysis methods;
- Sensitivity and reproducibility of the test systems;
- Dosing period and measurement points;
- Heart rate and other effects that confound interpretation of QT interval data;

- Inter-species and gender differences.

In circumstances where results among non-clinical studies are inconsistent and/or results of clinical studies differ from those for non-clinical studies, retrospective evaluation and follow-up non-clinical studies can be used to understand the basis for the discrepancies. Results from follow-up studies can be a significant component of an integrated risk assessment.

The integrated risk assessment is the evaluation of non-clinical study results including the results from follow-up studies and other relevant information. The integrated risk assessment should be scientifically based and individualized for the test substance. Evidence of risk is the overall conclusion from the integrated risk assessment for a test substance to delay ventricular repolarization and prolong QT interval in humans.

1.2.7 Clinical cardiac safety evaluation

Evaluation of the safety of the investigational drug via clinical and electrocardiographic evaluations is the most pivotal part of the global safety strategy during clinical research and drug development. High among the concerns during the development and testing of any new drug is the fear of cardiac toxicity, and particularly of lethal cardiac arrhytmias (25).

The “primary goal” of electrocardiology in R&D is to evaluate the effect of the investigational drugs on the electrical functions of the heart, beginnig with early phase studies in healthy volunteers and continuining through to later phase studies that further define drug effects in the target population. The primary objectives of the cardiac safety assessment in R&D are to determine the drug-associated ECG changes, and their relationship to the pharmacokinetic/pharmacodynamic pecularities of the investigational drug. This can only be ascertained based on a statistical analysis of a large ECG database comprising the study and control groups. The secondary objectives of the cardiac safety assessment in R&D are the detection and monitoring of ECG abnormalities identified in any subject who has been exposed to the investigational drug. As the result of the very low incidence of the life-threatening arrhytmias and/or sudden cardiac death, the detection of lethal ventricular arrhythmias is generally limited to post-marketing surveillance and not a main focus in earlier phases of R&D (25).

Given the clinical issues that had arisen with drug-induced TdP, there was an emerging consensus that there was a significant need for regulatory guidance to assist in the development of drugs and minimize these risks. In December 1997, the

European Committee for Propietary Medicinal Products (CPMP) issued the document "Points to Consider: The assessment of the potential for QT interval prolongation by non-cardiovascular Medicinal Products" (172). This document was the first formally issued regulatory document that specifically discussed how sponsors should address the potential of drugs to cause QT prolongation and TdP. By this time, it had become evident that not only antiarrhythmic drugs but also drugs used for a wide range of other diseases and indications had a propensity to cause QT prolongation and TdP. For a number of nonantiarrhytmic drugs QT prolongation has been observed at standard doses, particularly in persons with impaired clearance of the drug, who thereby were exposed to high plasma concentrations. The Points to consider document emphasized the need for nonclinical and clinical assessment of effects on cardiac repolarization and provided guidance as to how this could be achieved during drug development. In view of some major regulatory agencies, these measures have not, however, been able to provide sufficient protection against the approval of new drugs that can trigger proarrhytmias during extreme conditios (173). In March 2002, the Cardio-Renal division of the FDA developed the concept of defining a drug's effect on the standard ECG from a single clinical research trial in a robust, intense or thorough manner so that the results would be "definitive" (174). It's considered that the motivation for this new concept was to prevent the public's exposure to drugs with uncertain or unknown effects on the ECG (with particular focus on cardiac repolarization as determined by the QTc interval duration from the standard scalar ECG) (175). Specific details of this "intensive or definitive ECG trial" first appeared in the November 2002 FDA-Health Canada ECG concept paper, entitled "The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhytmic Potential for Non-Antiarrhytmic Drugs". The adjectives that have been used to characterize this trial, intensive, definitive and thorough are comparable, but since no single trial should be viewed as 100% definitive the best descriptor for now is "Thorough ECG trial" as used by the International Committee on Harmonization (ICH) in their discussions on this topic (25).

The 'International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use' (ICH) issued the E14 clinical guidance in May 2005: 'The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs' (ICH Harmonized Tripartite Guideline E14, 2005) (176). The ICH process for the developing of a new guidance brings together experts from regulatory authorities and the pharmaceutical industry in Europe, Japan and the United States to discuss scientific and technical aspects of

drug development. The purpose is to develop guidance to the industry, which will be followed in the three regions to achieve greater harmonization in regulatory requirements with the objective of reducing or obviating the need for duplicate testing during non clinical and clinical development of new drugs. The objective of such harmonization is a more economical use of human, animal and material resources and the elimination of unnecessary delay in the global development and availability of new medicines while maintaining safeguards on quality, safety and efficacy and regulatory obligations to protect public health. An Expert Working Group (EWG) was assigned to each accepted topic with the mission to develop guidelines. In some instances, there are regional differences in interpretation of scientific data that cannot be resolved by the ICH process. To adjust for these differences, a certain regional flexibility would have to be built into the document, and there is often a balance between meeting these differences in opinion and the generation of a useful document.

When consensus was reached within the EWG and all parties on the ICH Steering Committee had endorsed a draft for public comments, the document achieved step 2, which indicated the formal start of regulatory actions. At this point, the document was published on the ICH website and elsewhere, and comments were solicited during 6-months period (step 3). These comments were considered by the EWG, the document was accordingly revised, and when consensus was achieved, step 4 and endorsement of the final document by the ICH regulators was reached. During step 5, the guidance was implemented in all regions (173)(177).

The guidance was quickly implemented in Europe and in the United States (178)(179). The centrepiece of the guidance is the "thorough QT/QTc" study, which is a dedicated study with the primary objective to quantify the effect of a new molecular entity on the QT interval (173)(180). This guidance provides recommendations to sponsors concerning the design, conduct, analysis, and interpretation of clinical studies to assess the potential of a drug to delay cardiac repolarization. This assessment should include testing the effects of new agents on the QT interval as well as the collection of cardiovascular adverse events. The investigational approach used for a particular drug should be individualized, depending on the pharmacodynamic, pharmacokinetic, and safety characteristics of the product, as well as on its proposed clinical use (176).

In 2007, the US Food and Drug Administration (FDA) formed an Internal Review Team (IRT) with responsibility to oversee the clinical assessment of QT prolongation for all

drugs that the agency reviewed. The IRT acts in an advisory function to the therapeutic divisions and reviews all protocols and reports for "thorough QT/QTc" studies and advises on QT assessment in other clinical trials (181). A similar advisory function that industry can interact with on a continuous basis does in practice not exist in Europe or in Japan (180).

There is no request per se on when the 'thorough QT/QTc' study should be performed but it is advisable to conduct the "thorough QT/QTc" study well before pivotal trials are undertaken to establish whether additional ECG monitoring is needed. It is also imperative to have sufficient knowledge of the pharmacokinetic profile of the drug, as the exposure in the "thorough QT/QTc" study should substantially exceed that observed in patients, including patients with impaired clearance of the drug. The timing of the "thorough QT/QTc" study within a clinical development program will also be influenced by other factors, such as (180):

- The outcome of the non-clinical safety assessment: In line with ICH S7B, all candidate drugs undergo a careful non-clinical evaluation, which at a minimum includes an *in-vitro* hERG channel assay and an *in-vivo* evaluation of ECG effects in, for example, dogs on non-human primates. At present, a negative non-clinical package does not obviate the need for a "thorough QT/QTc" study, even though this may change based on ongoing initiatives to evaluate the predictive value of non-clinical assays performed at today's standards. For these drugs, it will in most cases be sufficient to perform the "thorough QT/QTc" studies during the latter part of phase 2. For a drug with an unambiguous non-clinical signal, it may be important to exclude that a non-clinical signal translates into a clinical effect relatively earlier, to avoid costly investments into a program that may become non-viable with a clinical QT liability;
- Severity of indication: A small QT effect may be acceptable for life-threatening disorders, particularly when no other therapy exists. In these cases, it may be preferable to conduct the "thorough QT/QTc" once some data on the clinical benefit of the drug has been obtained.
- Previous experience with the pharmacological class versus novel mode-of-action.

1.2.7.1 Objective of the "thorough QT/QTc" study

The "thorough QT/QTc" study is intended to determine whether the drug has a threshold pharmacological effect on cardiac repolarization, as detected by QT/Qtc prolongation. The threshold level of regulatory concern is around 5 ms as evidenced by an upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms. The rational for choosing these threshold values was based on the observation that drugs which prolong the mean QT/ QTc interval by around 5 ms or less do not appear to cause torsade de pointes (178)(182). It is not to establish to which extent a drug is proarrhythmic but to identify those drugs, which need a more careful assessment of this liability in the targeted patient population. Drugs with a positive "thorough QT/QTc" study, that is, for which an effect exceeding 10 ms cannot be exclude, therefore need additional ECG monitoring in patients (173)(183). While drug-induced QTc prolongation of less than 5 ms is considered as not pro-arrhythmic, a prolongation of more than 20 ms is considered a definite risk factor for TdP (176)(182)(184).

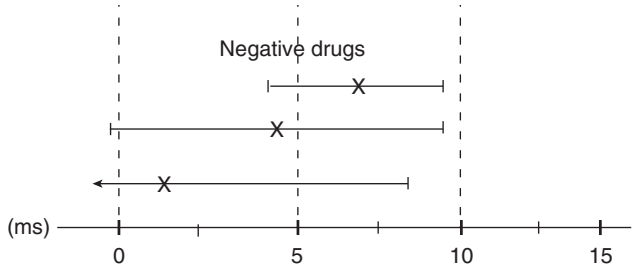

Figure 14: The effect of three drugs with negative result in a "thorough QT/QTc" study (Adapted from (173))

1.2.7.2 Subjects inclusion in the "thorough QT/QTc" study

The "thorough QT/QTc" study is performed in healthy normal volunteers in the age group of 18–45 years to avoid confounding because of underlying disease, comorbidities or concomitant medications (184). The "thorough QT/QTc" study with these drugs may then be performed in patients with the disease against which the drug is targeted. Cardiac safety of rotigotine was evaluated in patients with advanced Parkinson's disease rather than in healthy subjects (185) and combretastatin A4 phosphate was studied in patients with advanced carcinoma (186). However, designing a "thorough QT/QTc" study in patients, especially for oncology drugs, poses definite medical and ethical challenges (187).

Physiological conditions that affect QT interval are important confounding factors that must be accounted for in the study design. The most important of these is the effect of heart rate on the QT interval, which is discussed separately later.

QT interval corrected for heart rate (QTc) is longer in females probably because androgens shorten the QT interval in males (188). The density of potassium ion channels differs in males and females and QTc-prolonging drugs produce greater QT prolongation in women (189). As a result, women account for 70% of reported cases of TdP (190). The ICH-E14 states that all major clinical studies should have an adequate representation of female subjects and that in a positive "thorough QT/QTc" study, data from vulnerable subgroups like females are of particular interest (176).

Other issues that have to be taken account are related with the status of the subjects. There is a small but significant circadian variation in the QTc interval ranging from 75 to 115 ms, which increases during sleeping hours and is longest in the early morning (191). Moreover, QT interval increases after consumption of a meal (192). Consumption of protein supplements, caffeine, xanthine-containing products, chocolate, cocoa-containing drinks, alcoholic beverages or grapefruit juice also affect the QT interval and should be avoided by subjects in a "thorough QT/QTc" study (193). Sympathetic and vagal activities affect both heart rate and the QT interval (194). Therefore, subjects are required to rest for 5 min in supine position before all ECG recording time-points in "thorough QT/QTc" studies. Moreover, blood is drawn only after the ECG has been recorded to avoid any confounding autonomic changes.

1.2.7.3 Study design of the "thorough QT/QTc" study

The "thorough QT/QTc" study should be adequate and well controlled, with mechanisms to deal with potential bias, including use of randomization, appropriate blinding, and concurrent placebo control group (176)(178).

Treatment arms/groups

As within-subject variability is lower than between subjects, a crossover-designed ' 'thorough QT/QTc" study is more efficient than a parallel-designed and requires a smaller sample size to exclude an effect. In certain instances, such as with drugs that need to be dosed for a week or more either to obtain steady state or because of dose-titration, a parallel-designed study is, however, preferable (180).

Negative control (placebo)

Placebo control is included in the ‘‘thorough QT/QTc’ ’ study, to account for the spontaneous diurnal and day-to-day variation in QT interval. It is common to observe ‘significant’ QT/QTc interval prolongation because of spontaneous variability even with placebo treatment (195). Therefore, QT/QTc change from baseline (ΔQTc) observed with a new drug, is adjusted for the time-matched change seen with placebo by appropriate statistical modelling, to obtain a ‘placebo-adjusted change from baseline’ in the QTc interval (ΔΔQTc).

Role of the positive control (PC)

The role of the PC is to demonstrate the study’s ability to detect a small effect on the QT interval. In an overwhelming majority of ‘‘thorough QT/QTc’ ’ studies, moxifloxacin, a fluoroquinolone antibiotic with a mild QT prolonging effect (196)(197) has been used, even though individual examples of other drugs, such as low-dose infusion of ibutilide and sparfloxacin, exist.

Moxifloxacin is the most commonly used positive control. It produces a peak QTc prolongation of approximately 10-14 ms after a single oral or intravenous dose of 400 mg (198). In most studies, moxifloxacin has caused a larger peak effect than 5 ms, more in the range of 8 to 15 ms. How assay sensitivity should be established based on the QTc effect caused by moxifloxacin has been widely discussed and was clarified through the E14 Q&A document (199).

The answer on Question 1 states that there are two conditions that need to be fulfilled to establish assay sensitivity:

the first is that the PC must cause a significant effect on the QTc interval, that is, the lower bound of the one-sided 95% confidence interval (CI) of the placebo-corrected QTc must be above 0 ms. This confirms that the ‘‘thorough QT/QTc’ ’ study can detect an increase in QTc, which is essential for the conclusion that a negative finding for the new drug is meaningful. The second condition is that the study should be able to detect an effect of about 5 ms (the QTc threshold of regulatory concern), in case there is such an effect.

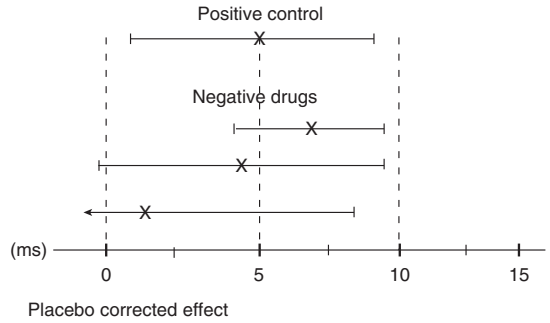

Figure 15: The effect of positive control in a "thorough QT/QTc study (Adapted from (173))

Two approaches can thereby be taken: 1)the most widely used approach has been to use of a PC with an effect size greater than 5 ms, such as moxifloxacin, whereby an effect significantly greater than 5 ms must be demonstrated (i.e. lower bound of one-sided 95% CI should be above 5 ms in the peak effect) (200). The document, however, cautions against using PCs with too large an effect, which may lead to the questioning of the study's ability to detect a 5 ms QTc prolongation. If this is the case, the effect of the PC can be examined at times other than the peak effect to determine whether an effect close to the threshold of regulatory concern could be detected. 2) The other approach is to use a PC with a peak effect close to 5 ms, in which cases the lower bound of the one-sided 95% CI must be >0. When using this approach, the sponsor needs to be confident that this small effect can be repeatedly reproduced, that is, to have a reasonably precise estimate of the drug's usual effect. Lastly and importantly, the answer states that the effect of the PC should be 'reasonably similar to its usual effect' in other ''thorough QT/QTc'' studies, in terms of peak effect (size and time point) and time course of the effect. If, as an example, the peak effect of moxifloxacin is around 5 ms, which would suggest an underestimation of the effect, questions regarding assay sensitivity can be raised and the interpretability of a negative finding for the new drug could be questioned.

In practice, assay sensitivity based on the QTc effect of 400 mg oral moxifloxacin has been established if (180):

1. the largest baseline-adjusted, placebo-corrected effect on QTcF is about 8 to 15 ms;
2. the peak effect is observed between 1 and 3 h post-dose;
3. the lower bound of the one-sided 95% CI of the peak effect exceeds 5 ms; and
4. the QTcF thereafter declines.

In most cases, thorough studies will be performed in a controlled setting, such in a phase I unit, with in-house healthy volunteers, using modern telemetry and safety surveillance. The proarrhytmic risk induced by agents that only midly prolong the QT interval is extremely low in healthy volunteers and will therefore be ethically acceptable in most regions. There are, however, exceptions, and in Japan, for example, it can be anticipated that nonpharmacological positive controls might be used more frequently (173).

Dose of new drug

Two doses of the new drug are often studied - the therapeutic dose, and a supra-therapeutic dose which would simulate levels seen in hepatic or renal failure or with metabolic inhibitors - usually 2–6 times the therapeutic dose (182). The selection of the supratherapeutic dose is one of the most critical features in defining whether the "thorough QT" study was adequately designed. The exact dose used depends on the maximum tolerated dose of the drug determined during early studies. In a recently published "thorough QT/QTc" studies, the supra-therapeutic dose of tolterodine (4 mg) was just two times the therapeutic dose because of dose-limiting anti-cholinergic side effects (201) while a fivefold higher dose was used for tadalafil (202) and levetiracetam (203) and a sixfold dose was used for levoceterizine (204) and maraviroc (205). Some authors propose as a rough guideline, the minimal clinical dose compared to the supratherapeutic dose is at least 3-5x apart and for certain agents such as antihistamines or antibiotics may over 10x apart (25). If for some reason a supra-therapeutic dose cannot be used, use of metabolic inhibitors to achieve the supra-therapeutic concentrations of study drug is an alternative option. Ketoconazole, which inhibits cytochromes oxidase enzymes that metabolize many drugs, should be avoided as ketoconazole itself can prolong the QT interval (206).

The decision to use a single dose or multiple doses of the drug in the "thorough QT/QTc" study depends on the pharmacokinetic characteristics of the parent drug and its metabolites. Single dosing has the advantages of being cost-effective by reducing the duration of the study as well as allowing use of a convenient crossover design. Drugs with short half-lives and no active metabolites could be administered as a single dose. Drugs with a longer half-life, like brivaracetam, which has an elimination half-life of 7–8 h, require multiple dosing (207). Multiple dosing should also be used for drugs with accumulation of active metabolites. Generally, any drug that is used for chronic therapy or at least for multiple dasy is likely to need a

multiple dose trial design unless the sponsor has definitive data to show that there is no difference in exposure of parent/metabolites after such multiple dosing vs a single dose. In multiple dosing studies, it is not necessary to administer multiple doses of the positive control; a single dose of moxifloxacin preceded by multiple doses of placebo will suffice (208).

Blinding of the study

The experimental conditions of the ''thorough QT/QTc'' study must be stringently controlled and study procedures identical between treatment arms/groups. As an example, blood draws (which always should be performed immediately after the ECG recording to avoid confounding stress) should be performed in all treatment periods, even though the samples from the placebo and PC may not be analysed. A rationale for storing samples from the PC arm can be that pharmacokinetic data sometimes can help explain unexpected results, such as a small moxifloxacin effect based on low peak plasma levels (as has been the case in some studies utilizing encapsulation of the drug). Awareness of treatment may introduce a confounding effect on the QT interval and double-blind administration of placebo/new drug is therefore an absolute requirement that has remained. Initially, the recomendations also mandated blinding of the PC. As an illustration of the analyses that FDA can perform across ''thorough QT/QTc'' studies, data from these studies that had used blinded or open-label moxifloxacin were presented at the April 2008 DIA meeting (209). The conclusion from this analysis was that assay sensitivity using moxifloxacin could be achieved with either approach; that is, the QTc effect was very similar. In some cases with over-encapsulated moxifloxacin, the QTc effect was, however, unexpectedly small, which likely was caused by altered pharmacokinetics of the drug due to the encapsulation. Thereafter it was accepted the use of open-label moxifloxacin in "thorough QT/QTc" studies and the same recommendation was later made in the Q&A document by the ICH E14 Implementation Working Group (IWG) (199).

ECG acquisition data

The E14 guidance has specified the limit of regulatory concern to be 5 ms, which is equal to one-eighth of a millimeter of a standard ECG tracing. The 12-lead ECG is one of the most common diagnostic procedures used today. It is adquire in a wide range of clinical care settings from pre-hospital sites to critical care units, from primary care

offices to home healthcare visits. Across all these settings, the defining characteristics of this procedure are the acquired leads and the electrode placement utilized to generate them. The 12-leads of a 12-lead ECG are leads I, II, III, aVR, aVL, aVF, V1, V2, V3, V4, V5 and V6.

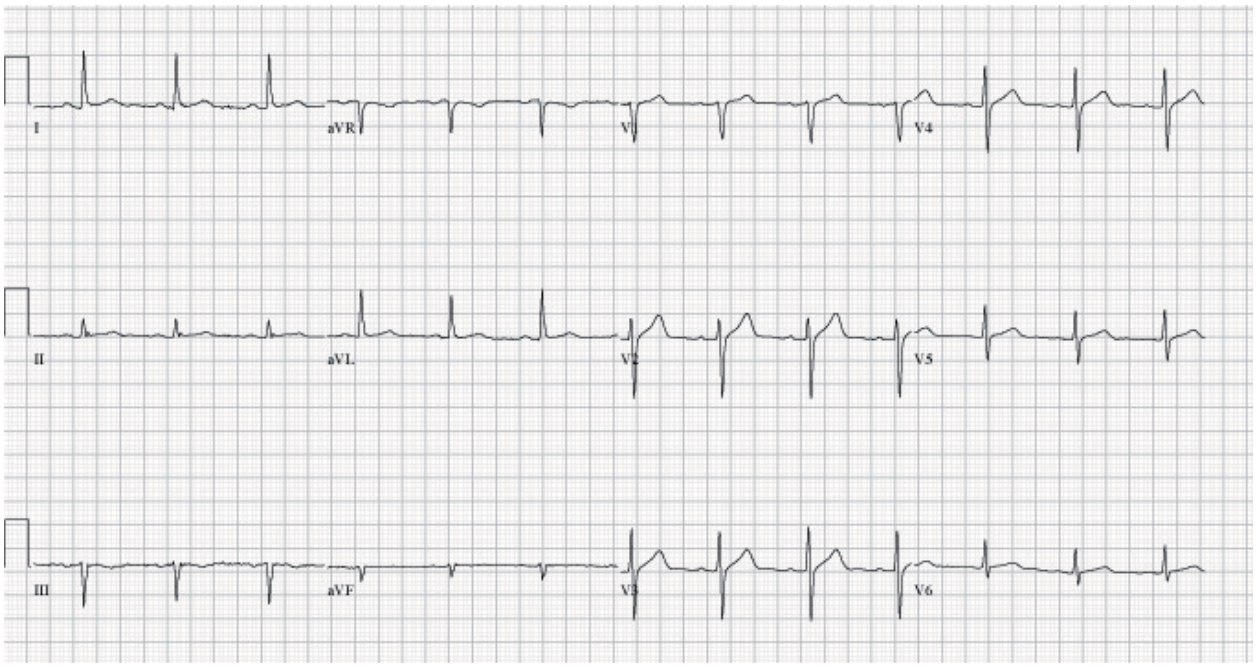

Figure 16: 12-lead ECG standard

To understand what a "digital" ECG is, it is important to distinguish digital from analog. The heart signals measured from the body surface are continous analog signals. These signals can be converted to a series of quantized, time ordered numbers or "digitized".

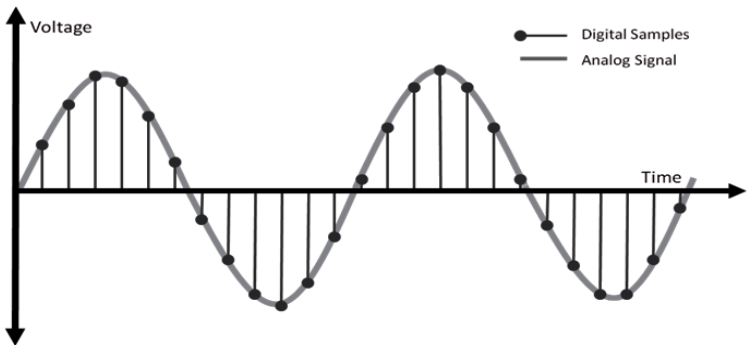

Figure 17: 12-lead ECG digitized

The continuous line represents the analog signal and the bars represent the digital conversion of that analog signal. The conversion process quantizes both the amplitude

and sampling rate. The result is a series of time ordered numbers that correspond to particular amplitudes at particular moments in time. Converted to a digital signal, the ECG can be transmitted and analyzed as digital data within various computational environments.

The 12-lead digital ECG is the extension of the digital ECG concept to incorporate all 12-leads. Hence, a digital 12-lead ECG is defined by two fundamental aspects: the recorded signals are obtained with 10 electrodes and 12 leads as previously defined, and the recorded data is digital in nature. The 12-lead digital ECG data itself can be of variable duration from a few seconds to several hours. Different 12-lead acquisition technologies yield variable duration recordings for use in different subject environments and drug development phases (25).

Therefore, digital ECG recorders are used for acquiring the ECG in "thorough QT/QTc" studies. Standard 10-s recordings of the 12-lead ECG are often used. However, this involves repeated connection and disconnection of the patient cable. A digital 12-lead Holter ECG device can be used instead, which records the digital ECG continuously over a 24-h period. Ten second snapshots of the ECG are then extracted from the recording at specified time-points. This has the advantage of permitting the selection of tracings, which are free of artifacts, at a stable heart rate, and at timepoints of interest that are identified retrospectively. Holter ECGs have been found to be as accurate as 12-lead digital ECGs in the assessment of drug-induced change in QT and RR intervals (210). Until recently, sampling rate of Holter ECGs was considerably lower than that of 12-lead ECGs because of technological limitations. Newer Holter recorders with sampling rates similar to those of 12-lead ECG devices are now available (184).

Moreover, it's recommended that it would be worthwhile to consider recording 3 to 5 replicate ECGs at each time point within a 2 to 5 minute period. Replicate ECGs are defined as single ECGs recorded within several minutes of a nominal time. A rationale for recording replicate ECGs is that the QT interval is assumed to be a continuous parameter that is measured with error. The clinical assumption is that even under stable conditions, an individual's true QT/QTc interval can vary over several minutes. How much of the variability observed under stable conditions over several minutes is due to natural biological variability and how much is due to measurement error is an open question. Using the mean from several ECGs recorded over a few minutes would be one way to reduce potential measurement error and obtain a more precise estimate of the subject's true response at a nominal time. Single ECGs distributed

appropriately over time may be adequate it the primary objective of the study is to estimate the QTc response profile (211)(212).

ECG measurement central laboratory

There is considerable variability in identifying the end of the T wave, as it gradually merges with the baseline. To maintain consistency in QT measurement, and to prevent reader bias, the E14 guidance states that electrocardiographs should be read in a central laboratory by a small group of trained readers blinded to both treatment and patient identity (176)(199). All ECGs from a single subject should be read by the same cardiologist, thereby avoiding the problem of inter-reader variability. Some ECGs should be reread to quantify inter- and intra-reader variability and these results are submitted to the regulatory authorities (176). High reader variability also contributes to large within-subject and between-subject variability in QT interval, which in turn increases the calculated sample size of a "thorough QT" study. Therefore, central ECG laboratories have stringent quality control processes to maintain high standards of ECG interpretation. Automated QT interval measurements by computer algorithms have been explored as an alternative to manual measurement, since machine-read ECGs would show greater consistency. However, manual QT intervals measurements or manual over-read of automated annotations are still preferred over automated algorithms (199)(212).

QT measurement

Traditionally, lead II has been used for QT interval measurement because in this lead, the vectors of repolarization usually result in a long single wave rather than discrete T and U waves (213). The highest accuracy for arrhythmic events is predicted by the longest QT interval in the 12-lead ECG (214), which is usually found in precordial leads V3 and V4 (211) (215). Nonetheless, lead II is still commonly used for QT interval measurement in "thorough QT" studies. Whether repolarization time includes the entire Q–TU complex is a subject of controversy (216). When a U wave interrupts the T wave before it returns to baseline, the QT interval is measured as the nadir between T and U waves (217). Where the end of the T wave is obscured by a U wave, QT interval measurement by the tangent method may be more reliable. An alternative to the single lead approach is to generate a computer-averaged representative beat for each of the 12 leads. These beats can then be superimposed

on-screen and the earliest onset of the Q wave and the latest T offset identified (199).

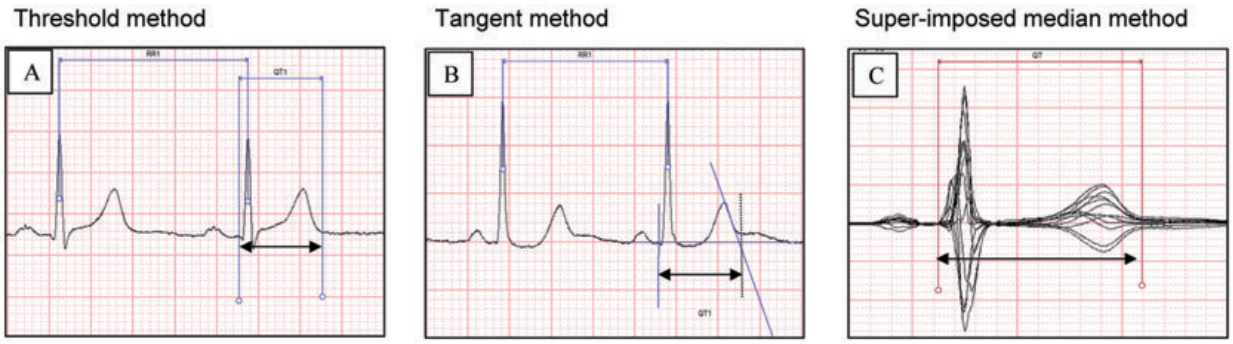

Figure 18: Methods of QT interval measures. With permission (184)

QT vs QTc and how to derive a QTc

The usual prolongation of the QT interval, that is considered to be as a proarrhytmic threshold, is higher than 500 ms. Nevertheless, the QT interval duration varies inversely with the magnitude of the heart rate (as the heart rate slows, the QT increases), and therefore it is very important to correct the measured QT interval duration for heart to derive the ECG interval of interest-the corrected QT interval or QTc. The Bazett correction formula (QTcB=QT/square root of the RR interval in seconds), used almost exclusively in clinical practice, overcorrects at eleated heart rates and under corrects at rates below 60. When corrected by Bazett's formula, on historical and epidemiological grounds, the widely accepted upper limits of normal QTc interval are 450 ms in adult males, 470 ms in adult females and 460 in children between 1 and 15 years of age (regardeless of gender) (182). Since 1999, in drug development the Fridericia formula (QTcF=QT/cube root of the RR interval in seconds) has been used widely because this correction tends to be more resistant to the effets of heart rate changes. However, in drug development sudies using the QTc correctd by a defining a population-based exponent is recommended. A population-based QTc formula is determined by taking the QT and heart rate paired data in the population studied at baseline or on placebo and defining the best correction exponent that provides as flat a slope a possible between heart rate and the resultant QTc (population). Subject specific or individual defined correction formulae are by design the most accurate method in correcting for the heart rate effect on the QT duration. The individual corrected QT (QTcI) is determined by taking each individual in a trial and calculating that individual's exponent that best eliminates the influence

of heart rate on QT duration. This requires at least 35 to 50 or more ECGs on placebo/baseline in which these ECGs encompass a range (usually 50-80 beats per minute) of spontaneous heart rate changes to have enough power to accomplish this task.

Application of linear regression techniques to plots of QT/RR data for the placebo or baseline study population allows for the estimation of the slope (b), which can be used for standardizing the data form both the drug and control groups to a normalized heart rate of 60 beats per minute, using the equation QT= a + b(1-RR) (199).

The individual QTc has been routinely employed in the "thorough QT" study and should be considered the primary endpoint for this trial's determination of the effect of the new drug on cardiac repolarization in such cases. QTcF is a secondary endpoint and the QTcB data are provided for historical reasons (25) (218).

Timing of ECGs

Electrocardiographs are recorded at several time-points decided by the pharmacokinetics of the study drug and its metabolites. It is important to record several ECGs close to the Tmax in order to study the peak effect on the QT/QTc interval. ECG recording should continue even after the Tmax to study any delayed effects of the drug or its metabolites on cardiac repolarization. ECGs are also recorded close to the Tmax of the positive control so as to demonstrate assay sensitivity. In order to retain double-blinding of the positive control, ECGs should be recorded at the same time-points in all treatment groups (184).

In crossover-designed "thorough QT" studies, a baseline assessment can be made either through time-matched recordings on a full baseline day before each treatment period (time-matched baseline) or through a limited number of recordings (e.g. three time points) before each period (predose baseline). Based on the experience from several sponsors and core ECG laboratories (197), it is usually not necessary to use a time-matched baseline in crossover studies, because adjustments for subject- and study- specific diurnal variation are accounted for in the assessment of time-matched drug-placebo differences in QT/QTc effect. The 'pre-dose' baseline is therefore regarded as adequate for crossover studies (199). For parallel-designed TQT studies, a full baseline day is still the most widely used approach and baseline-adjusted QTc is then calculated by comparing the QTc value for each time point at baseline and post-dosing ('time-matched'). There are some data (219), however, suggesting that results

would be the same and the variability lower if baseline was generated through averaging of all values from a full baseline day.

Regarding the presentation of data from clinical trials, historically the only data presented were the mean baseline values and mean changes from baseline. While some studies have reported mean change from baseline to maximum QTc interval (peak effect), others have reported mean change in QTc interval averaged across the dosing interval. Since drug metabolites may also mediate blockade of potassium channels, the peak effect on QTc interval and not necessarily the effect at peak concentration of the parent drug is also relevant (182).

Other electrocardiographic data issues

While the primary focus of most concern with regard to cardiac safety is the effect on the cardiac repolarization (QTc interval), it is important not to forget that the standard ECG provides additional information that must also be analyzed and considered. Drugs that affect heart rate (HR) have potential implications whether the slow the rate or increase the rate. Cardiac conduction as manifested by the PR interval (atrio-ventricular (AV) conduction) and QRS interval (intraventricular conduction) should be carefully considered, because drugs that affect calcium and sodium channels may show such effects by prolonging these intervals. Induction of bradyarrhythmias from effects on AV conduction and increases in proarrhytmic responses from sodium channel blockade has important safety implications. Morphological changes in the ECG waveforms may be signals of myocardial cell integrity disturbance by a new drug, and the effects on T-U wave complexes may be an important determinant of an affect on cardiac repolarization(25). Some authors propose the following approach to standardized these changes (173):

- T wave morphology: normal; abnormal: flattened, inverted, biphasic, notched
- U wave: absent or normal; abnormal

Pharmacokinetic adquisition data

Pharmacokinetic properties of the new drug should be well known when the ‘ ‘thorough QT/QTc” study is conducted and ECG adquisition and blood samples should be timed to ensure that the peak plasma concentration of the drug (and of the positive control) and major metabolites are covered. In many cases, this can be achieved with six to eight timepoints, and it is worth bearing in mind that the

objective of false positive findings increases with the number of assessed timepoints, which should be factored into the decision.

The collection of blood samples in close temporal proximity to the ECGs is encouraged to permit an exploration of possible pharmacokinetic-pharmacodynamic relationships; however, such samples should be drawn after the ECG recordings have been completed to avoid the possible confounding effects of pain or anxiety related to venipuncture (220). Although there may theoretically be an advantage to demonstrate assay sensitivity at the same time of the day as when the peak effect of the new drug is expected, this has not been a requirement. In most ‘‘thorough QT/QTc" studies, moxifloxacin has been dosed in the morning and the peak effect has been observed 1 to 3 h thereafter (180).

1.2.7.4 Statistical issues for the "thorough QT/QTc" study

Sensitivity test

A negative study would be one where the 95% one-sided upper confidence bound of the placebo-adjusted QTc prolongation is <10 ms at all time-points at which ECGs are recorded (176). This definition is chosen to provide reasonable assurance that the mean effect of the study drug is not greater than around 5 ms (184). While statistical testing for this effect requires multiple comparisons, adjustment for multiplicity is not performed in this analysis as the test relies on the intersection-union test (184).

When analyzing the effect of the positive control, the objective is to prove its "superiority" when compared to placebo. To establish assay sensitivity and reject the null hypothesis, at least one lower bound of 95% one sided CI with moxifloxacin should be above 5 ms. If each time-point-specific comparison between moxifloxacin and placebo is tested separately, it is called a local test of significance (221). As local tests will be performed at many time-points, loss of alpha because of multiplicity of tests needs to be adjusted for. Using a more conservative value of alpha would increase the likehood of a type II error. Hence, one can anticipate a priori a few time-points at which maximum QTc prolongation occurs with the positive control (221)(222). After a single dose of 400 mg of moxifloxacin, the maximum QTc effect of around 1 and 4 h after oral administration (198). Selection of three time-points falling within this time period would minimize the extent of correction of alpha. Another approach involves the use of a so-called global test instead of performing multiple local test with alpha adjustment (221)(223).

Sample size

The sample size of a study depends on the estimated difference between the means and the variability between observations. The difference between the means has been defined by the E14 guidance to be 5 ms. Therefore, efforts are made to decrease all possible sources of variability in QT interval measurement in order to reduce sample size; ECG are recorded only after adequate rest, at precise time-points, using high-quality digital ECGs and are read in a central laboratory by a small group of trained individuals (224). Precise on-screen techniques also increase accuracy of QT interval measurement. QT values may vary by as much as 25 ms over 10 consecutive complexes. Moreover, there is considerable minute to minute variation in the QT interval. Hence, recording three to five replicate ECGs at each time-point and measuring QT intervals in three or more complexes in each ECG helps decrease variability further (225).

For a parallel study, the between subject variability (usually 9-14 ms) is important, while within subject variability (usually 5-10 ms) is important in crossover studies (226). The sample size selected should also be adequate to demonstrate assay sensitivity with the positive control as the value of alpha to be used for analysis of positive control data may require multiplicity adjustment (221).

When calculating the sample size for the ‘‘thorough QT/QTc” study, it is important to evaluate at the variability of similar studies. It also seems prudent to assume that the new drug has some effect on the QTc interval, for example 2 to 3 ms and to use 90% power. With these assumptions and the variability obtained from a trained clinical site and an experienced central ECG laboratory using a standard mesurement technique, sample sizes between 32 and 48 subjects for a crossover study or per group in a parallel designed ‘‘thorough QT/QTc” study has often been sufficient(180).

Outliers

Besides looking for mean QTc prolongation, QT/QTc prolongation in individual subjects beyond specified cut-off limits is also important. Previous studies have shown that the 40 year risk of cardiac arrest or sudden death is <20% in individuals with a QTc>450 ms, and 80% in persons with QTc≥500 ms (227)(228)(229).

There is also general agreement that QTc prolongation by >30 ms should raise concerns, with greater concern wht the QTc exceeds >60 ms (230). The ICH E14

guidance specifies that all subjects with QTc interval between 450 to 479 ms and those with QTc interval ≥500 ms must be reported. Similarly, individuals with QTc prolongation of 30-59 ms and ≥60 ms must be reported as outliers (176).

Concentration - QTc analysis

Regulatory review of a ‘‘thorough QT/QTc” study is not complete without an assessment of concentration-QTc relationship (184). An evaluation of the concentration-QTc relationship takes into account individual responses instead of averaging the QT response at each timepoint (231). In addition to this, concentration-QTc analysis helps in predicting QT prolongation at doses of study drug other than those used in ‘‘thorough QT/QTc” study. It is also useful in situations where a new drug may show marginal QTc prolongation at a single timepoint as a type I error; concentration-QTc modelling can confirm that the drug is devoid of a positive QTc effect. Concentration-QTc analysis showed that the QTc prolonging effect of ranolazine in patients with hepatic insufficiency is about thrice that seen in healthy normal individuals (181). The drug was therefore considered to be contraindicated in liver disease. Concentration-QTc modelling cans also help to differentiate whether excessive QT prolongation observed in a few subjects is due to high drug concentration or due to differential susceptibility in these subjects (187). Usually a linear mixed effect model is used to assess concentration-QTc relationship. Most studies have fitted a simple linear model to the concentration-QTc data, giving a line of best fit, wit 90% confidence ranges (232). Concentration-QTc analysis has also been used to identify the QT prolonging effect of an chemotherapeutic drug in cancer patients using QT and concentration data from phase I studies because a formal ‘‘thorough QT/QTc” study was not feasible in these subjects (181).

Analysis of QT/QTc interval data

Although increases from baseline in the QT/QTc interval constitute signals of interes, interpretation of these differences is complicated by the potential for changes not related to drug therapy, including regression toward the mean and choide of extreme values. Regression toward the mean refers to the tendency of subjects with high baseline values to have lower values at later time points, while subjects with low baseline values tend to experience increases. The direction of regression depends on initial selection criteria. The process of choosing the highest of multiple observed

values will also almost invariable cause an apparent change form any single baseline value, a phenomenon found in both drug and placebo-treated groups.
The QT/QTc interval data, according ICH E14 requirements, should be presented both as analysis of central tendency and categorical analyses due to both can provide relevant information on clinical risk assessment (199).

Analysis of central tendency

The effect of an investigational drug on the QT/QTc interval is most commonly analyzed using the largest time-matched mean difference between the drug and placebo (baseline-adjusted) over the collection period. Additional approaches to the assessment of central tendency could include analysis of changes occurring around the Cmax for each individual. This last analysis would be especially important if the drug has large between-subject variability in the rate of absorption or metabolism. The intent is to capture the largest time-based population effect that the drug caused on the QT interval. This effect can be analyzed by comparing change from baseline values at corresponding time points on placebo and on drug, and a time-matched, baseline-adjusted, placebo-corrected effect can therey be calculated. A compound is negative in the thorough QT study if "the upper bound of the 95% one-sided confidence interval for the largest time-matched mean effect excludes 10 ms". The value is calculated for each nominal time point by subtracting the placebo QT interval (baseline adjusted) from the drug QT interval. These placebo-substracted values are then averaged at each time point across the study population, and the time point with the largest mean effect is used for the central tendency analysis.
The number of subjects, which will allow the study to meet the stringent criteria for assay sensitivity, depends on the effect size and on the variability of the QT interval. The QT variability is lower in healthy volunteers than in patients, and a residual standard deviation between 10 and 15 ms is often seen in carefully controlled studies. Measures that decrease this variability will have substantial impact on the required sample size (173).

Categorical analysis

Categorical analyses of QT/QTc interval data are based on the number and percentage of patients meeting or exceeding some predefined upper limit value. Clinically noteworthy QT/QTc interval changes might be defined in terms of absolute QT/QTc intervals or changes from baseline QT/QTc intervals. As with all QT/QTc

interval analyses, categorical analyses are most informative when it is possible to compare the rate of supra-threshold readings in the treatment and control groups.

There is no consensus concerning the choice of upper limit values for absolute QT/QTc interval and changes from baseline. While lower limits increase the false-positive rate, higher limits increase the risk of failing to detect a signal of concerns. In clinical trials, a prolongation of QTc >500 ms during therapy has been a threshold of particular concern. Multiple analyses using different limits are a reasonable approach to this uncertainty, including:

Absolute QTc interval prolongation:

QTc interval >450 ms

QTc interval >480 ms

QTc interval >500 ms

Change from baseline in QTc interval:

QTc interval increases from baseline >30 ms

QTc interval increases from baseline >60 ms

It is recognized that the "thorough QT/QTc" study is not adequately powered to provide statistically significant results for small increases of categorical outliers. Some regulators regard this analysis, however, as a useful exploratory tool to scrutinize any potential effects on cardiac repolarization in susceptible persons, even though the relative value is probably larger in studies in patients. One way to achieve some degree of protection against random effects is to include the placebo arm in the analysis. To enable the increase-from-baseline analysis, a baseline assessment (eg, during 1 hour before dosing) has to be included.

Whether there are drugs that cause an effect only in categorical response, without a corresponding mean effect, using sufficiently high exposure, is an area of debate. At the moment, it's not aware of any drug that has caused marked categorical changes withour affecting the central tendency (173).

Analysis of relationship between drug exposures and QT/QTc interval changes

As previously was commented, establishing the relationship of drug concentrations to changes in QT/QTc interval may provide additional information to assist the planning and interpretation of studies assessing cardiac repolarization.

Morphological analysis of ECG waveforms

While the predictive value of changes in ECG morphology, such as the development of U waves, has not been established, morphological abnormalities should be described and the data presented in terms of the number and percentage of subjects in each treatment group having changes from baseline that represent the appearance or worsening of the morphological abnormality.

Adverse events

Although drug-induced prolongation of the QT/QTc interval is usually asymptomatic, an increased rate of certain adverse events in patients taking an investigational agent can signal potential proarrhytmic effects. The rates of the following clinical events should be compared in the treated and control patients, particularly when there is evidence of an effect on the QT/QTc interval:

Torsade de pointes
Sudden death
Ventricular tachycardia
Ventricular fibrillation and flutter
Syncope
Seizures

Torsade de pointes is very infrequently captured in clinical databases, even those for drugs known to have significant proarrhytmic effects. Given this, the failure to observe an epidsode of TdP in a drug application database is not considered sufficient grounds for dismissing the possible arrhythmogenic risks of a drug when these are suspected on the basis of ECG and other clinical data. The other adverse events listed above, while less specific for an effect on cardiac repolarization, are more commonly captured in clinical trials, and an imbalance in their frequency between study groups can signal a potential proarrhytmic effect of the investigational agent (199).

MedDRA® the Medical Dictionary for Regulatory Activities terminology is the international medical terminology developed under the auspices of the International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (220).

1.2.7.5 Interpretation of the "thorough QT/QTc" study

The objective of the ''thorough QT/QTc" study is to confidently exclude that a drug prolongs the QTc interval 10 ms or more at the one- sided upper 95% confidence limit (176)(173). It is difficult to determine whether there is an effect on the mean QT/QTc interval that is so small as to be of no consequence. However, drugs that prolong the mean QT/QTc interval by around 5 ms or less do not appear to cause TdP. On that basis, the positive control (whether pharmacological or nonpharmacological) should be well characterized and consistently produce an effect on the QT/QTc interval that is around the threshold of regulatory concern (5 ms).

Based on similar considerations, a negative "thorough QT/QTc" study is one in which the upper bound of the 95% one-sided confidence interval for the largest time-matched mean effect of the drug on the QTc interval excludes 10 ms. This definition is chosen to provide reasonable assurance that the mean effect of the study drug on the QT/QTc interval is not greater than around 5 ms. When the largest time-matched difference exceeds the threshold, the study is termed 'positive.' A positive study influences the evaluations carried out during later stages of drug development, but does not imply that the drug is pro-arrhythmic (176).

1.2.7.6 The "thorough QT/QTc" study's impact on clinical program

The outcome of the "thorough QT/QTc" study has a major impact on the level of ECG monitoring in subsequent trials. For compounds with a negative "thorough QT/QTc" study, the collection of baseline and periodic on-therapy ECGs in accordance with the current investigational practices in each therapeutic field is almost always sufficient evaluation(176). In most cases, this means that additional data on ECG intervals, especially QT intervals, are not required, except in cases when these intervals are reported as part of an adverse event.

Regulatory decisions regarding approval and prescribing information should be based on a careful assessment of relevant data from all stages of drug development, with appropriate attention to evidence of dose dependency, concentration relationship, and trend over time, central tendency analyses of effect size; categorical analyses of outlier values; morphological abnormalities; discontinuation and dosage reductions caused by QT/QTc prolongation; and pre- or postmarketing adverse events suggestive of proarrhytmia.

Substantial prolongation of the QT/QTc interval, with or without documented arrhytmias, can be the basis for nonapproval of a drug or discontinuation of its clinical development. Failure to perform an adequate clinical assessment of the QT/QTc prolongation liability of a drug may likewise be justification for delaying or denying marketing authorization (220).

It should be noted that there are clear differences in opinion among regulators regarding the predictive value of the S7B nonclinical studies and the E14 Thorough QT/QTc study. The FDA would, in most cases accept that clinical data override nonclinical data and would regard a compound as devoid of proarrhythmic propensity if negative in the thorough QT/QTc study, regardless of the S7B nonclinical battery. This is not the case among European regulators, and this difference was met in the document by acknowledging that there may be "very unusual cases" in which nonclinical assays are strongly positive and the thorough QT/QTc study results are negative. If this discrepancy cannot be explained by other data and if the drug belongs to a pharmacologic class of concern, an expanded ECG assessment should be performed during late-stage development.

A positive thorough QT/QTc study result will, on the other hand, require that the QT effect be further evaluated in the targeted patient population. This evaluation could be achieved by the collection of QT interval data at baseline and on therapy with a limited number of ECG recordings in "substantial number of patients in late phase clinical trials. It would be important to ensure that on-therapy ECGs were recorde at the anticipated time of peak drug effect. The objective is to fully describe the QT effect in patients with emphasis on dose-related and concentration-related responses. Patient with risk factors for TdP (such female gender or cardiac disease) should be included in these analyses, which would not only focus on mean effects but also place an emphasis on outlier values in individual patients.

Once approved, contraindication for certain patient groups may apply, and the effect on the QT interval needs to be clearly described in the label, as well as in precautionary advisories for the prescribing physician and for patients (173).

The ICH E14 provides guidance to sponsors on how to clinically evaluate a new chemical entity's propensity to prolong the QT interval. Because most sponsorps wish to develop drugs for all major regions, these specialized studies will undoubtedly be conducted for the vast majority of drugs in development.

1.3 Rupatadine: Development of a novel antihistamine

Rupatadine belongs to the pharmaceutical group of the antihistamines, selective antagonists of the H1 histamine receptors.

Rupatadine is indicated for the treatment on symptoms associated with seasonal or perennial allergic rhinitis and chronic idiophatic urticaria (233).

1.3.1 Problem statement

H1 antihistamines are among the most prescribed medications in the world. Originally, studies of the relative potencies of H1 antihistamines were based on the capacity of different compounds to competitively inhibit the H1 receptor binding of histamine. Nevertheless, it has already been known for some time that, in addition to acting on H1 receptors, many H1 antihistamines, at appropriate doses, are capable of inhibiting not only the release of histamine by mast cells, but also mast cell activation itself. Depending on their action on the central nervous system (CNS), they are classified as "classic", or first-generation, and "non-classic", or second-generation. Over the last 20 years, second-generation H1 antihistamines, as rupatadine, were synthesized- compounds with high potency, long-lasting effect and minimal adverse effects: they are unlikely to cross the BBB and rarely cause sedation. They have efficacy for the treatment of patients with allergic rhinoconjunctivitis, urticaria and other allergic diseases, although they differ significantly in terms of their chemical structure, clinical pharmacology and toxicity potential.(234)

1.3.1.1 Allergic rhinitis

Rhinitis refers to disease involving inflammation of the nasal membranes (235). Symptoms include nasal discharge, sneezing, and congestion. Rhinitis may be infectious or noninfectious. The most common noninfectious rhinitis is allergic rhinitis (AR), but there is also another non-infectious type as non-allergic (vasomotor, "irritant") rhinitis, with different pathophysiology and treatments.

AR is associated with the epithelial accumulation of effector cells such as mast cells and basophils, and inflammation in the nasal mucosa. Immunologic activation of these effector cells induces the secretion of both newly generated and preformed mediators. Quantitatively, histamine is the most abundant preformed mediator and its implication in many of the symptoms of the disease has been clearly demonstrated. These effects are largely mediated through histamine H1 receptors. A

further newly formed lipid–derived mediator is the platelet-activating factor (PAF). The biological properties of this mediator include vasodilation and an increase in vascular permeability that may contribute to nasal blockage (236). PAF and histamine are known to complement each other in vivo, being histamine a mediator of early response and released from preformed reservoirs in mast cells, whereas PAF is mainly synthesized de novo. Each of these mediators is able to promote the release of the other in some tissues and cells.

AR, that may be seasonal (SAR) or perennial (PAR), is characterized by nasal mucous membrane swelling and blockage, reflex sneezing and hypersecretion, and ocular manifestations including itching, tearing, and conjunctival edema and redness. Persons with SAR, otherwise known as hay fever or pollinosis, have symptoms primarily in the spring, summer, or fall, during the pollinating season of the plants to which affected persons are sensitive, including trees, grass, or weeds. Persons with PAR, on the other hand, have year-round symptoms (although there may be some seasonal variation) related to allergens that are largely indoors (e.g., house dust mites {D. pteronyssinus}, animal dander, and mold spores)(237). Nevertheless, it is often difficult to differentiate between SAR and PAR, and because of this, the World Health Organization's Allergic Rhinitis and its Impact on Asthma Group has recommended instead that AR be classified as `intermittent' and `persistent'. Intermittent allergic rhinitis is defined as symptoms experienced for less than four days a week or for less than four weeks a year. Persistent allergic rhinitis is defined as symptoms experienced for more than four days a week and for more than four weeks a year (238)(239).

AR is a very common condition worldwide, with estimates of global prevalence ranging between 10 and 25% (240), and epidemiologic evidence suggests that its prevalence is increasing (241)(242). It is even more prevalent in younger populations, affecting up to 40% of children and adolescents (235)(240)(243)(244).

Besides clinical symptoms, health-related quality of life is impacted by AR, including effects on physical function, energy, social function, mental health, bodily pain, mood, learning ability, and workplace productivity (245)(246). If left untreated, AR can be associated with serious complications, including asthma, sinusitis, respiratory infections, and otitis media (247). In addition, AR appears to be linked to a number of other conditions (238)(248).

The objective of treatment of AR is to diminish symptoms and decrease progression to other sequelae and complications. Rhinitis is considered pathologic when symptoms

are severe enough to require therapy. Since this is a chronic condition, treatments must be safe, well-tolerated, and effective in the long-term. First-line treatments for AR include allergen avoidance and environmental control; however, the evidence for the effectiveness of these interventions is limited. Pharmacotherapy treatment recommendations depend on symptom severity and may include antihistamines, decongestants, corticosteroids, leukotriene-receptor antagonists, mast cell stabilizers, anticholinergics, and allergen-specific immunotherapy (249).

1.3.1.2 Chronic idiopathic urticaria

Urticaria is a condition characterized by transient, pruritic wheals, which are primarily the result of histamine release from mast cells. It is characterized by well-circunscribed, intensely pruritic, raised wheals (edema of the superficial skin) typically 1 to 2 cm in diameter on any part of the skin. Urticaria can occur with or whithout angioedema, which is localized, nonpitting edema of the subcutaneous or interstitial tissue that may be painful and warm.

Urticaria and angioedema are thought to have similar underlying pathophysiologic mechanisms, with histamine and other mediators being released from mast cells and basophils. The difference between the two conditions is whether the mast cells are in the superficial dermis, which results in urticaria, or in the deeper dermis and subcutaneous tissues, which produces angioedema. Immunoglobulin E (IgE) mediation of this histamine release is often ascribed, but non-IgE and nonimmunologic mast cell activation can also be a cause. Chronic urticaria may have a serologic autoimmune component in some patients, including antibodies to IgE and the high-affinity IgE receptor. However, the exact mechanism of action and significance of these antibodies remain unclear (250).

Urticaria, with or without angioedema, can be classified as acute or chronic. In acute urticaria, although individual wheals resolve within hours, they can recur for up to six weeks, depending on the etiology. Chronic urticaria is usually defined as recurring episodes of urticaria lasting 6 weeks or more (251). The etiology of chronic urticaria can be physical stimuli or may be idiopathic. Types of chronic urticaria that occur in response to physical stimuli include dermatographism (urticaria in response to stroking, friction, or rubbing), cholinergic urticaria (where stimuli that raise the core temperature of the body elicit urticaria), cold urticaria (where wheals occur after exposure to cold; this condition is rarely associated with underlying diseases), solar

urticaria (provoked by ultraviolet light), and aquagenic urticaria (precipitated by contact of the skin with water of any temperature). So-called "chronic idiopathic urticaria" (CIU), may be due to an autoimmune process in 40–50% of patients (252).
It is estimated that at least 50% of general populations have experienced urticaria at one time or another (253). Urticaria occurs across all age ranges and has a lifetime prevalence of approximately 20 percent in the general population, with the chronic form affecting 1 percent of the population.
Acute urticaria is much more common than the chronic form in both adults and children, accounting for 70% of cases. Although often it is not possible to identify the specific cause of CIU, an increasing amount of evidence suggests that the symptoms of CIU, including oedema, erythema and pruritus, are primarily elicited as a consequence of histamine released from activated dermal mast cells and basophils binding to the H1 receptors. This has consequently advocated the use of oral H1 receptor antagonists (H1 antihistamines) as the mainstay drugs licensed for therapeutic intervention in CIU (239).

1.3.2 Antihistamines, a therapeutic option

Many drugs are available for the treatment of AR and CIU. Apart from anthistamines, agents with anti-allergic actions include sodium chromoglycate and nedocromil, ipratropium bromide, topical decongestants, and topical and oral corticosteroids.
Antihistamines became widely used in the middle to late 1940s. They quickly became established in the treatment of various allergic disorders, particularly rhinitis and urticaria.
Antihistamines, historically known as histamine H1-receptor blockers or antagonists, are specific for the H1-receptor of histamine. In addition, some H1-antihistamines inhibit transmission through the muscarinic, α-adrenergic, and serotonin receptors and through ion channels. The H1-antihistamines have been reclassified as inverse agonists, rather than as H1-receptor antagonists, which is consonant with an increased understanding of their molecular pharmacologic features. Histamine, a natural body constituent, is a low-molecular-weight amine synthesized from l-histidine exclusively by histidine decarboxylase, an enzyme that is expressed in cells throughout the body, including central nervous system neurons, gastric-mucosa parietal cells, mast cells, and basophils. Histamine has an important role in human health, exerting its diverse biologic effects through four types of receptors. Histamine

plays a pivotal role in allergic inflammation, which is a complex network of cellular events that involve redundant mediators and signals. Histamine is released from the granules of FceRI+ cells (e.g., mast cells and basophils) along with tryptase and other preformed mediators, as well as leukotrienes, prostaglandins, and other newly generated mediators, after the cross-linking of surface IgE by allergen or through mechanisms that are independent of IgE. After allergen challenge in sensitized persons, histamine is found locally in relatively large (microgram) quantities per 1 million cells, in contrast to leukotrienes and other mediators, which are found in picogram quantities. Although most of the effects of histamine in allergic disease occur through H1 receptors, hypotension, tachycardia, flushing, and headache occur through both the H1- and H2-receptors in the vasculature, whereas cutaneous itch and nasal congestion may occur through both the H1- and H3-receptors. In addition to its role in the early allergic response to antigen, histamine acts as a stimulatory signal for the production of cytokines and the expression of cell-adhesion molecules and class II antigens, thereby contributing to the late allergic response (234).

Side effects occurred commonly with all of these antihistamines. Those seen most often were sedation and dry mouth (atropine-like). The occurrence of side effects limited their efficacy by constraining the degree of H1 blockage that could be achieved clinically. The search for new non-sedative antihistamine compounds was not achieved until four decades later in the 1980s when non-sedative anthistamine astemizole and terfenadine were introduced (254).The evolution of newer antihistamines, having less central sedative actions and fewer antagonistic effects on other amine receptors, has led to a series of more selective but non specific drugs, as evidenced by their effects on cardiac potassium channels.

The ability of antihistamines to influence other cellular mechanisms, either by receptor effects or by actions on the release of mast cells, and basophil-derived mediators including PGD2, LTC4, PAF or cytokines, provides them with the possibility of having beneficial effects which may extend their usefulness. PAF, like histamine, is known to cause bronchoconstriction and increased vascular permeability, but it could also be responsible for bronchial hyperreactivity, a common feature of asthma (255). PAF is also a very potent chemotactic stimulus for eosinophils and is believed to be an active agent in the induction of shock states and to act as a mediator of inflammatory disease (256). Moreover, it is known that PAF and histamine complement their activity *in vivo*, since histamine is a mediator of early response, being released from preformed reservoirs in mast cells. On the contrary PAF, which could be regarded as a

late-response mediator, is synthesised mainly de novo (257). Furthermore, each mediator is able to promote the release of each other in some tissues and cells (258). Thus, it seems reasonable to consider that a drug which prevents the blockage of both PAF and histamine effects can probably have advantages over the blockage of a single mediator.

1.3.3 Product therapeutic rationale

Rupatadine fumarate (RUP) is a new chemical entity which possesses a potent PAF antagonist and antihistamine activity and selected from a series of N-alkylpyridine derivatives.

On the basis of available preclinical data it was anticipated that rupatadine would be useful in the management of diseases with allergic inflammatory conditions. Its anti-inflammatory actions had been shown in appropriate studies of inhibition of three key processes: mast cell degranulation, eosinophyl chemotaxis and cytokine release. It has also demonstrated to inhibit platelet-activating factor (PAF)-induced effects *in vitro* and in animal species including man. This dual antagonism of histamine and PAF together with its proven anti-inflammatory actions may add advantages over other currently available antihistamines.

Because of this J. Uriach y Compañía, S.A. decided to develop the complete preclinical and clinical dossier to market rupatadine in Europe, following the European Medicines Agency (EMA) requirements.

Rupatadine belongs to the pharmaceutical group of the antihistamines, selective antagonists of the H1 histamine receptors, with ATC code of R06A X28. Its chemical designation is 8-chloro-11-[1-[(5-methyl-3-pyridinyl)methyl]piperidin-4-ylidene]-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b] pyridine fumarate. The empirical formula is C26H26ClN3 and molecular weight is 415.958 g/mol (259).

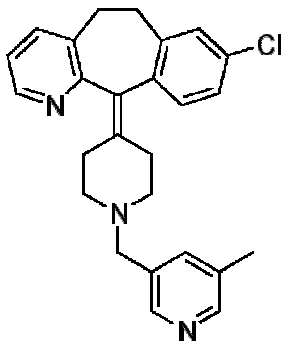

Figure 19: Molecular structure of rupatadine fumarate

Rupatadine is indicated for the treatment on symptoms associated with seasonal or perennial allergic rhinitis and chronic idiophatic urticaria (233).

1.3.4 The development programme

The clinical development of rupatadine was initiated in 1995 focussing on the seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR) indications. In the absence of any established allergic guideline at that time, the initial clinical developmental plan was based on the results of an exhaustive literature review. This clinical development plan included a total of 6 studies in SAR and 4 studies in PAR.

A first Mutual Recognition Procedure (MRP) to two European countries concluded with the marketing authorisation being granted in Portugal and Greece, and then it was submitted and approved in other Concerned Member States (Spain, Belgium, Luxemburg and Ireland).

Afterwards, two new clinical trials were conducted in order to fulfill the new guideline recommendations: Guideline on the clinical development of medicinal products for the treatment of allergic rhino-conjunctivitis (260).

Also, in 2002, it was initiated the clinical development of rupatadine in the inidication of chronic idiophatic urticaria (261) with two studies.

Furthermore, two studies were designed to obtain additional data on rupatadine's long-term safety in compliance with the recommendations of the Guideline on population exposure required to assess clinical safety (262).

Finally, in the initial clinical development plan, the cardiac safety of rupatadine was evaluated according to the Guideline CPMP/986/96 Points to consider: The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products (172). No alteration in QT/QTc was noted.

The safety pharmacology and toxicology studies were performed in compliance with Good Laboratory Practices (263). The rupatadine clinical plan was designed and conducted in compliance with the World Medical Association Declaration of Helsinki (264) and current standards, regulations and requirements for Good Clinical Practice (GCP), as set out in the Guidelines of the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, including study conduct, record keeping, data collection (18).

1.3.4.1 Quality aspects

Drug substance

Rupatadine fumarate, an N-alkylpiperidine derivative, is a drug substance not had been described in any Pharmacopoeia Structural formula.

Rupatadine fumarate is a white or slightly pink crystalline powder, with a particle size between 10 µm and 100 µm. It is slightly soluble in water; its solubility in aqueous solutions depends on the pH, showing maximum solubility at pH 1.4 and is practically insoluble at pH 7.0.

Rupatadine fumarate is not hygroscopic and it is stable in solid state. It should be stored in well-closed containers protected from light.

The excipients are controlled by European Pharmacopoeia.

Drug Product

The dosage form is a conventional tablet containing 12.8 mg of rupatadine fumarate (equivalent to 10 mg of rupatadine free base). The particle size specification for rupatadine fumarate is in accordance with the particle size found in all batches used in the manufacture of the tablet bio-batches and the criteria stated in ICH Q6A (265).

Since the rupatadine fumarate molecule is stable to temperature and humidity and in view of its poor flowability, it was decided to produce the tablets by wet granulation.

In the stability studies the batches, storage conditions and duration of studies comply with the ICH (Q1A) guideline (266). Moreover, a photostability study was also conducted on the tablets, according to the ICH (Q1B) guideline (267).

The proposed shelf life for Rupafin 10 mg tablets is 3 years. It is recommended to keep the blister units in the outer carton.

1.3.4.2 Non clinical studies

Pharmacodynamics

Rupatadine displays an antagonist activity toward H1 histamine receptors(268). Pre-clinical studies performed with rupatadine include pharmacodynamic studies *in vitro* and *in vivo* in several animal species (mice, rats, guinea pigs, rabbits and dogs) related to its main mechanism of action, its histamine and also PAF antagonist properties, and, as additional support, *in vitro* and *in vivo* experimental models related to other activities(269).

Rupatadine exhibits an H1 antihistamine activitiy similar or even superior to that of other second-generation antihistamines both *in vitro* and *in vivo* experimental

models. Some metabolites of rupatadine may contribute to the antihistamine effect, probably increasing the duration of action. Moreover, rupatadine presents an additional PAF (Platelet-Activating-Factor) antagonist activity although weaker than as antihistamine in several models studied(270).

Allergic and inflammatory pathologies are complex and depend on the formation or release of different mediators. The fact that rupatadine blocks the actions of both histamine and PAF may produce beneficial effects in these diseases, greater than they would be if each mediator were inhibited separately. In several different types of cells both PAF and histamine are in fact known to induce the release of the other mediator (271). It is, however, difficult to separate the two effects in the context of pathological models, in which the overall effect may be due to the sum of the specific effects deriving from the blockage of the two types of receptor plus other effects that are independent of any interaction with them. In any case, the effects of rupatadine in the allergy models tested in various species, both *in vitro* and *in vivo*, suggest a very complete antiallergic and anti-inflammatory profile.

Pharmacokinetics

A series of studies in the same species used for toxicological evaluation, which have permitted to evaluate the absorption, distribution, metabolism and excretion of rupatadine were conducted. It is rapidly absorbed when administered orally in all species examined. In mice, there is a greater rate of metabolisation than that seen in rats and humans. An important first effect is also observed in the rat and another one lower in the dog. No specific accumulation is observed in tissue distribution studies. Rupatadine is highly bound to plasma proteins and it is extensively metabolised in the liver, mainly by CYP3A4. Therefore, interactions with CYP3A4 inhibitors may be expected. Main metabolites and metabolic pathways have been identified. There are two metabolites in humans, UR-12788 and UR-12335 (inactive, but the main metabolite found in plasma), which are minoritary in the rat and not found in the dog.

The main elimination route is in the bile; metabolites are excreted in the faeces in all-animal species and humans, although there is also renal excretion. Rupatadine plasma half-life varies from 1 h in mouse to 2 h in rat and dog. Metabolites half-lives are longer. RSA (product of degradation of rupatadine fumarate) shows negligible oral bioavailability.

No drug-interaction pharmacokinetic *in vivo* studies have been performed (272).

Toxicology

It was conducted a battery of rupatadine toxicology studies which, includes single and repeated dose toxicity, reprotoxicity, genotoxicity and carcinogenicity studies. Studies with two metabolites, UR-12788 and UR-12335, have also been performed. All these studies were conducted in accordance with GLP.

The single dose toxicity studies performed after oral and intraperitoneal administration, showed that rupatadine orally administered shows a low toxicity (rat and mouse). From rat oral repeated dose toxicity studies, the Non Observed Adverse Effects Level (NOAEL) has been established in 30 mg/kg/day. The main findings observed were considered as a adaptive response related with hepatic microsome induction of rupatadine, and others, as an exaggerated pharmacological response and similar to those obtained with other durgs for this species. Wide safety ratios were obtained from toxicokinetics at 3 mg/kg/day.

A dog chronic tocicity study was conducted and the NOAEL as 20 mg/kg/day. Wide safety margins were also obtained from AUC values of rupatadine at 1 mg/kg/day.

The Non Observed Effects Level (NOEL) dose in respect of fertility may be estimated at 25 mg/kg/day (rat), but some fertility impairment has been detected, although at doses several times those proposal in clinical use.

At 5 mg/kg/day, no toxic effects on maternal or foetuses or litter were recorded in rats and at 5-25 mg/kg/day, no toxic effects on maternal or foetuses in rabbits were reported. No embryotoxic effects and apparent teratogenicity have been observed at doses of up to 120 mg/kg/day (rats) and 100 mg/kg/day (rabbits).

On the basis of the genotoxicity and carcinogenicity studies available, no genotoxic and oncogenic potential have been detected (272).

Safety pharmacologic studies (Effects less related to the proposed indications)

A battery of safety pharmacology studies to assess the effects of RUP on different systems, such as cardiovascular, renal and respiratory, CNS and autonomous nervous system, coagulation, gastrointestinal motility and behaviour in several models and species (mouse, rat, rabbit and dog) were also conducted, which basically conform to the Note of Guidance on Safety Pharmacology Studies for Human Pharmaceuticals, CPMP/ICH/539/00 (162). Moreover, additional studies are provided focusing on the most limiting aspects of antihistamine therapy, that is, anticholinergic, CNS and cardiovascular side effects.

On the basis of the results from these studies, it can be concluded that no risk of peripheral anticholinergic effect may be expected at therapeutic doses.

In relation to its possible CNS depressing effect, it is important be noted that, in monkeys, rupatadine presented no sedative effects at the dose 50 times greater than therapeutic dose although the sedative effect of rupatadine has not been compared *in vivo* to those of other second-generation antihistamines. From data of a study *ex vivo* on guinea pig, rupatadine shows a reduced capacity to cross the blood-brain barrier.

Taking in account the pharmacological class, the risk of rupatadine to provoke a prolongation of the QT segment of the electrocardiogram and the development of ventricular arrythmias known generically as torsades de pointes, TdP was evaluated by the following *in vitro* and *in vivo* studies:

In vitro study

Rupatadine and some of its human main metabolites were tested to assess the effect on cardiac action potential in isolated canine Purkinje fibres, such as it is recommended in CPMP "Points to consider" (CPMP/986/96) (172). Reference substances were cisapride and astemizole.

Rupatadine and these metabolites did not statistically modify the physiological parameters studied related to cardiac action potential, especially they did not display any significant prolongations of the parameter APD90 at concentrations of up to 10-5 M (rupatadine and UR-12788) and 10-4 M (UR-12335), under physiological heart rate conditions (60 ppm). In case of rupatadine, this concentration was 2080 fold higher than the maximum plasma concentration (4.8 nM) obtained after repeated administration of rupatadine in humans at 10 mg/kg. Likewise, the safety factor for UR-12788 (Cmax = 3.9 nM) and UR-12335 (Cmax = 29.8 nM) were 2580 and 3350, respectively.

Other parameters as APD30, APD50, APD70, resting potential (RP), amplitude of action potential (APA) and maximal rate of depolarisation (Vmax) were not modified at 60 ppm, suggesting no effect on ionic channels responsible for cardiac depolarisation and repolarisation processes (sodium, potassium, calcium channels). When a strong bradychardia was simulated (12 ppm), rupatadine at 10-5 M showed only a slight decrease in action potential duration to 50 % repolarisation (APD50), suggesting a possible reduction in the plateau of action potential. Neither rupatadine nor UR-12788 showed any effects at 10-6 M, nor UR-12335 at any concentration studied. No early after depolarisations due to either RUP or its metabolites were observed in any conditions. Both cisapride and astemizole induced, as expected, their

typical electrophysiological effects, being these ones more marked, also as expected, under low stimulation rate.

The favourable cardiac safety profile of rupatadine even under risk conditions was confirmed in another study aimed to evaluate the effect of rupatadine and its metabolites in Purkinje fibres under hypokalaemic (2 mM) conditions. Rupatadine, UR-12788 and UR-12335, at concentrations 200, 250 and 330 times the Cmax at clinically effective doses, did not have any effect on action potential duration.

Finally, a new study was conducted to assess the potential of RUP (0.01, 0.1, 1, 3 and 10 μM), UR-12788 (1 μM) and UR-12335 (1, 3 and 10 μM) to inhibit HERG currents by examining their effects on the ion channel in an appropriate in vitro test system. Effects of vehicle (0.1 % DMSO) and reference substance (E-4031), as positive control, were also evaluated. Rupatadine inhibited outward current by 45% at 10 μM (2080 times the therapeutic Cmax, 4.8nM). When outward and tail currents were corrected for mean vehicle rundown the IC25 value for rupatadine inhibition of HERG outward current was 0.9 μM, and the IC25 and IC50 values for HERG tail current were 0.7 and 8.1 μM, respectively. The IC50 value is 1685 times the Cmax of RUP achieved in humans at therapeutic doses.

Treatment with 1 μM UR-12788 was associated with a reduction of HERG current of around 16 %. This was not significantly different from the rundown of the current in the presence of vehicle (≈11.5 %). It had no effect at concentrations 258 times the Cmax found after rupatadine therapeutic dose administration (3.9 nM).

Treatment with 10 μM UR-12335 (335 times the therapeutic Cmax, 29.8 μM) resulted in a small inhibition (approximately 23 %) of HERG currents compared to vehicle treated cells. Concentrations of 1 and 3 μM had no effect on HERG currents.

All these ratios are more favourable than those obtained from open literature for others antihistamines (273)(274). Therefore, it can be concluded that the potential of rupatadine for QT widening due to HERG channel blockage seems to be very low.

In vivo studies

Studies in vivo have seen conducted in rat, guinea pig and dog. Of these species only guinea pig and dog can be considered good predictive models. This is due to the fact the relative contribution of different potassium currents responsible for cardiac repolarisation may vary with species (275).

In spite of the fact the rat is a very controversial species for evaluating QT interval variation, both rupatadine and loratadine were intravenously administered at the

dose of 30 mg/kg in rats anaesthetised. Changes in blood pressure and heart rate were observed, but the electrocardiographic parameters measured (QTc, PR and QRS) were not significantly altered, in contrast with terfenadine.

In anaesthetised guinea pigs, RUP at 30 mg/kg iv does not produce any evident alterations in the haemodynamic and electrocardiographic parameters studied. Loratadine, at the same dose, did not affect the electrocardiogram, but provoked a significant increase in heart rate and a fall followed of a pronounced rise in blood pressure. Terfenadine and astemizole at dose of 10 mg/kg iv caused bradychardia and alterations of PR (both compounds, suggesting atrioventricular conduction anomalies) and QRS (astemizol) segments of the electrocardiogram.

In a cardiovascular system study on anaesthetised Beagle dogs when rupatadine was administered at doses levels of 0.2 and 2 mg/kg iv no effect was observed on mean blood pressure or heart rate, and relatively small changes in left ventricular dp/dt. However, at 20 mg/kg, it caused a slight drop in mean blood pressure (recovering in 2 minutes) and a small rise and persistent in heart rate, and a sustained increase in dp/dt. All the three doses studied caused no effect on any of the electrocardiograph parameters measured.

In addition, rupatadine does not have any cardiac tropism from the results of distribution studies, since rupatadine levels in heart tissue are undetectable 24 h after administration.

Other study was conducted in conscious guinea pigs to evaluate possible interaction, both pharmacokinetic and pharmacodynamic of rupatadine with ketoconazole (potent inhibitor of its hepatic metabolism and in addition, with known effect on the QT prolongation by itself). Rupatadine orally was administered at 120 mg/kg, 2 h after of ketoconazole (60 mg/kg, p.o) and was compared to loratadine and terfendadine at the same dose. Loratadine did not modify either the ketoconazole-induced increase in QTc or the decrease in heart rate, whereas terfenadine did. Rupatadine did not increase QTc but decreased QT prolongation caused by ketoconazole. The mechanism, by which this effect is produced, it is not clear.

From this battery of studies it can be concluded that the safety pharmacology of rupatadine do not identify causes of concern and it seems to be similar to that expected for other marketed second-generation antihistamines. In relation to cardiovascular effects, *in vitro* and *in vivo* studies do not identify any potential for QT prolongation by administration of rupatadine alone or in combination with

ketoconazole. Especially effects of rupatadine and main human metabolites on expressed HERG channels were evaluated and compared with those of other antihistamines, and not potential of rupatadine for QT widening due to HERG channel blockage was observed. Moreover, no effects on action potential duration (APD) in dog Purkinje fibres and on QT prolongation in animal models were seen (272).

1.3.4.3 Clinical studies

Clinical pharmacology

Pharmacokinetics

Pharmacokinetic parameters of rupatadine and some of its metabolites have been characterised in 14 clinical pharmacology studies in which healthy volunteers received single or multiple doses ranging from 10 mg to 100 mg. Examinations of the disposition of 14C-rupatadine, relative bioavailability, the pharmacokinetics in elderly and the effect of concomitant food, alcohol intake and a number of drugs was also investigated.

Rupatadine is rapidly absorbed exhibiting a relevant first-pass metabolism effect. Maximal plasma concentration is achieved approximately one hour after dosing. Food had no significant effect on the extent of rupatadine absorption although delayed the time to peak plasma concentration in approximately one hour. Linear pharmacokinetics was demonstrated over the range of 10 mg to 40 mg although at higher doses AUC and Cmax increase in a non-linear manner. The volume of distribution was over 143 L/kg reflecting distribution into the tissues. The mean terminal half-lives ranged from 4.6 (10 mg) to 12.8 hours (40 mg).

Rupatadine is extensively metabolised and less than 1% of the dose is excreted as unchanged drug. Several active metabolites have been identified, the major being UR-12790, that seem to contribute to rupatadine activity. The metabolites showed longer mean elimination half-lives than rupatadine, ranging from 14 to 41 hours.

In vitro studies indicated that rupatadine is primarily metabolised by hepatic cytochrome P-450 enzyme CYP3A4. This finding was confirmed in *in vivo* studies showing increased plasma concentrations of rupatadine following co-administration with erythromycin and ketoconazole, known selective and potent CYP3A4 inhibitors.

There are no relevant age differences in pharmacokinetic parameters and no dose adjustments are required in the elderly. No data about the pharmacokinetics of

rupatadine in subjects with impaired hepatic or renal function are available and therefore rupatadine is not recommended in these populations (270)(276).

Pharmacodynamics

Pharmacodynamycs of rupatadine were characterised in 5 phase I studies. Two other pharmacokinetic trials have provided additional pharmacodynamic data.

Clinical pharmacology studies provided evidence about the pharmacodynamic effect of rupatadine. It resembles to that of other second-generation molecules. Antihistamine activity has been evaluated using standard procedures and doses from 2 mg to 80 mg exhibited significant inhibition of intradermal histamine administration. This action was shown to be dose related and it outlasts detectable plasma levels. Some studies explored aspects of secondary pharmacology such as the lack of anticholinergic activity and preliminary data about antiallergic properties are provided.

In studies of antihistamine activity in humans, both the magnitude and duration of wheal and flare suppression by rupatadine in response to intradermally injected histamine or PAF are dose-related.

Onset of antihistamine action was evident within 1-2 h after rupatadine, with a peak effect between 6-24 h, and lasting up to 24-36 h after 10 mg and for at least 48 h after 20 mg.

Rupatadine inhibited PAF-induced platelet aggregation *ex vivo* (up to 100 % inhibition, depending on PAF concentration) 2-6 h after administration (compared with pre-dose response). Inhibition reached a maximum at 4 h, whereas no effect was found at 24 h.

Rupatadine doses of 10 and 20 mg present a significant and sustained peripheral H1 antihistamine effect without changes in the objective evaluation of CNS function and objective performance measurements (272)(270)(276)(269)(277).

Clinical efficacy

The clinical development of rupatadine was initiated in 1995 focussing on the Seasonal Allergic Rhinitis (SAR) and Perennial Allergic Rhinitis (PAR) indications. In the absence of any established allergic guideline at that time, the initial clinical developmental plan was based on the results of an exhaustive literature review. This clinical development plan included a total of 2213 patients treated at different doses of rupatadine and 955 patients treated with placebo. A total of 6 studies in SAR and 4 studies in PAR were performed (270)(276).

Afterwards, two new studies were conducted in order to fulfill the new guideline recommendations Guideline on the clinical development of medicinal products for the treatment of allergic rhino-conjunctivitis (CHMP/EWP/2455/02) (260), especially in terms of treatment duration (4- and 12- weeks for SAR and PAR, respectively) and main efficacy variable definition (Total Symptoms Score). Furthermore, two studies were designed to obtain aditional data on rupatadine's long term safety in compliance with the recommendations of the Guideline on population exposure: the extent of population exposure required to assess clinical safety (CPMP/ICH/375/95) (262).

At last, the clinical development program plan for rupatadine in the SAR and PAR indications consist of 6 studies in PAR (phase II - IV) and 9 studies in SAR (phase I- IV). All studies had a multicentre, double-blind, randomized and parallel group design except for a Camera Exposure study and an Allergen Challenge Exposure study which had a randomized, double-blind, placebo-controlled, crossover design. Five studies in SAR and four studies in PAR were active-drug controlled. Active comparison agents were among the most commonly used second-generation antihistamines (cetirizine 10 mg, loratadine 10 mg, ebastine 10 mg and desloratadine 5 mg). The population enrolled in these studies is considered to be representative of the general population. The selection criteria referred to patients with presence of qualifying symptoms to ensure the inclusion of patients suffering from acute episodes of at least mild-moderate intensity of nasal symptoms. The assessment of efficacy in Phase II and III studies, was based on accepted endpoints. Placebo and second-generation antihistamines with proven efficacy in both indications were used as controls. The primary analysis was based on the intention-to-treat population although a per protocol analysis was also performed.

The dose finding studies assessed the 2.5, 5, 10 and 20 mg dosages and based on the results the 10 mg and 20 mg doses were selected to be tested in pivotal trials. Lower doses were also effective in the relief of symptoms of allergic rhinitis. However, the 10 and 20 mg dose obtained the best symptom scores and, taking into account some other relevant secondary endpoints such as overall impression of efficacy and safety evaluation, the 10 mg dose was concluded to be significantly superior to the remaining groups (268)(270)(276).

Initially, four phase III pivotal trials support the indication of SAR. In terms of primary response measure, rupatadine 10 mg was significantly better than placebo in the relief of allergic symptoms. When compared with other second-generation antihistamines (ebastine, cetirizine, loratadine and desloratadine) no statistical

differences could be detected between the treatment groups (272). A Phase IV study was conducted in order to fulfil the CHMP/EWP/2455/02 guideline requirements (260). According to the guideline a superiority trial with a randomized, double-blind, three arm parallel groups design comparing rupatadine 10 mg with placebo and desloratadine 5 mg, as an active comparator, was considered adequate. Duration of treatment and selected efficacy endpoints also complied with the guideline. The results on the primary endpoint, assessed as change from baseline in the total patient symptom-score over the four week treatment period, showed superiority of rupatadine 10 mg versus placebo. The observed effect was similar to the obtained with desloratadine 5 mg. The results obtained in this study provided reassurance of the efficacy of rupatadine in SAR indication.

Initially, three phase III pivotal studies supported the indication of PAR. Patients treated with rupatadine 10 mg and 20 mg showed to be asymptomatic approximately 50% of days in the 4 weeks treatment period. There were no differences in the relief of allergic symptoms when rupatadine was compared to loratadine, ebastine or cetirizine(272). Also in this condition, a new study has been performed complying with the Guideline (260). This study has confirmed that rupatadine 10 mg was statistically better than placebo in reducing the mean total symptom score over a 12 week treatment period.

Globally, sufficient data were available to support the efficacy of rupatadine in SAR and PAR indications. A consistent statistically significant effect over placebo was demonstrated and the observed effect with rupatadine is as expected for a second-generation antihistamine. It was concluded that rupatadine 10 mg and 20 mg have shown efficacy in the relief of symptoms associated with allergic rhinitis, both seasonal and perennial. When compared with second-generation antihistamines such as ebastine, cetirizine, desloratadine and loratadine a similar efficacy was demonstrated. The 10 mg dose obtained the best benefit/risk profile and therefore, it was recommended for approval.

The Clinical Development of rupatadine in the indication Chronic Idiopathic Urticaria (CIU) was started in 2002 and comprises of two randomised, multicenter, double-blind, parallel group, placebo-controlled studies (278)(279). The design and population was similar in both studies. Treatment duration was different, being 4 weeks for the phase II study and 6 weeks for the confirmatory study, despite the main efficacy variable was set up at 4 weeks. The dose-response study assessed the clinical efficacy of 5 mg, 10 mg and 20 mg of rupatadine compared with placebo. Rupatadine

10 mg and 20 mg were statistically different to placebo in the change from baseline in mean pruritus score (MPS). The impact in the patient´s quality of life by means of a validated questionnaire such as the DLQI was also measured. Rupatadine 10 and 20 mg were better than placebo in the change from baseline in MPS. The effect was observed after 1 week of treatment and maintained over the six week period.
Based on the results of theses studies, it was considered that the risk-benefit relationship is favourable to the 10 mg dose. Comparing the data with published studies for other antihistamine agents used in CIU, even recognising its limitations, the effect of rupatadine is highly similar to the effect reported. In conclusion, rupatadine´s effect is within of what is expected for an antihistamine agent in CIU.

Clinical safety

The safety population was that included in the clinical pharmacology studies and controlled Phase II/III clinical trials. A total of 3490 patients and healthy volunteers aged from 12 to 65 years were involved, the majority of them aged between 18 and 65 years. 2025 subjects have received rupatadine 10 mg; this represents 58% of the total subjects. Reported adverse events were similar between rupatadine 10 mg and the other second-generation antihistamines used as active comparators. The most frequent related adverse events were headache (6.9%), somnolence (9.5%), fatigue (3.2%), and asthenia (1.2%) and the majority of them were mild to moderate in severity.
No sex or age related relevant differences were detected in the adverse event frequency when the population was analysed according to sex (females versus males) or age (adolescents versus adults) subgroups.
Six serious related adverse events were reported in subjects receiving the study drug, three aminotransferases increase and three creatine phosphokinase increases (270)(276)(280).

In conclusion, the global safety profile of rupatadine can be considered similar to that described for second-generation antihistamines. Headache, somnolence and fatigue are the most frequently reported adverse events with the 20 mg dose show higher frequencies than 10 mg dose. Therefore, it was considered that 10 mg dose obtained the best benefit/risk profile and it was recommended for approval (272).
Nevertheless, taking account the data about the cardiotoxicity of non sedant antihistamines were published, with reports about the appearance of “Torsade de

pointes" (281)(282), the cardiac safety of rupatadine was assessed according to CPMP/986/9612 the ´Points To Consider - The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products' (172).

Even though there were no preclinical findings indicating any QT interval prolongations, it was applied a conservative approach to cardiac safety assessment, taking into account the characteristics of the therapeutic group. This approach generated data from over 500 volunteers/patients (many more than the minimum number of 100 cases in early phase I/II studies that is recommended in the CPMP guideline 986/96 (172), and data from phase III ECGs on more than 900 patients, without any clinically significant QTc interval prolongation. On this matter, it is interesting to highlight that the cardiac safety evaluation with rupatadine, included interaction studies with rupatadine 20 mg/day, in which volunteers had maximum concentrations of rupatadine up to 8 times larger that without interaction concentrations. There was no prolonged QTc up to the recommended limits.

During the development phase of rupatadine both FDA and EMA provide recommendations for sponsors concerning the design, conduct, analysis and interpretation of clinical studies to assess the potential for delaying cardiac repolarisation (179)(178). These papers recommended performing a 'through Qt/QTc' study to expand ECGs safety evaluation during later stages of drug development. It was recommended that the study be randomized, blind and placebo controlled. Moreover, as high sensitivity is necessary in order to detect differences in the QTc measure, it was essential that a positive control be included. On that basis, the positive control should be well characterized and consistently produce an effect corresponding to the largest change in the QT/QTc interval that is currently viewed as clinically unimportant to detect (a mean change of around 5 ms). Taking account the published data, moxifloxacin is the most frecuent positive control used. Following dosing with 400 mg of moxifloxacin, the mean change in QTc from the pre-dose value at the time of maximum drug concentration was 6 msec (283).

In this type of study, as recommended by the guidelines, there should be a characterization to ensure that the dose-response and generally the concentration-response relationship for QT/QTc prolongation, including exploration of concentrations that are higher than those achieved by following the therapeutic doses. In the general population we can find patients, which for many circumstances could present drug overexposure and therefore, present a greater risk of QT/QTc

prolongation. The guidelines recommended, if not precluded by considerations of safety or tolerability due to adverse effects, that the drug should be tested at substantial multiples of the patient maximum therapeutic exposure.

Then, following the publication of the new guidelines (176), an additional study was designed to comply with the guideline.

2. OBJECTIVE AND HYPOTHESIS

- To assess whether administration of a repeated dose 10 mg/day and at dose of 100 mg/day (10 times the authorised approved dose of rupatadine) on the ECG parameters with a special focus on its effect on cardiac repolarization (QTc interval duration) in healthy volunteers.

  The primary hypothesis is that rupatadine 100 mg doesn't show significant prolongation on QT interval vs placebo in steady state.
  The secondary hypothesis assessed in single dose and in steady state were: that rupatadine 10 mg doesn't show significant prolongation on QT interval vs placebo in steady state.

- Furthermore, the study was performed to assess the pharmacokinetic-pharmacodynamic relationship between plasma concentrations of rupatadine (and its metabolites) in the effects, if any, on cardiac repolarisation mainly in the QTc interval.

  The working hypothesis is that the large QTc prolongation should be with the large plasmatic concentration levels. Pharmacokinetic profile of rupatadine 10 mg and 100 mg was also described.

- Safety was evaluated by the incidence of treatment emergent adverse events and by changes in physical examination, vital signs, clinical laboratory test and ECG during the study.

  Our hypothesis is that rupatadine is safe and well tolerated.

2.1 Primary endpoint

To evaluate the rupatadine effects on the QTc interval (individual subject corrected: QTcIX).
When the protocol was designed, the analysis of the QTc was based on the previous bibliography and on the drafts of the guideline (Step 2, June 2004 and Step 3, December 2004). It was specified that: "a negative thorough study is one where the largest difference between the drug and placebo (baseline substracted) for the QTc

interval is around 5 ms or less, with a one-sided 95% confidence interval that excludes an effect >8.0 ms". In the updated guideline (May 2005) this definition was modified: to evaluate the drug effects on the QTc interval (individual subject corrected) the largest time-matched mean difference between the drug and placebo (baseline-subtracted) for the QTc interval must be used. A negative "thorough QT/QTc study" is one which the upper bound of the 95% one-sided confidence interval for the largest time-matched of the drug on the QTc interval excludes 10 ms (178). Then the protocol was amendment according it.

2.2 Secondary endpointss

To evaluate the rupatadine effects on the QT and QTc interval (QTcB and QTcF corrected) using the largest time-matched mean difference between the drug and placebo (baseline-subtracted) for the QTc interval.

To evaluate the rupatadine effects on the QT and QTc interval (QtcIX, QTcB and QTcF corrected) using the largest time-average mean difference between the drug and placebo (baseline-subtracted) for the QTc interval.

To evaluate the maximal change for each individual, the outliers values and the morphological changes between treatments.

To compare gender differences for the central tendency analysis in change from baseline ECG intervals for QTcIX

Furthermore, the study was performed to assess the pharmacokinetic-pharmacodynamic relationship between plasma concentrations of rupatadine (and its metabolites) in the effects, if any, on cardiac repolarisation mainly in the QTc interval. The working hypothesis is that the large QTc prolongation should be with the large plasmatic concentration levels. Pharmacokinetic profile of rupatadine 10 mg and 100 mg was also described.

Safety was evaluated by the incidence of treatment emergent adverse events and by changes in physical examination, vital signs, clinical laboratory test and ECG during the study.

3. SUBJECTS AND METHODS

3.1 Ethics

3.1.1 Independent Ethics Committee (IEC)

Before performing the study the protocol was approved by the Ethical Committee for Clinical Research of the 'Hospital de la Santa Creu i Sant Pau' in Barcelona. It was approved on the 23th of November 2004 with assigned number EudraCT: 2004-003899-11.

The study protocol was also authorised by the Spanish Drug Agency (Ministry of Health), being approved after stated authorisation given on December 22th 2004, expenditure date 11-01-05.

No relevant amendment nº 1 was submitted to the Ethical Commite on 4th of February 2005 and to the Spanish Drug Agency on the 9th of February 2005.

Relevant amendment nº 2 was submitted to the Ethical Committee on 4th of July of 2005 and further approved on 26th of July 2005. The EC approval was submitted to the Spanish Drug Agency on the 2nd of September 2005, and it was approved by the procedure of administrative silence after 60 days from the submission.

3.1.2 Ethical conduct of the study

The study was conducted following the international recommendations for clinical research gathered in the declaration of Helsinki and its updating (Edinburgh, Scotland – 2000, Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002 (264)) and following the EC Note for Guidance on Good Clinical Practice (CMPM/ICH/135/95) (Guideline for Good Clinical Practice – ICH Topic E6. Step 5, Consolidated Guideline) (18). The study was performed following the Standard Operating Procedures of the 'Centre d'Investigació de Medicaments' and of the J. Uriach y Compañía,S.A.

3.1.3 Patient information and consent

All subjects were thoroughly informed on the nature of the study (aims, type of treatment, methodology) and the drugs tested (expected risks/benefits, possible side effects and adverse reactions). They were clearly informed that they could withdraw from participating in the study at any time and that this would have no effect on the medical care they received. They were also informed that the main investigator could

exclude them from the study if he/she considered it necessary.
In addition to being fully informed verbally so that they could ask any questions in relation to the study, this information was also provided in written form to the volunteers.
All the subjects included gave their consent to participate in the study and signed an informed consent form.
A model of the informed consent form and of the information for the volunteers can be found in Annex 1.

3.1.4 Access to patient data

The patient, by signing the informed consent form, authorized to the investigators, the sponsor and its representatives, the Ethics Committee and competent authorities to have direct access to the medical records and other source data or medical documents. All patient recorded data are protected by LOPD legislation (Ley 15/1999 del 13 de dicembre; BOE 14-12-1999 and regulatory framework of Ley 15/1992, del 29-10-1992 and BOE 14-12-1999).
All the staff who participated in this research project expressly agreed not to disclose the patients' identity and to observe the confidentiality rules regarding data and information made available to them for participating in this study.
The investigator kept complete identification of each patient and agreed to provide the monitor, auditor and/or competent authorities with any necessary information. This information was treated in a strictly confidential manner following the actual laws about data protection.
All the information obtained as a result of the study was the property of the study sponsor and was confidential until deemed appropriate by the sponsor. The investigator could only report the study progress and results to the sponsor, the EC of his site and the applicable regulatory authorities. Any other communication regarding the study progress or results should be previously authorized by the sponsor in writing.

3.1.5 Insurance policy

J. Uriach y Compañía, S.A. took out a legal liability insurance policy, under the conditions and limitations set forth by the Royal Spanish Decree (Real Decreto Español) 223/2004(284) .

3.2 Overall study design and plan - description

This was a study included in the clinical development of the rupatadine.

This study was performed following a single blind (blinded volunteers and evaluators and unblinded investigators), randomised, placebo and active control (moxifloxacin) and parallel design, in 160 healthy volunteers. Subjects were randomly allocated to one of the 4 treatment groups: rupatadine 100 mg (R100); rupatadine 10 mg (R10); moxifloxacin (Moxi) and placebo (Pb).

Table 4: Treatment allocations

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Treatment 1	Pb	R100	R100	R100	R100	R100
Treatment 2	Pb	Pb	Pb	Pb	Pb	Pb
Treatment 3	Pb	Moxi	Pb	Pb	Pb	Moxi
Treatment 4	Pb	R10	R10	R10	R10	R10

Placebo was administered on Day 0 in all the groups in order to get an intraindividual control of the spontaneous variability in the ECG changes.

Moxifloxacin was only administered on D1 and D5 in order to avoid at the maximum the appearance of some adverse events or discomfort in the volunteers.

3.3 Active and control groups

The rupatadine doses were selected to provide evidence of a dose response or concentration response relationship for QT/QTc interval analysis. The recommended dose of Rupatadine 10 mg once day is the therapeutic dose authorised and marketed for controlling the symptoms of allergic rhinitis in adult and adolescent patients. Otherwise, the trial also evaluates a supratherapeutic dose of rupatadine since the study population is in healthy volunteers rather than the target population to eliminate the heterogeneous conditions that affect the QT duration, as the guidelines recommended. Taking into account antihistamine characteristics and the previous preclinical toxicity and interaction data in humans, we use the criterion to administer 10 times the therapeutic dose (100mg/day), because it is not predicted that there would be patients with a greater overexposure than this dose.

In order to assure an adequate and well-controlled study, with mechanisms to deal a potential bias, a placebo group also was included

Furthermore, a 'thorough study' should have a high degree of confidence in the ability of the study to detect differences of clinical significance. This confidence is greatly enhanced by the use of a concurrent positive control group (to establish the assay sensitivity). The positive control selected should have an effect on the mean QT/QTc interval of about 5 ms. Detecting the positive control's effect would established the ability of the study to detect such an effect of the study drug. On these bases, moxifloxacin 400 mg was selected as the positive control.

A multiple dose design was selected on the basis of the metabolism of rupatadine, which has two active metabolites. The choice of a parallel trial was made in light of the chronic dosing required to reach steady state and the elimination of any chance of carryover or period effects which could occur in a crossover model. To more robustly define the ECG effects of rupatadine, a full set of endpoint ECGs were obtained not just at baseline and at steady state but also after single dose on day 1 for comparisons. A validated central ECG laboratory using digital ECG processing techniques was employed with robust fully manual measurements as recommended by regulatory guidances.

3.4 Selection of study population

The subjects participating in the study were selected from the panel of volunteers of the Drug Research Centre of Hospital de la Santa Creu i Sant Pau.- CIM Sant Pau. All subjects included in the study met the following inclusion/exclusion criteria.

3.4.1 Inclusion criteria

1. Male or female between 18 and 45 years old (inclusive).
2. In good health as determined by the principal investigator based on medical history, physical examination, ECG, and clinical laboratory tests.
3. Body Mass Index between 19 and 27 kg/m^2.
4. Non-smokers (refrained from any tobacco usage, including smokeless tobacco, nicotine patches, etc., for 6 months prior to the administration of the study medication).
5. Negative serology Hepatitis B, C or HIV in the 3 previous months.
6. Female subjects must have a negative serum pregnancy test result prior to

enrolment into the study. Female subjects of childbearing potential (including peri-menopausal women who had had a menstrual period within one year) must be using appropriate birth control (defined as a method which results in a low failure rate, i.e. less than 1% per year, when used consistently and correctly, such as implants, injectables, some intrauterine contraceptive devices [IUDs], sexual abstinence, or a vasectomised partner) during the entire duration of the study. Oral contraceptive medications were prohibited in this study.

7. Capable of understanding and complying with the protocol, and had to have signed the informed consent document prior to the performance of any study-related procedures.

3.4.2 Exclusion criteria

Subjects presenting with any of the following were not be included in the study:

1. Currently abusing drugs or alcohol (> 30 gr/day; or > 1/2 drinks/day for females/males, or with a history of drug or alcohol abuse within the past two years
2. Subjects abusing any xanthine-containing food and beverages (e.g., 2 or more coffee tea or chocolate cups, and cola beverages).
3. Had taken any grapefruit or grapefruit juice during 14 days prior to the screening visit.
4. Unwilling or unable to comply with the protocol or reside in the study unit during the study period or to cooperate fully with the principal investigator and site personnel.
5. Had used any prescription medication within 14 days prior to Day 0, or over-the-counter (OTC) medication, herbal preparations, and/or vitamins within 48 hours prior to the start of study drug administration on Day 0.
6. A 12 lead ECG obtained at screening with: PR > 240 msec, QRS >110 msec and QTc > 430 msec in males or QTc > 450 msec in females, bradychardia (<50 bpm) or clinically significant minor ST wave changes on the screening ECG, or any other changes on the screening ECG that would interfere with measurement of the QT interval.
7. Had a serum potassium, sodium, calcium, or magnesium level that is not within normal limits or has other laboratory values outside the normal range at the screening visit that are deemed by the principal investigator to make the subject an inappropriate candidate for the study.

8. Had taken any other investigational drug during the 2 months prior to screening visit.
9. Had donated or lost more than 250 cc of blood within 30 days prior to screening visit.
10. Pregnancy or breast-feeding women.
11. Had any condition(s) that in the investigator's opinion would: a) warrant exclusion from the study or b) prevent the subject from completing the study.
12. Unable to understand, verbally and/or in written form, the informed consent.
13. History of hypersensitivity or allergic reaction to moxifloxacin or any other member of the quinolone class of antibiotics.
14. History of hypersensitivity or allergic reaction to Rupatadine or any other antihistamine compounds.

Furthermore, all subjects that showed any waiver to the inclusion or exclusion criteria were excluded from the study, at the discretion of principal investigator and/or medical monitor.

3.4.3 Removal of patients from therapy or assesment

Because the participation was voluntary the subjects could drop out from the study without having to specify their reasons. In turn, the investigator could withdraw a subject from the study whenever he considered it appropriate. Furthermore, it was specified that a subject would be withdraw from the study for the following reasons:

- Mistake in treatment allocation
- QTc interval >500ms
- Presence of any of the exclusion criteria occurring or evidenced after subject has been randomised at the discretion of the Investigator
- If an adverse reaction (including a concomitant illness)would have developed, which was considered by the Investigator incompatible with the continuation of the study
- If the administration of a drug, which was not permitted by the exclusion criteria, would have been necessary
- Subject failure to comply with the requirements of the protocol

All discontinuations were recorded and clearly specified in the Case Report Form (CRF). Subjects who discontinued the study were replaced.

3.5 Treatments

3.5.1 Treatments administered

The administered treatments were the following:

- Experimental drug: Rupatadine 10 mg and 100 mg, tablets, once daily, orally. Antihistaminics - H1-antagonist, ATC code: R06AX28.
- Placebo control: Placebo tablets, once daily, orally
- Positive control: Moxifloxacin 400 mg, once daily, orally. Antibacterial fluoroquinolones, ATC code: J01MA

On day 0, the subjects were randomised to one of the four treatment groups. Investigator or authorised staff administered the drug to the subjects, in one box, which contain 6 bags with the diary dose of one of the four study treatments in order to the randomization list. The study drug dose was administered with 240 ml of water, with at least 10 hours to fast. Each day, subjects intook the study drug dose under supervision of the investigator.

All treatments were administered once daily during 6 consecutive days.

The 10 mg rupatadine group, took on day 0: placebo, and on days 1-5: rupatadine 10 mg/day.

The 100 mg rupatadine group, took on day 0: placebo, and on days 1-5 the respective rupatadine dose.

The moxifloxacin group took on day 0: placebo, on days 1 and 5 moxifloxacin 400 mg, and on days 2,3 and 4 days: placebo as positive control, in such away that the adverse events or discomfort produced by multiple dose of moxifloxacin will be minimised.

The placebo group took 6 days placebo treatment.

The study drug was exclusively used for the present clinical study and only was administered to the subjects enrolled in the study

For pharmaco-technical reasons, it was not possible to make an only tablet for the 100 mg dose. Then, the 100 mg dose was administered as the sum of three tablets (40 mg + 40 mg + 20 mg). In order to maintain the blinded conditions the other groups of treatment got three tablets (active treatment + two placebo tablets)

3.5.2 Identity of investigational products

Experimental Treatment:

Rupatadine, Lab J.Uriach y Compañía, S.A.

Pharmaceutical form: tablets 10mg

Administration route: oral

Batch num: 0904. Expiry date: January 2006

Manufactured by: Lab. J. Uriach y Compañía, S.A.

Experimental Treatment:

Rupatadine, Lab J.Uriach y Compañía, S.A.

Pharmaceutical form: tablets 20mg

Administration route: oral.

Batch num: 0904. Expiry date: January 2006

Manufactured by: Lab. J. Uriach y Compañía, S.A.

Experimental Treatment :

Rupatadine, Lab J.Uriach y Compañía, S.A.

Pharmaceutical form: tablets 40mg.

Administration route: oral.

Batch num: 0904. Expiry date: January 2006

Manufactured by: Lab. J. Uriach y Compañía, S.A.

Reference Treatment:

Control:

Placebo (lactosa), Lab J.Uriach y Compañía, S.A.

Pharmaceutical form: tablets.

Administration route: oral

Batch num: 0904. Expiry date: January 2006

Manufactured by: Lab. J. Uriach y Compañía, S.A.

Active control:

Moxifloxacin (Actira®), Bayer Healthcare, S.A.

Pharmaceutical form: capsules.

Administration route: oral.

Batch num: 0904. Expiry date: January 2006

Manufactured by: Lab. J. Uriach y Compañía, S.A.

The study sponsor keeps the manufacturing and quality control protocols of the treatment lots manufactured for this clinical study. Further, samples of the lots will be kept until twelve months after the expiry date.
Medications were prepared individually for each volunteer. Investigator or authorised staff will administered the drug to the subjects, in one box, which contain 6 bags with the diary dose of one of the four study treatments in order to the randomization. Each treatment unit was identified with a label that contained the following information:

Sponsor: J. Uriach y Compañía, S.A.
Polígon Industrial Riera de Caldes
Avda. Camí Reial, 51-57.
08184 Pala-Solità i Plegamans. Barcelona
Technical Director: Dr J.C. Gibert
Protocol IC012RUP/1/04
Volunteer nº:
Investigator: RM.Antonijoan; M.Barbanoj
Day 0-5
Oral administration
Batch num.: 0904
Expiry date: 01 2006
DRUG FOR CLINICAL STUDY ONLY
KEEP AWAY FROM CHILDREN'S REACH

3.5.3 Method of assigning patients to treatment groups

The subjects were allocated to one sequence of treatment administration following a randomisation list previously generated. The randomization was stratified according to gender to assure that each treatment group would have the same proportion of males and females. An unbalance of 5% according to gender was accepted.
Treatment was randomly assigned to each of the active codes by personnel from the Quality Assurance Unit of J. Uriach y Compañía Research Centre, not involved in the conduction of the study, which guaranteed the confidenciality of this information. Treatment was allocated to patients according to a computer-generated randomized

list produced by the Production Quality Management of J. Uriach y Compañía Research Centre.

There were two copies of the study randomization list. One of them was properly sealed and kept in the Production Quality Management of J. Uriach y Compañía. The other copy was given to the investigators and stored in the Center File.

3.5.4 Selection of doses in the study

As it was commented previously, the selected rupatadine doses were the therapeutic dose and supratherapeutic dose. Rupatadine 10 mg orally once day is the authorised and marketed dose. Otherwise, the trial also evaluated a supratherapeutic dose of rupatadine since the study population is in healthy volunteers rather than the target population in order to eliminate the heterogeneous conditions that effect QT duration, as the guidelines recommended. Taking into account the antihistamin characteristics and the previous preclinical toxicity and interaction data in humans, we use the criterion to administer 10 times the therapeutic dose (100mg/day), because it is not predicted that there would be patients with a greater overexposure than this dose.

In order to assure an adequate and well-controlled study, with mechanisms to deal with potential bias, a placebo group also was included

Moxifloxacin 400 mg was used as the positive control for determination of the cardiac repolarisation effect. The 6 ms prolongation QTc was produced after a single dose of moxifloxacin, and because of this the protocol envisages administration on days 1 and 5, and the placebo administration on days 2,3 and 4, in order to reduce discomfort and adverse events for a continuous treatment, without interference in the correct design of the study.

Furthermore, in order to control spontaneous variability in ECG effects, all the groups intook placebo on Day 0.

Subjects were randomized and they received a single oral dose of either rupatadine 10 mg or 100 mg, placebo or moxifloxacin that were administered onde daily in the morning for 6 days in all cases.

3.5.5 Blinding

As usual in this type of studies, moxifloxacin was given open label.

In order to blind the rupatadine and placebo treatments to the volunteers, double dummy techniques were applied.

Individual envelopes identified with the patient's assignment number contained the identity of the treatment assigned in each case were prepared. These envelopes, properly closed and sealed were provided to the investigator. At the end of the study, all envelopes were returned to the study monitor who checked that no envelope was opened for an unjustified reason.

The study allowed the investigators to remain unblended in order to have a rapid response in case of a fatal adverse event.

The centralized laboratory as well as statistical unit were blinded for the ECG analysis. Digital 12-lead Holter ECGs were recorded as noted on a digital flash card. The subject's unique identification number and demographic information were recorded for each card. The central ECG laboratory and the Statistical Unit received the data without knowledge of subject treatment assignment, and generated and evaluated the 12-Lead ECG digital packet at each time point required by the protocol.

3.5.6 Prior and concomitant therapy

No other medication was allowed during the course of the study. Exceptionally, paracetamol was allowed if the investigator considered it appropriate, and this had to be correctly recorded in the case report form (CRF).

3.5.7 Treatment compliance

The study medications were always administered in the presence of an investigator. A drug dispensation list was recorded and at the end of the treatment the drug accountability was done.

3.6 Efficacy and safety variables

3.6.1 Efficacy and safety measurements assessed and flow chart.

3.6.1.1 Flow Chart

Table 5: Flow chart procedures

	Screening	Day -1	Day 0 (Baseline Period)												
			0h	30´	1h	1h30’	2h	3h	4h	6h	8h	12h	14h	16h	20h
Informed consent	X														
Demographics	X														
Clinical history	X														
Physical examination	X	X													
Vital Signs	X	X													
Haematology /Biochemistry	X	X													
Urinalysis	X	X													
Pregnancy test[a]	X	X													
Toxicology	X	X													
Serology	X														
Genotype CYP2D6	X														
ECG standard[b]	X	X													
Flashcard ECG: 3ECGs			X[c,d]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Previous/concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization			X												
Study drug			X												
Pharmacokinetics															

a: Females only
b: Recorded with Standard Paper ECG
c: Insertion Flashcard ECG
d: Basal

	Day 1 (Active treatment period)												
	0h	30´	1h	1h30’	2h	3h	4h	6h	8h	12h	14h	16h	20h
Informed consent													
Demographics													
Clinical history													
Physical examination													
Vital Signs													
Haematology /Biochemistry													
Urinalysis													
Pregnancy test$_a$													
Toxicology													
Serology													
Genotype CYP2D6													
ECG standard$_b$			X										
Flashcard ECG: 3ECGs	X$_{c,d}$	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Previous/concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization													
Study drug	X												
Pharmacokinetics	X$_d$	X	X	X	X	X	X	X	X	X	X	X	X

	Day 2 (+23h Day1)	Day 3	Day 4
Informed consent			
Demographics			
Clinical history			
Physical examination			
Vital Signs			
Haematology /Biochemistry			
Urinalysis			
Pregnancy test[a]			
Toxicology			
Serology			
Genotype CYP2D6			
ECG standard[b]	X	X	X
Flashcard ECG: 3ECGs	X[d]		
Adverse events	X	X	X
Previous/concomitant medication	X	X	X
Randomization			
Study drug	X	X	X
Pharmacokinetics	X[d]		

	Day 5													Day 6	Day 7 (Check-out)
	0h	30´	1h	1h30’	2h	3h	4h	6h	8h	12h	14h	16h	20h	23h	
Informed consent															
Demographics															
Clinical history															
Physical examination															X
Vital Signs															X
Haematology /Biochemistry															X
Urinalysis															X
Pregnancy test[a]															X
Toxicology															
Serology															
Genotype CYP2D6															
ECG standard[b]			X												X
Flashcard ECG: 3ECGs	X[c,d]	X	X	X	X	X	X	X	X	X	X	X	X	Xd	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Previous/concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization															
Study drug	X														
Pharmacokinetics	X[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X

3.6.1.2 Procedures for visits

The following procedures were performed in the different visits:

Screening period

In the 4 weeks before the beginning of the experimental phase all the candidates for participation in the study (after being informed verbally and in writing and once they had given their written informed consent to participate in the study) underwent an examination to verify their health condition and compliance with the inclusion/exclusion criteria: clinical record; complete physical examination including body mass index, vital signs and ECG; laboratory tests (haematology, biochemistry, urinalysis and serology including HIV, hepatitis B and C) and drug screening in urine (cocaine, amphetamines, opiates, ethanol, benzodiazepines and cannabis) and, in the case of women, a pregnancy blood test. The expected number of healthy volunteers to be included was 160, 40 healthy volunteers in each group of treatment

Day –1 (Check in at the Clinical Unit)

The eligible subjects checked in at the Clinical Unit on Day –1.

The following procedures were done:

- Physical examination and vital signs.
- Check the inclusion and non-inclusion criteria.
- Record paper 12-lead electrocardiogram using standard ECG recorder (HP Pagewriter 300pi).
- Collect blood and urine samples for safety clinical laboratory tests.
- Collect urine sample for drug abuse.
- Pregnancy test in female subjects of childbearing potencial.
- Records adverse events.
- Record concomitant medications.

Dinner was served at 8 pm. Volunteers remained at night in the Clinical Unit.

Day 0 (Baseline period)

On day 0, the subjects were randomised to one of the four treatment groups following the randomisation table . The treatement groups were :

Group Rupatadine 10 mg comp: day 0 (placebo), and on days 1-5 rupatadine 10 mg/day.

Group Rupatadine 100 mg comp: day 0 (placebo), and on days 1-5 rupatadine 100 mg/day.

Group Moxifloxacin 400 mg: day 0 (placebo), days 1 and 5 moxifloxacin 400 mg/day, and on days 2,3 and 4 placebo.

Group Placebo: 6 days placebo treatment.

A flashcard was inserted into the Holter ECG device (Mortara Instrument H-12 ECG continuous recorder) and assigned to each volunteer. Recording 12-lead ECGs by Holter was done at baseline, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h and 23h.

Assessment of adverse events and changes in concomitant medication were registered at the same timepoints as well as after spontaneous communication).

Breakfast, lunch and dinner were given at 2h (10 a.m.), 6h (2 pm.) and 12h (8 pm) postdose, respectively.

Volunteers remained at night in the Clinical Unit

Day 1 (Active treatment period)

A new flashcard was inserted into each Holter ECG device and recording 12-lead ECGs (three in each point) was done at baseline, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h and 23h.

Blood samples were collected for pharmacokinetic at baseline, 30', 1h, 1h30', 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h and 23h.

Active treatments were administered according to randomisation code.

Record safety ECGs standard (between 1h and 2h post-treatment).

Assessment of adverse events and changes in concomitant medication were registered at the same timepoints as well as after spontaneous communication).

Breakfast, lunch and dinner were given at 2h (10 a.m.), 6h (2 p.m.) and 12h (8 p.m.) postdose, respectively. Volunteers remained at night in the Clinical Unit.

Day 2 to Day 4 (Active treatment period)

Baseline three 12-lead ECGs was recorded using flashcard the Day 2 (+23h of Day 1).

Baseline blood samples were collected for pharmacokinetics the Day 2.

Active treatment according to randomisation code were administered.

Assessment of adverse events and changes in concomitant medication were registered at the same timepoints as well as after spontaneous communication.

Baseline 12-lead ECGs were done using a standard ECG machine from 1 hour after the intake of the drug and before they get out of the Unit.

Subjects checked in at the Clinical Unit in the afternoon on Day 4 and they remained at night Day 4 in the Clinical Unit.

Dinner was served at 8 pm.

Day 5 (Active treatment period)

A new flashcard was inserted into the Holter ECG device.

Record three 12-lead ECGs with flashcard at baseline, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h 16h, 20h and 23h.

Blood samples were collected for pharmacokinetics at: baseline30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h and 23h.

The last dose of active treatments was administered according to randomisation code.

Record safety ECGs standard (between 1h and 2h post-treatment).

Assessment of adverse events and changes in concomitant medication were registered at the same timepoints as well as after spontaneous communication.

Volunteers remained at night Day 5 in the Clinical Unit.

A breakfast, lunch and dinner were given the day 6 at 2h (10 a.m.), 6h (2 p.m.) and 12h (8 p.m.) postdose, respectively.

Day 6 (24 h after dosing)

ECG Holters were removed after the last recording (23 hours after the last dose).

A blood sample was collected (23h after the last dose).
Assessment of adverse events and recording changes in concomitant medication were registered.

Day 7 (48 h after dosing and check out of the study)
12 lead ECGs using a standard ECG recorder was register.
Blood sample (48 h after the last dose) was collected.
Assessment of adverse events and recording changes in concomitant medication were registered.
Physical examination and vital signs were performed as well as blood and urine samples were collected for clinical laboratory tests (haematology, biochemistry, urinalysis and pregnancy test (only in woman).
Pathologic changes, if any was followed until their normalisation

3.6.1.3 Evaluation of procedures

Laboratory Tests: The laboratory tests performed were: haematology, biochemistry, urinalysis and serology including HIV, hepatitis B and C) and drug screening in urine (cocaine, amphetamines, opiates, ethanol, benzodiazepines and cannabis) and, in the case of women, a pregnancy blood These tests were analysed in the Central Laboratory of Hospital de la Santa Creu i Sant Pau and were evaluated by the investigators.

Standard ECGs: One safety ECG standard was performed, using a HP Pagewriter 300pi machine, at the screening (day –21 to –2), upon check-in (day –1), on day 2, day 3, day 4 and day 7. These ECGs were performed only by safety reasons, but they have not been used in the formal statistical analysis of the endpoint ECG data.

Digital ECGs: Twelve-lead ECG data were digitally obtained using a twenty-four hours of continuous recording on Day 0, Day 1 and Day 5.
Digital ECGs were analized by eResearch Technology (eRT), a global company specializing in centralized cardiac safety services in drug development

The quality of the ECG data was assure by the use of modern equipment with the capacity for digital signal processing and correctly calibrated (Mortara Instrument H-12 ECG continuous recorder).

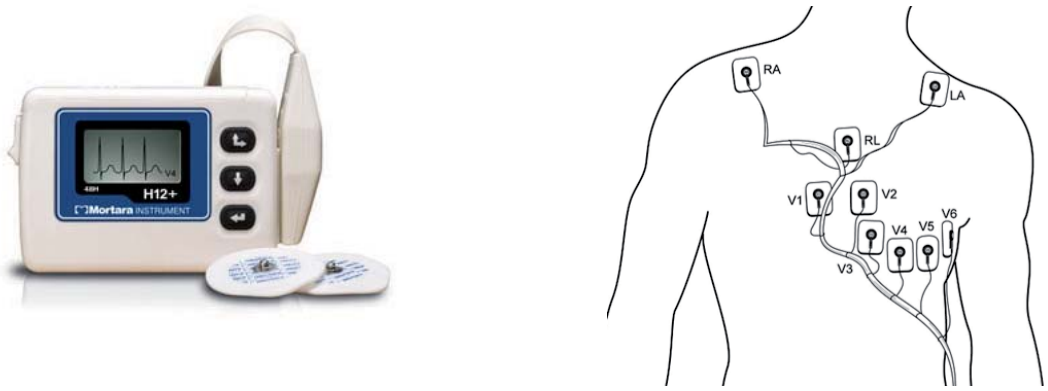

Figure 20: Mortara Instrument H-12 and leads distribution

The H12+ Holter recorder stores 12 leads continuously for a 24-hour period. A keyboard is available to set up system configuration, to enter patient's ID, to check lead quality during hook-up, and to start the recording. During the recording, the keyboard can be used to enter event markers. H 12+ has a large LCD screen to allow ECG display during the hook-up, lead quality check, system configuration and various warning messages for the hook-up technician. H12+ uses one AA battery, and a removable Compact Flash card as a memory support Digital 12-lead Holter ECGs were recorded on a digital flash card. The subject's unique identification number and demographic information were recorded for each flash card. The flash cards recorded during the study were sent to the eRT central laboratory in Philadelphia (USA) for a treatment blinded manual analysis. The central ECG laboratory received the data and they converted them to individual XML files ("XML"). XMLs were loaded by eRT into its data management system, EXPeRTTM ("EXPeRT"), and were available for analysis and measurement within EXPeRT.

Training sessions were realized to ensure consistency of operador technique and data adquisition practices.

The ECGs were stored on a flashcard about every 10 seconds and were not available for review until the card was received by the central ECG laboratory and analyzed.

For both baseline (Day 0) and treatment (Day 1, Day 5) period, 3 ECGs will be downloaded at each of the following pre-specified time points:

Day 0 (baseline period): 0h, 30´,1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h, 23 h

Day 1 (after single dose): 0h, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h, 23h.

Day 5 (steady state day): 0h, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h, and 23h.

The timepoints were selected on the basis of the pharmacokinetic profile of rupatadine to get a complete evaluation of its effects.

Three ECGs for each of 13 time points at baseline, Day 1 and Day 5 analyzed a total of 117 ECGs in each subject. This totaled about 19000 ECGs in this trial.

In the event of the holter recording ending before recording is complete, eRT was permitted to extract timepoints before the scheduled time under guidelines stated according to the pharmacokinetic profile of treatments.

Each fiducial point (onset of QRS, offset of T wave, etc.) was electronically marked and the original ECG waveform and such annotations were separately saved in an xml format available for independent review.

Three 12-lead ECGs were downloaded from the H-12 flash card within 1 minute to provide 3 ECGs for each time point. Three complexes from lead II were analyzed using a high-resolution manual on screen caliper method with annotations to define ECG interval durations. Standard ECG intervals including RR, PR, QRS, and QT were determined. There were three types of corrections from QT to QTc determined: QTcI (individually defined QTc), QTcB (QTc obtained by the Bazett's formula), and QTcF (QTc obtained by the Fridericia's formula). Changes in ECG intervals from baseline were calculated. Baseline was defined as the mean of the values for the ECG measurements taken on

Table 6: Performed measurements for each ECG by eRT

Interval	Measure
Three R-R	mean R-R is reported.
Three PR	mean PR Interval is reported
Three QRS	mean QRS Width is reported
Three QT	mean QT Interval is reported

The following calculations were made from the intervals measured for each ECG.

Three QTc, Bazett's formula

QTcB1 = QT1/√RR0

QTcB2 = QT2/√RR1

QTcB3 = QT3/√RR2

Three QTc, Fridericia's formula

QTcF1 = QT1/3√RR0

QTcF2 = QT2/3√RR1

QTcF3 = QT3/3√RR2

Mean QTcF = (QTcF1 + QTcF2 + QTcF3)/3

Three (3) Heart Rate

HR1 = 60 / RR0

HR2 = 60 / RR1

HR3 = 60 / RR2

Mean Heart Rate = (HR1 + HR2 + HR3)/3

Statistical Department of J. Uriach y Compañía,S.A. calculated the individually defined QTc as determined by:

QTcIX = QT / (RR) b

where b is the estimated slope of the linear regression model of log(QT)=a+b*log (RR). This is considered the most accurate method to correct QT for heart rate. Calculation of QTcIX will be accomplished using a SAS program.

Physician Electrocardiographers from eRT interpreted all ECGs generated from this project and such assessments will include standard comments on normal/abnormal, rhythm, arrhythmia, conduction, morphology, myocardial infarction, ST segment, T wave and U wave observations.

Figure 21: Procedure for ECG adquisition

Pharmacokinetic data:

Blood was drawn at for pharmacokinetics on day 1 and on day 5 at the following points: baseline, 30´, 1h, 1h30´, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 20h and 23h. after the intake of the medication. Pharmacokinetic sample collections were taken following ECG measurements. Blood samples of 8 ml were collected from all subjects on all treatments to maintain comparable trial conditions and the blind. Only those plasma samples collected following administration of rupatadine were analyzed. Parent rupatadine and its metabolites (BCP and BCP-OH) were measured.

Blood samples were collected in tubes containing lithium heparin and were centrifuged at 3000 r.p.m. during 10 minutes at 4º C. Supernatant plasma was separated in 2 aliquots that were kept at -20º C until they were analysed.

Plasma samples were identified with the following label:

* ICO12RUP/1/04
* Volunteer: (num. 1...160)
* Treatment: (1.....4)
* Day: (1 or 5)
* Time: (Baseline...+ 48 h)
* Date: (dd/mm/yy)

The plasma levels of rupatadine, BCP and BCP-OH were measured using a validated LC/MS/MS analytical method with a calibration range from 0.1 to 10 μg/L and a lower limit of quantification of 0.1 μg/L for each compound.

Adverse events: Adverse events (Aes) were register by direct open question at the specified timepoints as well as by spontaneous notification in any time. All the adverse events were evaluated by the investigators and followed until its resolution.

Adverse events were coded using the MedDRA dictionary (version 8.0). Each of the adverse events recorded were associated with a low level term, a preferred term and an organ class. Adverse events have been summarized by organ class and preferred term in two tables, the former for all reported AEs and the last for related (possible, probable and definite) AEs

3.6.2 Primary variable

The primary analysis of QTc was based on individually corrected QT (QTcIX). Treatment effects on QTcIX were assessed using the largest time-matched mean difference between the drug and placebo (baseline-subtracted) for the QTc interval.

Treatment comparison was conducted for each ECG interval between rupatadine and placebo. An analysis of covariance (ANCOVA) of change from baseline ECG interval, with treatment and time as model terms, was performed for each ECG interval. From this ANCOVA, two-side 90% confidence intervals from the following treatment differences in least squares means were calculated: 10 mg rupatadine versus placebo, 100 mg rupatadine versus placebo, 10 mg rupatadine versus 100 mg rupatadine, and moxifloxacin versus placebo.

Statistical analysis would be performed to evaluate the gender differences only if the positive "thorough QT/QTc study" is reached. An analysis of covariance (ANCOVA) of change from baseline ECG interval, with treatment, gender, treatment by gender interaction as model terms, was performed for each ECG interval. The test will be made at the 0.10 significant level.

3.6.3 Secondary variables

3.6.3.1 Pharmacodynamic – ECG analysis

Treatment comparison and gender differences for the central tendency analysis in change from baseline ECG intervals for QTcB, QTcF and QTcIX were done through tests similar to those applied to assess the primary variable.

All outliers were summarized for each treatment group on the basis of subject incidence rates. The outlier summary tables include total number of subjects, number of outlier subjects, and percentage of outlier subjects. Treatment comparison between Rupatadine doses and placebo, and between moxifloxacin and placebo were tested using Chi-square analysis or Fisher exact test.

3.6.3.2 Pharmacokinetic-Pharmacodynamic relationships

The primary pharmacokinetic-pharmacodynamic (PK/PD) analysis explored the relationship between changes from baseline in QTcIX (QT corrected for each individual using about paired QT and RR data sets prior to treatment) and plasma concentrations of rupatadine. The PK/PD relationship is an important component of a totality of evidence assessment of the risk of QT prolongation. Model characteristics were based on knowledge of the pharmacology of rupatadine and its metabolites.

The following plots are provided using data from individual subjects and mean data for each treatment group:

- Plot QTcI changes from baseline versus time overlaid with the plot of rupatadine plasma concentrations at corresponding time points.
- Plot of maximum QTcI changes from baseline versus rupatadine plasma concentrations collected at the corresponding time.

The secondary analysis was based on QTcF and QTcB, respectively. The plots were generated for rupatadine and their metabolites (BPC and BPC-OH). Moreover, plots were generated for single dose and steady state periods.

3.6.4 Drug concentration measurements

A traditional approach was used to collect and analyze data to assess the pharmacokinetic profile of rupatadine. Plasma levels of rupatadien and its main metabolites (BCP and BCP-OH) were measured using a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) analytical method with a calibraciton range from 0.1 to 10 μg/L. and a lower limit of quantification of 0.1 μg/l for each compound. The results of the rupatadine, BCP and BCP-OH concentrations in the study samples have been tabulated in concentration units (μg/l, equivalent to ng/ml) by treatment, in order to be used to the appropriate pharmacokinetic analysis.

The pharmacokinetic parameters were derived individually for each subject from the plasma levels using (WinNonlin 4.1, Pharsight Corporation) with a non-compartmental analysis. The arithmetic mean, standard deviation, mean standard error, coefficient of variation, median, maximum, minimum, range and geometric mean of these parameters were also calculated. The following kinetic parameters were evaluated:

3.6.4.1 Single dose:

- Cmax: maximum observed plasma level achieved after the administration.
- Tmax: time to reach the observed maximum concentration.
- AUC0-∞ : area under the concentration-time curve from zero to ∞ with extrapolation of the terminal phase. This parameter was determined using the following equation: AUC0-∞ = AUC0-t + Ct/λz, being Ct the observed plasma concentration at the last experimental time (t) with a concentration value above the LOQ and being the first order rate associated with elimination phase. AUC0-t was calculated with linear log-trapezoidal method.
- AUC0-t: area under the concentration-time curve from zero to time t hours calculated by linear trapezoidal rule, being time t the last experimental time with a concentration value above or equal to the LOQ.
- t1/2: terminal half-life obtained using the formula ln2/λz. This constant is estimated via linear regression of time versus logarithm of concentration.

• Vz/F: apparent volume of distribution based on the terminal phase. It is calculated from Vz/F= Dose/ λz AUC0-∞. This parameter was reported only for rupatadine.

• CL/F: apparent total plasma clearance after an extravascular administration. It is obtained using the formula CL/F= Dose/ AUC0-∞. This parameter was reported only for rupatadine.

• Mean residence time, MRT: was calculated by the relation AUMC0-∞ /AUC0-∞ being AUMC0-∞ the area under the first moment of the concentration-time curve from zero to infinite with extrapolation of the terminal phase.

• Elimination constant, λz: first order rate constant associated with the terminal (log-liner) portion of the curve. This constant is estimated via linear regression of time versus logarithm of concentration.

3.6.4.2 Steady state:

For the multiple dose, at least the next pharmacokinetic parameters have been calculated:

• Cmax: maximum observed concentration during a dose interval and Tmax; time of maximum observed concentration. These parameters were obtained from observed data on the last day treatment

• Cavg: mean concentration at steady-state. Computed as (AUC0-τ)/τ, being τ the dosing interval.

• AUC0-∞: area under the concentration-time curve from zero to ∞ with extrapolation of the terminal phase. This parameter was determined using the following equation: AUC0-∞ = AUC0-t + Ct/λz, being Ct the observed plasma concentration at the last experimental time (t) with a concentration value above the LOQ and being the first order rate associated with elimination phase. AUC0-t was calculated with linear log-trapezoidal method.

• AUC0-τ: area under the concentration-time curve from 0 to τ, being τ the interval of dosing (24 hours). This parameter was calculated with linear log-trapezoidal method.

• t1/2: terminal half-life obtained using the formula ln2/λz , being λz the first order rate constant associated with the terminal (log-linear) portion of

the curve. This constant is estimated via linear regression of time versus logarithm of concentration.

- Vz/F: volume of distribution based on the terminal phase. Computed as: Dose/ (AUC0-τ *λz). This parameter was only calculated for rupatadine.
- CLss/F: apparent total clearance computed as CLss/F =(Dosis/AUC0-τ). This parameter was only calculated for rupatadine.
- MRT: mean residence time extrapolated to infinity. For steady-state data this parameter was calculated as [AUMC0-τ+τ(AUC0-∞ - AUC0-τ)]/ AUC0-τ, being AUMC the area under the first moment curve extrapolated to infinity.
- Cmin: the minimum observed concentration between 0 and τ.
- Tmin: time of minimum concentration based on samples collected during a dosing interval.
- PTF (%): Peak through fluctuation over one dosing interval at steady-state. Computed as: 100 (Cmax- Cmin)/Cavg
- Rtheor: theoretical accumulation ratio. Computed as: 1/(1-e -λz*τ)
- Elimination constant, λz : first order rate constant associated with the terminal (log-liner) portion of the curve. This constant is estimated via linear regression of time versus logarithm of concentration.

For extravascular administration the fraction of dose absorbed cannot be estimated, therefore volume of distribution and clearance were actually Vz/F and CLss/F where F is the fraction of dose absorbed.

3.7 Data quality assurance

Data Quality assurance includes the steps to assure the accuracy and reliability of data included in the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and associated personnel in the study, and monitoring visits by the Quality Assurance Unit of the Research Institute.

3.7.1 Monitoring procedures

The monitors of J. Uriach y Compañía, S.A. conducted the visits to the study facilities for the purpose of monitoring the study. The investigator agreed to allow these monitors access to the clinical supplies dispensing and storage area and to study documentation for the above-mentioned purpose and agreed to assist the monitors in their activities, if requested. The investigator agreed to allow inspectors from Regulatory Agencies to review records and to assist the inspectors in their duties, if requested.

Source documents were the physician's or hospital's subject records maintained at the study site. In most cases, the source documents were the physician's or hospital subject's chart. Periodically, the J.Uriach y Compañía,S.A monitors visited the study site for the purpose of directly comparing the data in the CRF with the source. The investigator agreed to make source documents available for this prupose. It was investigator's responsibility to ensure accurate completion of the CRF and to approve the CRF. The CRF had to be signed by the investigator or a subinvestigator. These signatures served to attest that the information contained in the CRF was accurate and true.

Monitoring visits were performed before the beginning of the study (27/01/05) and (10/02/05), during the experimental phase (03, 11, 16 and 21/03/05), (12, 19 and 26/04/05), (03, 18 and 24/05/05), (03, 14, 21 and 29/06/05) after the end of the experimental phase (05, 08, 12, 15, 19, 21 and 27/07/05).

All the CRFs were revised following Good Clinical Practice guidelines.

3.7.2 Data management

All data management activities were described in a comprehensive Data Management Plan (DMP) which required the sponsor's approval before starting activities. This document described the Data Management process, Database Contents, annotated CRF, Pre-Entry Review List, Self-Evident Correction Conventions, Query Contacts, and Consistency Checks. All documents related to the clinical trial such as protocol, EC approvals, regulatory approval, individualCRFs, analytical certificates and quality control of batches, randomization code, adverse event forms, SOPs, statistical analysis, study

support and payments to investigators and the final report were filled at J. Uriach y compañia, S.A. offices in a study master file, ensuring storage for the lifetime of the tested product and available to the authorities requesting it.

A validation plan was set-up according to the protocol requirements, which described the validation rules to be applied. Actions that should be taken in case of data abnormalities were detailed. In case of missing values, out of range values, data inconsistencies or values that failed logical checks, correction forms (queries) were edited and transmitted to the investigator for clarification. A database was created in order to collect all clinical and other data from the clinical trial. The data management of demographic data and adverse events took place at Centre de Investigació de Medicaments del Institut de Recerca (Hospital de Sant Pau). The data management of ECG data took place in the facilities of the eRT.

3.7.3 Study audits

The Quality Assurance Unit of the Research Institute has audited the condunting of the study and the Final Report of this clinical trial and is responsible for keeping all the documents related with the study until two years after the last approval of a marketing application and until no applications are pending or planned in an ICH region, or until at least 2 years after the official suspension of the clinical development of the product studied. The sponsor, Lab. J. Uriach y Compañia, S.A. will keep a master file of the study during the lifetime of the product.

3.8 Statistical procedures planned in the protocol and determination of sample size

3.8.1 Statistical and analytical plans

A total of 160 subjects were planned to be randomised to receive one of the four treatments with approximately 40 subjects per treatment group.

The null hypothesis assumes that there is no relationship in the QTc change from baseline vs. dose of rupatadine. The positive control was used to determine “assay sensitivity” in that the study can detect a small positive

change in QTc duration from baseline using an agent, moxifloxacin, of about 5-10 msec. Changes on treatments (Rupatadine and moxifloxacin) were placebo corrected using the concomitant placebo group mean change from baseline.

A statistical analysis plan was prepared, approved and signed before the database closure meeting, and before doing the patients' evaluability assessment.

3.8.1.1 Population for analysis

The following analysis were defined:

All randomized patients: This subset included all subjects who were randomized to a treatment group, regardless of whether or not a particular patient received any study medication.

Safety patients: This subset included all randomized subjects who have received any study drug, independently of the degree of adherence to the protocol.

Per protocol/Main efficacy analysis: It included all randomized subjects who adhered to the protocol with no major deviations. The statistic analysis was based on this population. Also the descriptive analysis was based on this population.

3.8.1.2 Clinical endpoints

Hypothesis

The primary hypothesis assessed in steady state was:

H0: Rupatadine 100 mg= Placebo
H1: There are differences

The secondary hypothesis assessed in single dose and in steady state were:

H0: Rupatadine 10 mg=Placebo
H1: There are differences

H0: Moxifloxacin 400 mg= Placebo
H1: There are differences

H0: Rupatadine 100 mg= Placebo (in single dose period)
H1: There are differences

3.8.2 Determination of sample size

The sample size chosen for this study was based on precedents set by similar ECG safety studies and the requirements of having enough power to detect a 5-milisecond QTc effect with a one side 95%CI that excludes an 8 ms effect (as originally was established). This upper bound was chosen to reflect the uncertainty related to the variability of repeated measurements. This generally requires more than 30 subjects per arm. It's recommended at least 40 because to do a gender analysis you would have 20 women and 20 men, a size likely to provide accurate endpoint data. A total of 160 subjects were estimated ot recive one of the four treatments, with approximately 40 subjects per treatment group. This is an adequate sample size to define a 5 ms effect with a one side 95% CI that excludes an 8 ms effect (a originally was established).

In the last version of CHMP/ICH/2/04 (178) it was established that the one side 95% CI should exclude a 10 ms effect. The earlier calculated sample size was enough also to detect the new defined effect.

For individual missing values and placebo group, the mean of the available data in other days for the same time point will be applied. A sensitivity analysis was conducted to confirm that the conclusions of the study are not sensitive to the choice of imputation method. It was compared the results of the main efficacy variable (imputed data) in the main subset population with those observed in the same variable and population with the source data (not imputed data).

3.8.3 Primary analysis

For each subject in each treatment group, three ECGs were measured at each of the specified time points on both Day 0 (baseline period) and Day 1 to 5 (treatment period).

The primary analysis of QTc was based on individually corrected QT (QTcIX). Treatment effects on QTcIX were assessed using the largest time-matched mean difference between the drug and placebo (baseline-substracted) for the

QTc interval. A negative "thorough QT/QTc study" is one where the main variable is around 5 ms or less, with a one-sided 95% conficence interval that excludes and effect >10 ms.

Individually defined QTc as determined by

$$QTcIX=QT/(RR)^{b}$$

where b is the estimated slope of the linear regression model of log(QT)= a+b*log(RR). This is the most accurate method to correct QT for heart rate. Calculation of QTcIX was accomplished using a SAS program.

Treatment comparison was conducted for each ECG interval between rupatadine and placebo. An analysis of covariance (ANCOVA) of change from baseline ECG interval, with treatment and time as model terms, was performed for each ECG interval. From this ANCOVA, two-side 90% confidence intervals from the following treatment differences in least squares means will be calculated: 10 mg rupatadine versus placebo, 100 mg rupatadine versus placebo, 10 mg rupatadine versus 100 mg rupatadine, and moxifloxacin versus placebo.

Statistical analysis would also be performed to evaluate the gender differences, only if the positive "thorough QT/QTc study" would be reached.

3.8.4 Secondary analysis

3.8.4.1 Pharmacodynamics- ECG analysis

The secondary analysis was based on QTcF and QTcB, respectively. Treatment effects on QTc was assessed using change from baseline ECG intervals where baseline is defined as the mean of te values of ECG measurements taken on Day 0 within a given treatment group. Moreover, the presence of outliers was also explored.

The following ECG intervals were obtained from the ECG data: heart rate (HR), RR, PR, QRS and QT. QTc (QT interval corrected for heart rate)was determined by three methods:

QT interval corrected for heart rate by Bazzett's formula

$$QTcB= QT/(RR)^{1/2}$$

QT interval corrected for heart rate by Friedericia's formula

$$QTcF= QT/(RR)^{1/3}$$

Calculations of QTcB and QTcF were responsibility of the central ECG laboratory.

HR was calculated by RR based on the following formula:

HR (bpm)= 60.000/RR (msec)

The central laboratory's cardiologists detected ECG morphological changes.

"New changes" were defined as "not present on any baseline ECG but present on any on-treatment ECG".

All H-12 ECG interval parameters were subjected to the central tendency analysis and outlier analysis.

The central tendency analysis was performed to examine the difference in mean change from baseline ECG intervals between treatment groups (QTcB, QTcF, andQTcIX). For each subject, the following values were calculated:

- Mean of all baseline values, mean of all treatment values and mean change from baseline were calculated for each ECG interval. The time-average method subtract time-averaged baseline (one single value as mean of all baseline measurements) from the mean of all measurements on treatment (one single value).

- The time-matched QT/QTc interval: subtract hour X at baseline from hour X on treatment to obtain a change from baseline for each time-point.

The maximal change for each individual

Descriptive statistics, including mean, standard deviation, median, maximum and minimum were obtained for each ECG interval for baseline, on-treatment, and change from baseline by treatment group.

Treatment comparison and gender differences for the central tendency analysis in change from baseline ECG intervals for QTcB, QTcF and QTcIX were done through tests similars to those applied to assess the primary variable.

3.8.4.2 Pharmacokinetic-Pharmacodynamic analysis

The primary pharmacokinetic-pharmacodynamic (PK/PD) analysis explored the relationship between changes from baseline in QtcI and plasma concentrations of rupatadine and its metabolites for single dose and in steady state.The following plots were planned using data form individual subject and mean data for each treatment group of rupatadine:

Plot of maximum QTcI changes from baseline versus time overlaid with the plot of rupatadine and its metabolites plasma concentrations at corresponding timepoints.

Plot of maximum QtcI changes from baseline versus rupatadine and its metabolites plasma concentrations collected at corresponding timepoint.

Secondary analysis were also considered based on QtcF and QTcB, for reupatadine and its metabolites and for single dose and steady state.

3.8.4.3 Outlier analysis

The outlier analysis (categorical analysis) was performed to summarize the total counts and percentages of outlier subjects in each treatment group for each ECG interval. For each subject, the maximum change from baseline was calculated based on change from mean of all baseline to the maximum treatment value (the longest duration of ECG interval) on any of the ECGs obtained in each subject on treatment) for each ECG interval. A subject was determined as an outlier subject if the following criteria are met for each of the ECG intervals:

- QT: maximum treatment value of greater than 500 msec when not present at baseline (new onset).
- QTc:
  1. maximum treatment value of greater than 500 msec when not present at baseline (new onset).
  2. maximum change from baseline between 30 and 60 msec, or
  3. maximum change from baseline >60 msec.
- PR: maximum treatment value of >200 msec and more than 25% compared to baseline.
- QRS: maximum treatment value of >100 msec and more than 25% compared to baseline.
- HR: treatment value reflecting a 25% decrease from baseline to a HR <50 bpm or a 25% increase from baseline reflecting a HR>100 bpm.

If a subject experienced more than one episode of a particular outlier event over all time points, the subject have been counted once for that event.

Morphological analysis from the ECG waveform interpretation as defined by the centralized cardiologist was also conducted. New onsets, defined as percentage of subjects meeting the following new criteria, were summarized for each treatment group: third degree heart block, ST segment change (elevation and depression separately), T wave abnormalities (negative T waves only), myocardial infarction pattern and, importantly, any new abnormal U waves.

All outliers were summarized for each treatment group on the basis of subject incidence rates. The outlier summary tables included total number of subjects, number of outlier subjects and percentage of outlier subjects. Treatment comparison between rupatadine doses and placebo and between moxifloxacin and placebo were tested using a Chi-square analysis or Fisher exat test.

3.8.5 Incidence of adverse events

All adverse events occurred during the study period were grouped by treatment, with special mention of severe and treatment-related events. Adverse events were coded using the MeDRA dictionary. Each of the adverse events recorded were associated with a low level term, a preferred term and an organ class. Adverse events were summarized by organ class and preferred term in two tables, the former for all reported adverse events and the last for related (possible, probable and definite).

For each organ class the number of patients with at least one adverse event in that organ class were summarized. Only adverse events in different organ classes were counted for each patient, and an adverse event in the same organ class occurring twice in the same patient were only included once in the total for that organ class.

The number of patients with at least one adverse event and the preferred term for the event were summarized for each organ class. Only different preferred terms were conunted for each patient, and an adverse event with the same preferred term occurring twice in the same patient were only included onec in the total for that preferred term.

Descriptive analysis was performed for those treatment-emergent adverse events (that means starting after randomization) and for any possibly or probably related to the study drug. A table was drawn up with the proportion of patients reporting at least one adverse event and at least one related adverse event and with the number of adverse events and related adverse events by treatment groups. Chi-square tests on proportions were performed on the rates of patients reporting at least one adverse event and at least one related adverse event.

3.8.6 Clinical laboratory results

The number of patients with normal values, clinically relevant abnormal values or abnormal values which were not clinically relevant were tabulated.

3.8.7 Physical examination

The number of patients with normal and abnormal physical examination values were tabulated.

3.8.8 Patient characteristics

All descriptive variables were tabulated. Quantitative variables were described by the number of available and missing observations, mean, median,standard deviation, the range (minimum and maximum) and the first and third quartiles. Qualitative variables were described by frequency and percentage. Missing values were tabulated with their frequency but not included in the calculation of percentages.

All demographic and certain baseline variables were described by treatment groups. The following variables age, weight and height, were assessed as esploratory analysis by means of an ANOVA. The gender was assessed as exploratory analysis by means of Chi-Square test of Fisher exact test.

Homogeneity at baseline was tested in the per protocol population.

3.8.9 Concomitant medications

Concomitant drug usage was summarized by the number and proportion of subjects in each treatment group receiving each drug. Multiple drug usage by a patient was conted once only.

3.8.10 Reasons for discontinuation

The number and proportion of patients discontinuing were tabulated by treatment group. Investigators' reasons for discontinuation of patients were summarized (number and percentage) and were compared by means of a chi-square or Fisher exact test. This section was described for the randomized population.

3.9 Changes in the performance of the study in relation to the protocol

Prior to the start of the study, changes were introduced as amendments to the protocol. They are described bellow.

No relevant amendment nº 1, dated on 31/01/2005, consisted of some administrative changes and the confirmation of the supratherapeutic dose of rupatadine (100 mg/day)

Relevant amendment nº 2 was submitted to the Clinical Research Ethic Committee (CREC) on 04/07/2005 and further approved on 26/07/2005 (see Appendix 16.1.1). This amendment is refered to the update of the ICH 14 guideline on May, 2005. When the protocol was designed, the analysis of the QTc was based on the previous bibliography and on the drafts of the guideline (Step 2, June 2004 and Step 3, December 2004). It was specified that: "a negative thorough study is one where the largest difference between the drug and placebo (baseline substracted) for the QTc interval is around 5 ms or less, with a one-sided 95% confidence interval that excludes an effect >8.0 ms". In the updated guideline this definition was modified: " a negative thorough study is one in which the upper bound of the 95% interval confidence for the largest time-matched mean effect of the drug on the QTc interval excludes 10 ms"

4. STUDY VOLUNTEERS

4.1 Disposition of volunteers

Single centre study with a planned sample size of 160 healthy volunteers. A total of 207 subjects were selected and underwent the corresponding test to check compliance with the inclusion/exclusion criteria. 38 subjects were excluded due inclusion criteria and one subject was reserve.

Eight subjects were excluded from efficacy analysis (per protocol) because of missing values in some time points (n=7) and for a mistake in the administration of the treatment (n=1). These subjects were replaced.

Table 7: Disposition of volunteers

	Treatment				All
	Placebo	**Rupatadine 10 mg**	**Rupatadine 100 mg**	**Moxifloxacin 400 mg**	
Randomised/Safety population					
n	41	45	41	41	168
Excluded subjects Subject id	#47	#11, #44, #51, #97, #102	#83	#125	8
Replaced by Subject id	#161	#170, #176, #163, #165, #182	#166	#164	8
Main analysis/ Per Protocol population					
n	40	40	40	40	160

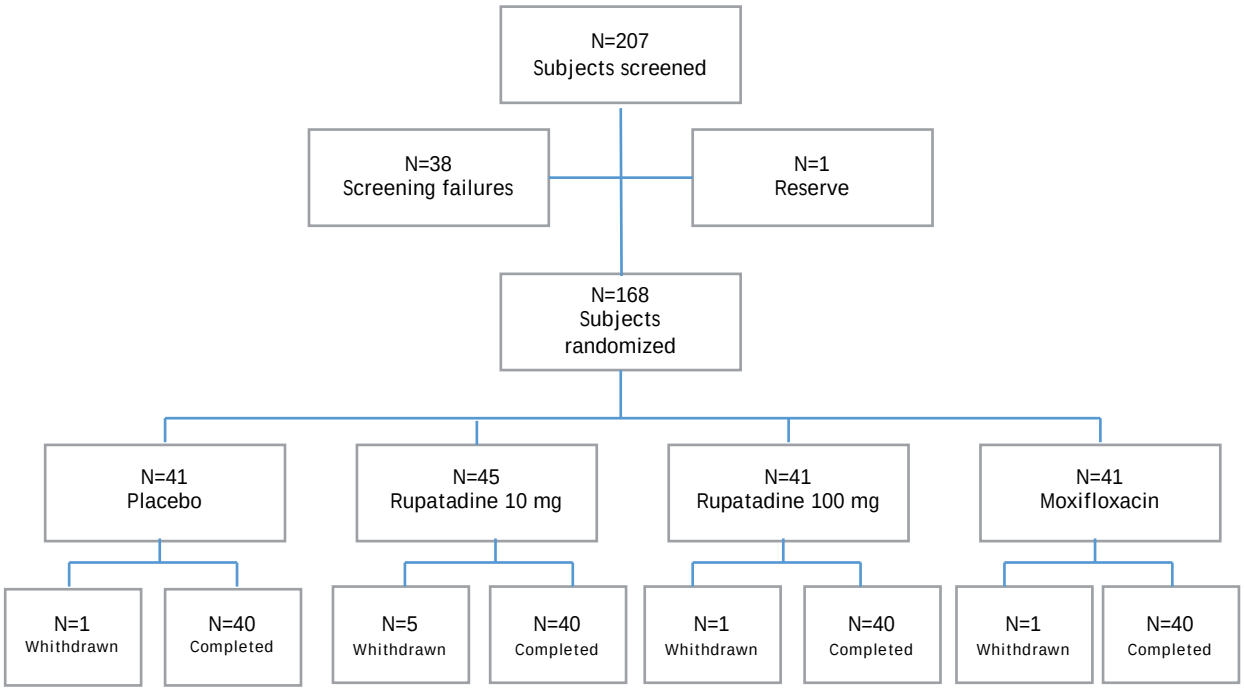

Figure 22: Disposition of volunteers

4.2 Deviations from the protocol

A major deviation was defined as a deviation that can interfere in the results and conclusions of the study.

A deviation major took place in the volunteer 47 based on a mistake in the treatment. Volunteer 47 (placebo) intook the drug with random number 48 (rupatadine 10) on day 3, because of this, the volunteer was withdrawal from the study.

The assigned medications for volunteers 37 and 38 were changed for the rest of the study. Then volunteer 37 was assigned to the moxifloxacin group and volunteer 38 was assigned to rupatadine 10 mg group.

5.RESULTS

5.1 Data set analysed

The study included 168 volunteers, both sexes and caucasians. Demographic and other baseline characteristics. Tables 8 and 9 summarises demographic and other baseline measurements.

Inclusion and exclusion criteria were reviewed at screening and at baseline. All subjects fulfilled all inclusion criteria and none of the exclusion ones.

Table 8: Demographic data

		TREATMENT			
		PLACEBO	RUPATADINE 10 MG	MOXIFLOXACIN 400 MG	RUPATADINE 100 MG
Gender					
Male	N	21	23	20	21
	%	51.22	51.11	48.78	51.22
Female	N	20	22	21	20
	%	48.78	48.89	51.22	48.78
Race					
Caucasian	N	41	45	41	41
	%	100.00	100.00	100.00	100.00
All	N	41	45	41	41

Table 9: Baseline assessment

		TREATMENT			
		PLACEBO	RUPATADINE 10 MG	MOXIFLOXACIN 400 MG	RUPATADINE 100 MG
Weight	Mean	66.56	67.45	63.55	67.42
	Std	9.87	8.82	10.11	10.25
	Min	50.00	52.00	52.00	51.20
	Max	91.20	84.50	88.00	88.70
Height	Mean	170.07	169.62	168.41	169.80
	Std	8.83	7.87	8.28	8.67
	Min	152.00	151.00	155.00	153.00
	Max	192.00	181.00	188.00	186.00
Age	Mean	25.66	26.96	25.90	26.68
	Std	5.28	5.11	5.43	6.09
	Min	18.00	19.00	20.00	19.00
	Max	41.00	43.00	44.00	44.00
BMI: Quetelet's index	Mean	22.93	23.37	22.29	23.15
	Std	2.25	2.06	2.23	1.94
	Min	19.20	19.10	19.10	19.10
	Max	27.00	26.80	26.80	27.00
	N	41	45	41	41
Systolic blood pressure (supine)	Mean	120.34	118.51	117.51	121.56
	Std	10.51	9.65	9.25	9.80
	Min	100	100	100	105
	Max	150	137	137	140
Dyastolic blood pressure (supine)	Mean	59.51	59.69	61.44	60.61
	Std	7.59	7.64	8.63	8.24
	Min	50	50	42	50
	Max	76	80	80	83
Heart Rate	Mean	72.37	70.47	78.85	71.00
	Std	8.00	9.00	9.73	11.19
	Min	58	50	51	50
	Max	91	89	89	95
Respiratory rate	Mean	14.76	15.47	16.05	15.41
	Std	2.35	3.23	1.97	2.11
	Min	11	12	12	12
	Max	20	32	20	20
Axilary temperature	Mean	36.10	36.04	36.12	36.14
	Std	0.30	0.39	0.30	0.40
	Min	35.4	35	35.5	35
	Max	36.6	37	36.8	37
	N	41	45	41	41

5.2 Measurement of treatment compliance

The volunteers took the study medication in the presence of a member of the research team. Compliance with treatment was 100% for both medication schedules.

5.3 Pharmacodynamic results

5.3.1 Primary analysis

Time-Matched analysis (Analysis added due to May 15, 2005 E14 Guidance)

A time-matched analysis was conducted as recommended by ICH E14 though the original protocol when designed contemplated that the customary time average analysis was to be employed. The following tables are included:

Details the mean change from baseline in milliseconds for each treatment at each time point on Day 1 and on Day 5 (Tables 10,11).

Details the 90% upper confidence boundary for each treatment at each time point showing the placebo and baseline corrected (delta analysis) for moxifloxacin and the Rupatadine dose groups: point on Day 1 and on Day 5 (Tables 12,13). The largest time matched mean increase (placebo substracted) for rupatadine 10 mg was 4 ms at 2h, 3h and 23h postdose on day 1, and 5 ms at 4h postdose on day 5. No increases in the QtcI change were observed for rupatadine 100 mg on day 1 and, on day 5, the largest time matched mean increase was 5 ms at 23h. For moxifloxacin, the largest time matched mean effect was 12 ms at 1h on day 1 and 9 ms at 1h30m on day 5. At hour 16 the placebo group had its most negative change from baseline, which will affect the upper confidence interval for the placebo corrected change from baseline.

Also, it was included a figure that display the placebo corrected mean for the QTcI duration by treatment effect at each time point (Figure 23).

Table 10: Details the mean change from baseline on day 1

TIME	RUPATADINE 10 MG	RUPATADINE 100 MG	MOXIFLOXACIN 400 MG	PLACEBO
0H30M	-2	-2	9	-2
1H	-5	-6	8	-4
1H30M	-3	-6	3	-2
2H	1	-4	6	-3
3H	1	-6	4	-3
4H	-1	-2	5	-3
6H	-1	-1	4	-2
8H	-2	-5	1	0
12H	-4	-5	4	0
14H	-4	-5	1	-3
16H	-8	-7	-2	-7
20H	-4	-6	0	-5
23H	4	-1	7	0

Table 11: Details the mean change from baseline on day 5

TIME	RUPATADINE 10 MG	RUPATADINE 100 MG	MOXIFLOXACIN 400 MG	PLACEBO
0H30M	4	4	11	6
1H	5	0	9	2
1H30M	1	-2	10	1
2H	3	-4	6	1
3H	2	-1	2	1
4H	4	0	6	-1
6H	4	0	6	3
8H	1	0	1	1
12H	3	0	6	2
14H	-1	-1	0	-1
16H	-2	-2	1	-8
20H	-3	-3	3	-3
23H	1	4	8	-1

Table 12: Day 1- 90% upper confidence boundary for QTcI delta analysis

TIME POINTS. DAY 1	RUPATADINE 10 mg	RUPATADINE 100 mg	MOXIFLOXACIN 400 mg
0H30M	5	5	16
1H	4	3	17
1H30M	4	1	10
2H	8	3	13
3H	8	2	11
4H	6	6	12
6H	5	5	10
8H	3	1	5
12H	1	0	9
14H	4	2	8
16H	4	5	10
20H	5	3	10
23H	8	3	12

Table 13: Day 5 – 90% upper confidence boundary for QTcI delta analysis

TIME POINTS. DAY 5	RUPATADINE 10 mg	RUPATADINE 100 mg	MOXIFLOXACIN 400 mg
0H30M	3	3	10
1H	8	3	12
1H30M	5	2	14
2H	7	0	10
3H	5	2	6
4H	9	5	11
6H	6	2	7
8H	5	4	5
12H	6	2	8
14H	4	4	5
16H	11	11	14
20H	4	4	10
23H	6	9	13

The upper bound of the confidence interval for largest time matched mean effect for rupatadine 10 mg was 8 ms on day 1, and 6 ms on day 5. For rupatadine 100 mg, the upper bound of the confidence interval was 6 ms on day 1 and 5 ms on day 5. For moxifloxacin, the upper bound of the confidence interval for the largest time matched mean effect was 17 ms on day 1 and 14 ms on day 5. At hour 16, both doses of rupatadine get an upper bound of 11 ms, but this value come from decreases on the QtcI value, not increases, and it is oversized by the most negative change in placebo group (see discussion)

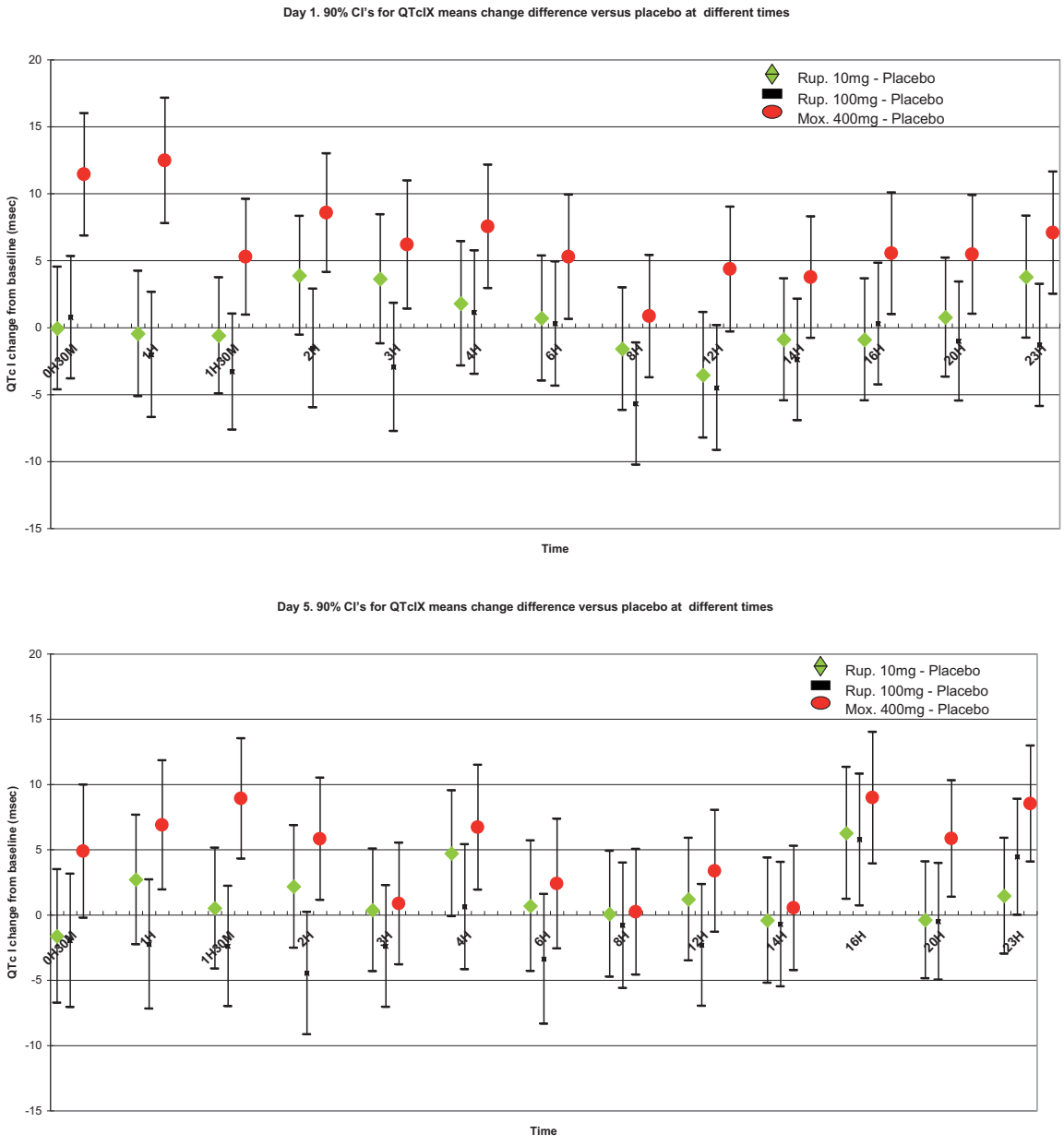

Figure 23: Placebo corrected mean for the QTcI duration by treatment effect at each time point

5.3.2 Secondary analysis

Time-averaged analysis:

A time averaged analysis was conducted as included in the original protocol. The following table details the mean change from baseline in milliseconds for each treatment (separately for Day 1 and Day 5) and the 90% upper confidence boundary.

Table 14: Details the results as mean change from baseline and confidence intervals

	RUPATADINE 10 MG		RUPATADINE 100 MG		MOXIFLOXACIN 400 MG		PLACEBO	
Study Day	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
QTcI in msec	-2	1	-4	0	4	6	-2	1
QTcI 90% CI – Min	-4	0	-5	-2	3	4	-4	-1
QTcI 90% CI – Max	-1	3	-3	2	5	7	-1	3

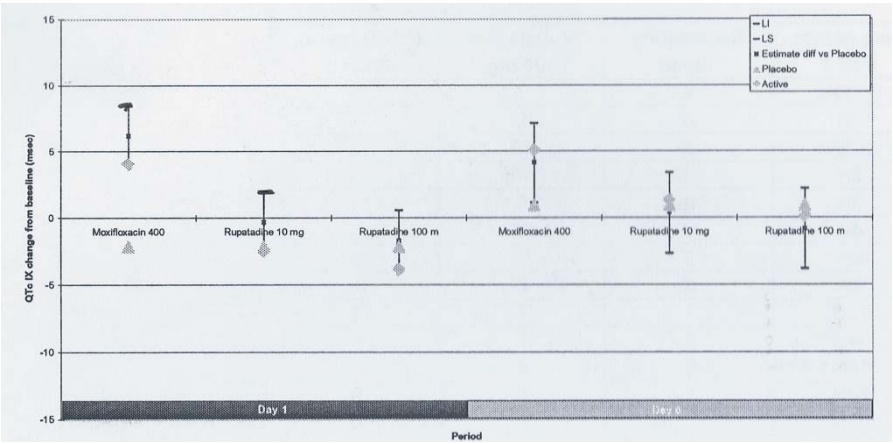

Figure 24: Time- averaged placebo corrected mean for the QTcI duration by treatment effect

There was no signal of any change in QTcI from baseline when placebo corrected for either the clinical dose or the supratherapeutic dose of rupatadine. The mean change from baseline was 0 ms and 0 ms for the therapeutic dose of rupatadine after one dose and steady state respectively. For the supratherapeutic dose, the mean change from baseline was –2 and –1 ms after one dose and steady state respectively. The upper confidence

interval for QTcI change was 0 and 0 ms for the clinical dose and -2 and -1 ms for the 100 mg supratherapeutic dose of Rupatadine after one dose and steady state respectively. To be certain that this negative result was not a false negative the use of a concomitant positive control group (studied in exactly the same manner as the other treatment arms) demonstrated the expected placebo corrected change from baseline at single dose of 6 ms and 5 ms on day 5 (upper confidence intervals were 6 and 4 ms respectively.

Pharmacodynamic analysis by QTcB and QTcF intervals

A time averaged analysis was conducted with QTcF and QTcB corrections and similar results were obtained. The following table details the mean change from baseline in milliseconds for each treatment (separately for Day 1 and Day 5) and the 90% upper confidence boundary.

Table 15: Details the results as mean change from baseline and confidence intervals for QTcF and QTcB corrections

	RUPATADINE 10 MG		RUPATADINE 100 MG		MOXIFLOXACIN 400 MG		PLACEBO	
STUDY DAY	DAY 1	DAY 5	DAY 1	DAY 5	DAY 1	DAY 5	DAY 1	DAY 5
QTcF in msec*	-2	2	-4	0	3	5	-2	1
QTcF 90% CI – Min	-3	0	-5	-2	2	3	-3	-1
QTcF 90% CI – Max	-1	4	-3	2	5	7	-1	2
QTcB in msec*	0	4	3	9	6	8	-1	3
QTcB 90% CI – Min	-1	2	2	7	4	6	-2	1
QTcB 90% CI – Max	2	6	5	11	6	10	1	5

There was no signal of any change in QTcF from baseline when placebo corrected for either the clinical dose or the supratherapeutic dose of

rupatadine. The mean change from baseline was 0 ms and 1 ms for the therapeutic dose of rupatadine after one dose and steady state respectively. For the supratherapeutic dose, the mean change from baseline was –2 and –1 ms after one dose and steady state respectively. The upper confidence interval for QTcI change was 0 and 2 ms for the clinical dose and -2 and 0 ms for the 100 mg supratherapeutic dose of Rupatadine after one dose and steady state respectively. The concomitant positive control group demonstrated the expected placebo corrected change from baseline at single dose of 5 ms and 4 ms on day 5 (upper confidence intervals were 6 and 5 ms, respectively).

The results when we analysed the change in QTcB from baseline when placebo corrected were more irregulars for either the clinical dose or the supratherapeutic dose of rupatadine. The mean change from baseline was 1 ms and 1 ms for the therapeutic dose of rupatadine after one dose and steady state respectively. For the supratherapeutic dose, the mean change from baseline was 4 and 6 ms after one dose and steady state respectively. The upper confidence interval for QTcI change was 1 and 1 ms for the clinical dose and 4 and 6 ms for the 100 mg supratherapeutic dose of Rupatadine after one dose and steady state respectively. The concomitant positive control group demonstrated the expected placebo corrected change from baseline at single dose of 7 ms and 5 ms on day 5 (upper confidence intervals were 5 and 5 ms; and 4 and 5 ms respectively).

Outlier analysis (categorical analysis)

Outlier analysis produce data as percentage of subjects in each treatment group that meet the criteria as defined for this analysis:

- For QT parameter: from mean baseline value to determine subjects who attain QT values > 500 msec when not present at baseline (new onset)
- For QTc: from baseline value to determine subjects who attain QTc values >500 msec when not present at baseline, as well as

categorizations of changes from baseline of 30-60 msec and >60 msec in QTC, as well as values >470 msec.

- PR change from baseline: more than 25% increase when PR>200 msec
- QRS change from baseline: more than 25% increase when QRS>100 msec.
- HR changes reflecting a more than 25% decrease from baseline to a HR< 50 bpm or a more that 25% increase from baseline reflecting a HR>100 bpm.

Morphological analyses were performed with regard to the ECG waveform interpretation as defined by the central ECG laboratory's cardiologist. New onset (presented as percentage of subjects meeting the new criteria) for the following variables: abnormal U waves, second degree heart block, third degree heart block, complete right bundle branch block, complete left bundle branch block, complete right bundle branch block, ST segment change (elevation and depression separately), T wave abnormalities (negative T waves only), and myocardial infarction pattern.

Using the specific criterion of a new >60 ms change in baseline of QTcI duration or a new >500 ms or a new abnormal U wave, no outliers were identified. The nonspecific criterion of a 30-60 ms change from baseline in QTcI duration revealed no evidence of any outliers in addition compared to placebo whereas there were more outliers as expected on moxifloxacin (the positive control) compared to placebo.

Table 16: Details the results as mean change from baseline and new outliers or morphological changes from baseline to day 1 and day 5.

	RUPATADINE 10 MG		RUPATADINE 100 MG		MOXIFLOXACIN 400 MG		PLACEBO	
STUDY DAY	DAY 1	DAY 5	DAY 1	DAY 5	DAY 1	DAY 5	DAY 1	DAY 5
Total N	40	40	40	40	40	40	40	40
Heart Rate in bpm*	2	2	8	10	3	3	2	3
Heart Rate tachycardic outliers N (%)	6 (15%)	3 (7.5%)	17 (42.5%)	18 (45%)	7 (17.5%)	5 (12.5%	4 (10%)	7 (17.5%)
HR bradycardic outliers N (%)	0	0	0	0	0	0	0	0
PR in msec*	-2	-1	-2	-1	-3	0	-2	-1
PR outliers N (%)	0	0	0	0	0	1 (2.5%)	0	0
QRS in msec N*	0	0	0	0	0	0	0	0
QRS outliers N (%)	0	0	0	0	0	0	0	0
QT in msec*	-6	-2	-17	-16	-2	0	-5	-4
QT new>500 msec N (%)	0	0	0	0	0	0	0	0
QT new>470 msec N (%)	0	0	0	0	0	0	0	1 (2.5%)
QTcI in msec*	-2	1	-4	0	4	6	-2	1
QTcI new >500 msec N	0	0	0	0	0	0	0	0
QTcI new >470 msec N (%)	0	0	0	0	0	0	0	0
QTcI 30-60 msec N (%)	2 (5%)	2 (5%)	3 (7.5%)	4 (10%)	5(12.5%)	11(27.5%)	4 (10%)	4 (10%)
QTcI >60 msec N	0	0	0	0	0	0	0	0
QTcF in msec*	-2	2	-4	0	3	5	-2	1
QTcF new >500 msec N	0	0	0	0	0	0	0	0
QTcF new>470 msec N (%)	0	0	0	0	0	0	0	0
QTcF 30-60 msec N (%)	2 (5%)	2 (5%)	3 (7.5%)	3 (7.5%)	4 (10%)	11 (27.5%)	2 (5%)	5 (12.5%)
QTcF >60 msec N	0	0	0	0	0	0	0	0
QTcB in msec*	0	4	3	9	6	8	-1	3
QTcB new >500 msec N	0	0	0	0	0	0	0	0
QTcB new>470 msec N (%)	0	2 (5%)	1 (2.5%)	0	0	1 (2.5%)	1(2.5%)	1 (2.5%)
QTcB 30-60 msec N (%)	10(25%)	16(40%)	12 (30%)	15(37.5%)	17(42.5%)	20 (50%)	9(22.5%)	17(42.5%)
QTcB >60 msec N (%)	1(2.5%)	0	0	0	0	0	0	1 (2.5%)
New abnormal U wave N	0	0	0	0	0	0	0	0
New ST segment depression changes N (%)	0	0	0	0	0	0	0	0
New T wave inverted N(%)	1(2.5%)	1(2.5%)	0	0	1 (2.5%)	0	0	0
New heart block, MI Complete RBBB&LBBB,N(%)	0	0	0	0	0	0	0	0

* Mean change from baseline

Bpm =beats per minute; msec=milliseconds; QTcI: Individual Correction; QTcB: Bazett correction; QTcF= Fridericia correction; RBBB=right bundle branch block; LBBB= left bundle branch block, MI= myocardial infarction; "new" means not present at baseline and only seen post baseline.

Heart Rate:

The time averaged analysis of heart rate change from baseline placebo corrected demostrated no change (within 1 bpm) in heart rate for the clinical 10 mg dose of Rupatadine (or moxifloxacin) after first dose or steady state. For the supratherapeutic dose of Rupatadine there was a 6-7 bpm increase which was not dose related but with more tachycardic outliers.

PR and QRS:

The placebo corrected change from baseline in PR interval duration after first dose or steady state of Rupatadine showed no change and there were no outliers. The same findings were present for QRS duration and its outlier criterion.

ECG morphology:

No morphological changes were noted in this trial except for occasional T wave inversions which is not considered clinically important in this setting.

5.3.3 Statistical/analitycal issues

Adjustments for covariates

The ANCOVA models were used to provide an assessment of baseline score value effects. This model followed the Points to Consider recommendation on the consideration that baseline values should be included as a covariate in the primary analysis.

Handling of dropouts or missing data

For individuals with missing values and placebo group, the mean of the available data in other days for the same timepoint was applied. For individuals with missing values and active treatment groups, eRT was permitted to extract timepoints before the scheduled time under guidelines stated according to the pharmacokinetic profile of treatments. When, it was

not possible complete all ECG recordings for a volunteer, this subject was replaced.

Interim analyses and data monitoring

No interim analyses were performed.

Examination of subgroups

Statistical analysis was also performed to evaluate the gender differences. Treatment comparison was conducted for each ECG interval. An analysis of covariance (ANCOVA) of change from baseline ECG interval, with treatment, gender and treatment by gender interaction as model terms and baseline value as a model covariate, were performed. The test was mede at the 0.10 significant level.

There was no difference between the QtcI results when comparing men and women (p=0.5097 on day 1 and p=0.1069 on day 5).The analysis were to be repeated by gender should an interaction of gender by treatment appear in the time-matched analysis. As the interaction treatment by gender term was not significant, this gender stratified analysis was not done.

5.3.4 Drug dose, drug concentration, and relationships to response

Pharmacokinetic-Pharmacodynamic relationships:

The following graphs depict the relationship of rupatadine parent and metabolites to the change in QTcI duration. The concentration-QTc response analysis was conducted using the QTcI in order to avoid the potential heart rate bias using QTcF or QTcB. The relationships between changes form baseline in QTcI relative to baseline and plasma concentrations are despicted for therapeutic and supratherapeutic dose of rupatadine and its metabolites.

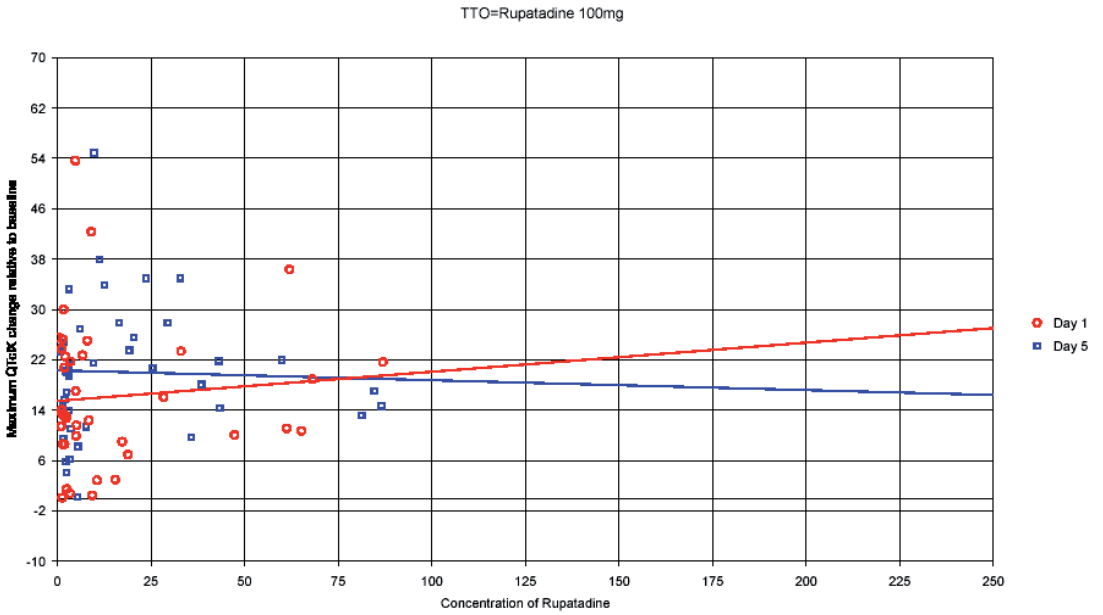

Figure 25: Maximum QTcIX change relative to baseline for rupatadine 100mg (with regression lines)

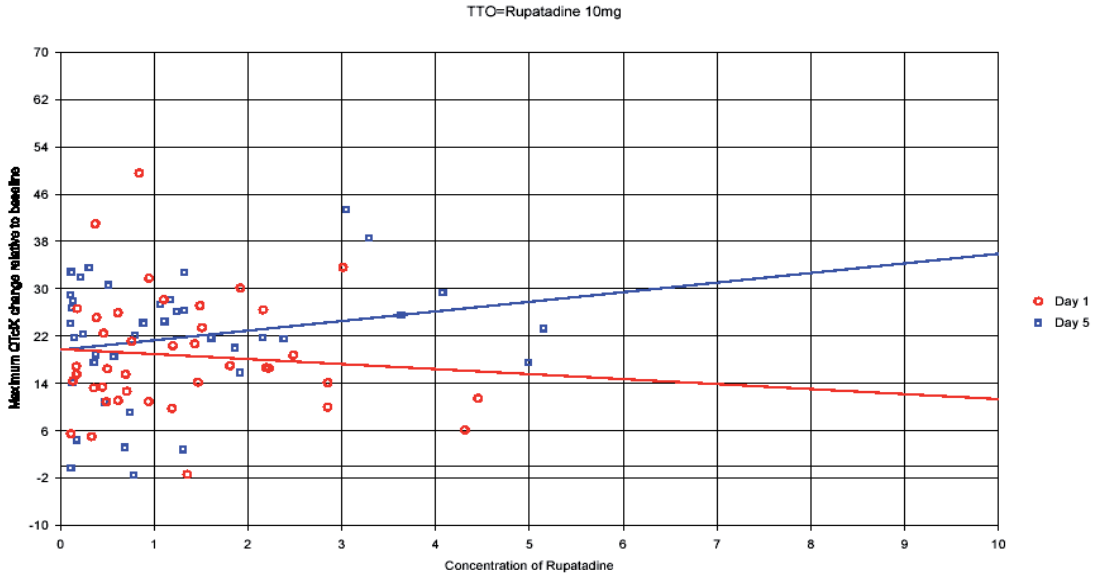

Figure 26: Maximum QTcIX change relative to baseline for rupatadine 10mg (with regression lines)

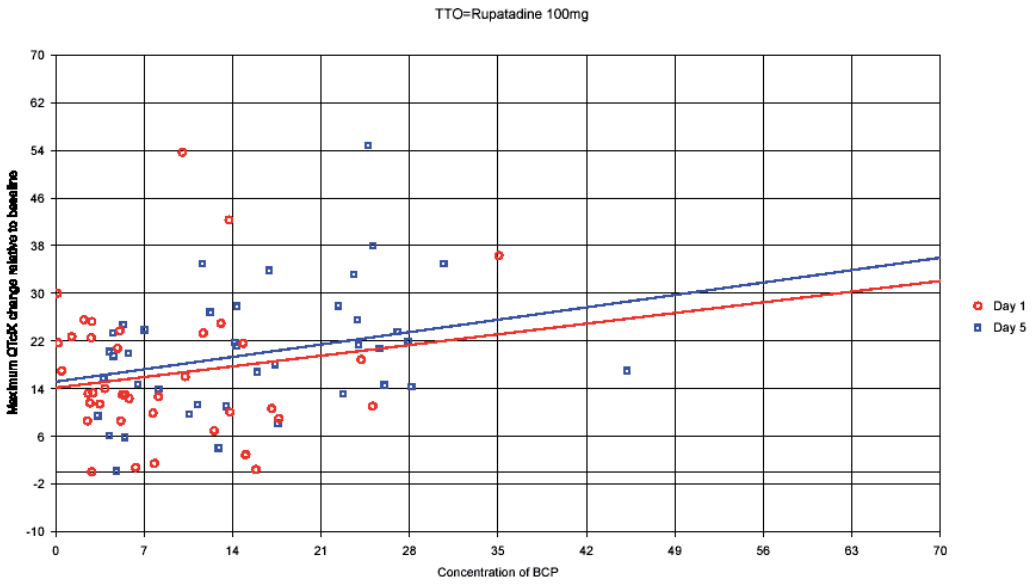

Figure 27: Maximum QTcIX change relative to baseline for BCP (RUP100mg) (with regression lines)

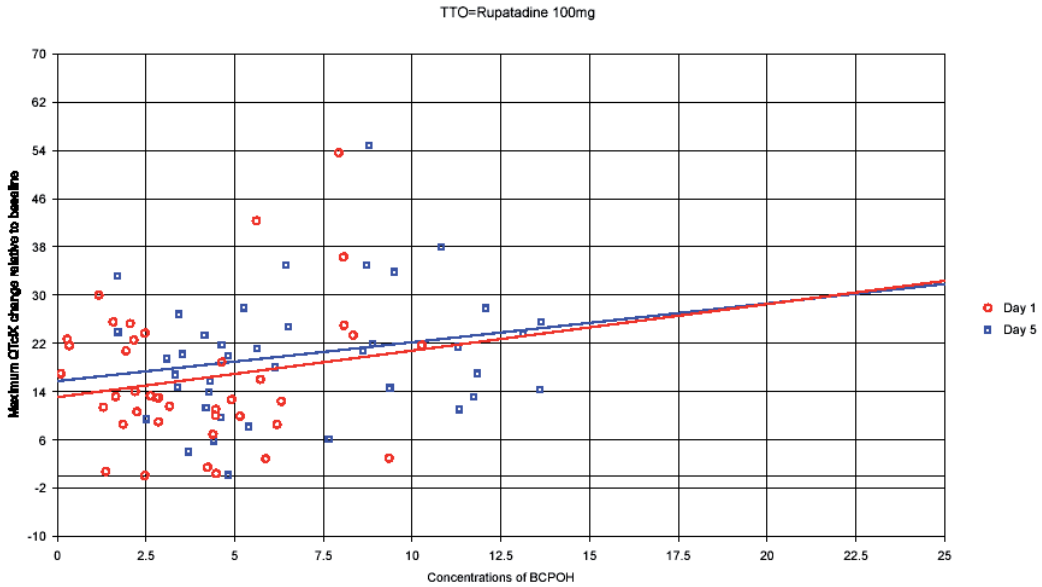

Figure 28: Maximum QTcIX change relative to baseline for BCPOH (RUP100mg) (with regression lines)

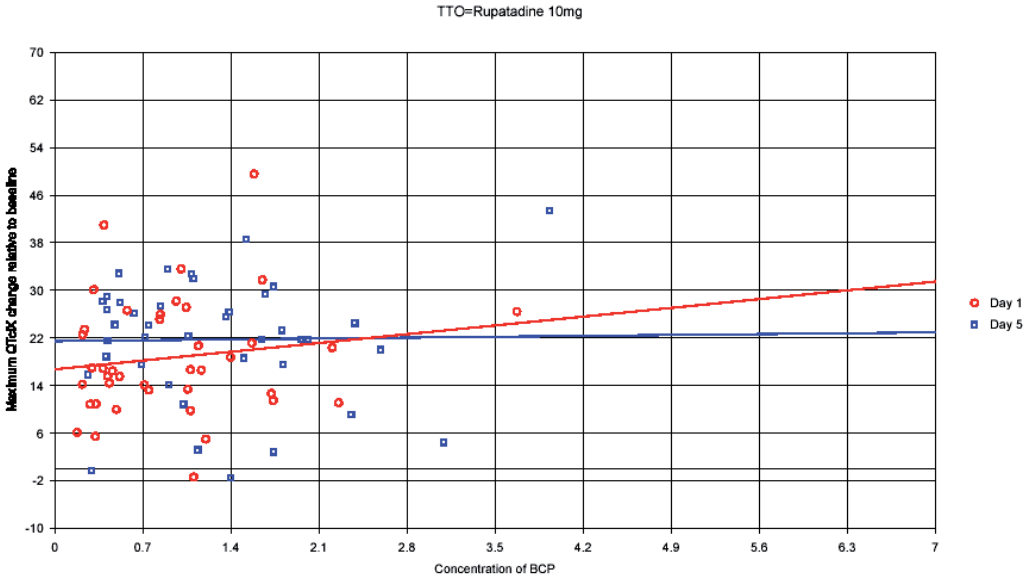

Figure 29: Maximum QTcIX change relative to baseline for BCP (RUP10mg) (with regression lines)

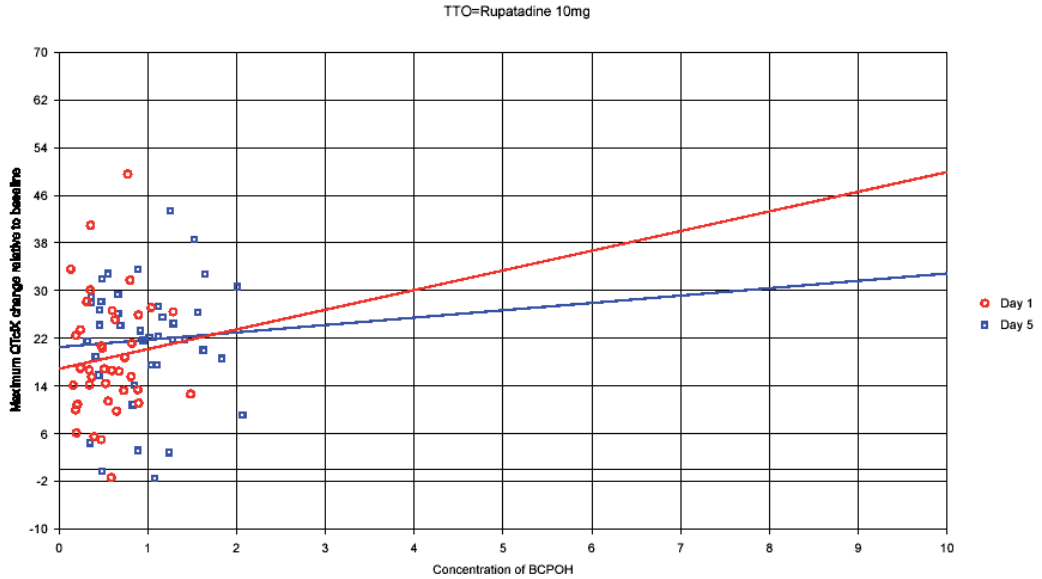

Figure 30: Maximum QTcIX change relative to baseline for BCPOH (RUP10mg) (with regression lines)

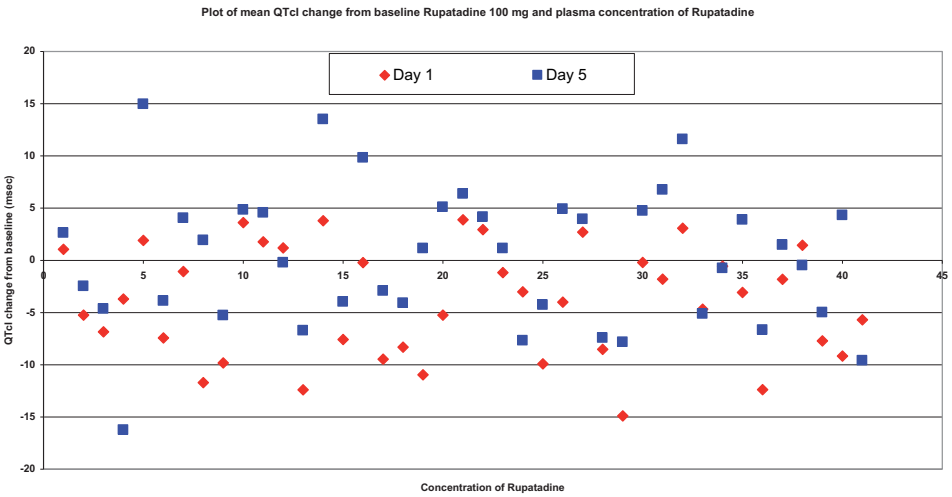

Figure 31:Rupatadine 100 mg dose group: QTcI vs parent rupatadine

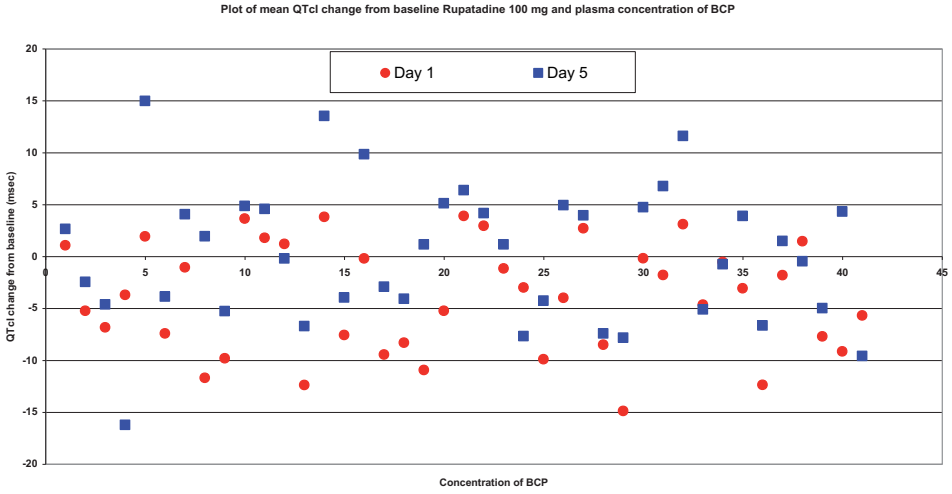

Figure 32: Rupatadine 100 mg dose group: QTcI vs BCP metabolite

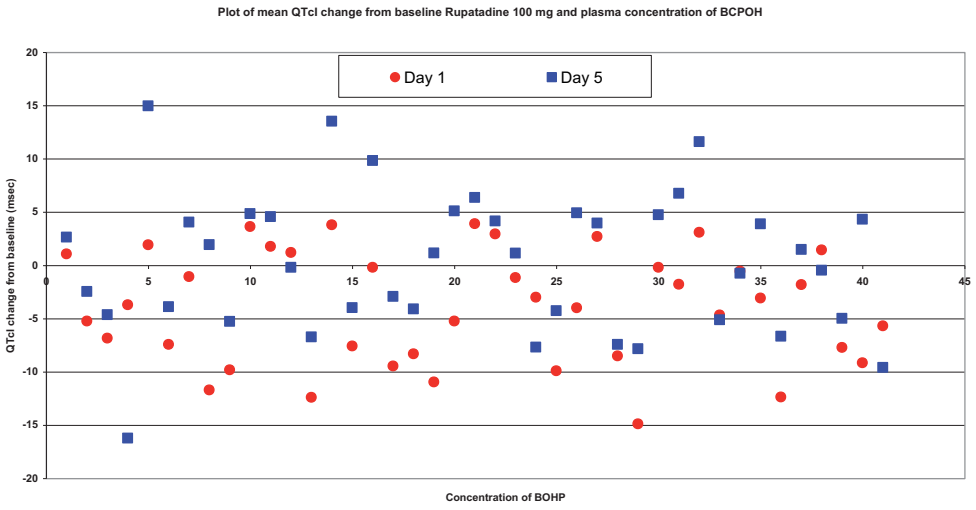

Figure 33: Rupatadine 100 mg dose group: QTcI vs BCP-OH metabolite

No significant or clinically relevant exposure-QTc response effects were observed at Day 1 od day 5 of rupatadine treatment.The relationship between the change in QTcI from baseline to plasma concentration of parent and metabolites of rupatadine (BCP and BCP-OH) demonstrated no signal of any positive effect to suggest that this antihistamine effects cardiac repolarization, nor with the therapeutic dose of 10 mg neither supratherapeutic dose of 100 mg. These data do not support the psoibility of any effect of rupatadine on QT interval prolongation.

Pharmacokinetic results:

The analytical method for rupatadine, BCP and BCP-OH analysis fit the concentration range found after repeated oral doses of rupatdine 10 o 100 mg, although 1/10 and 1/25 dilutions were needed for many samples of the highest dose. No interfering substances were detected.

Volunteers nº 11, 44, 51, 97 and 102 from the treatment group 1 (10 mg rupatdine tablet) and volunteer nº 83 from the treatment group 2 (100 mg rupatadine tablet) were replaced by the same number of volunteers due to problems on dynamics determination. These replaced volunteers were excluded from the pharmacokinetics calculations so that estimation of pharmacokinetic parameters was performed with forty volunteers in each treatment group, as initially planned.

The following figures show the mean plasma concentration versus time resulting from these data from each compound.

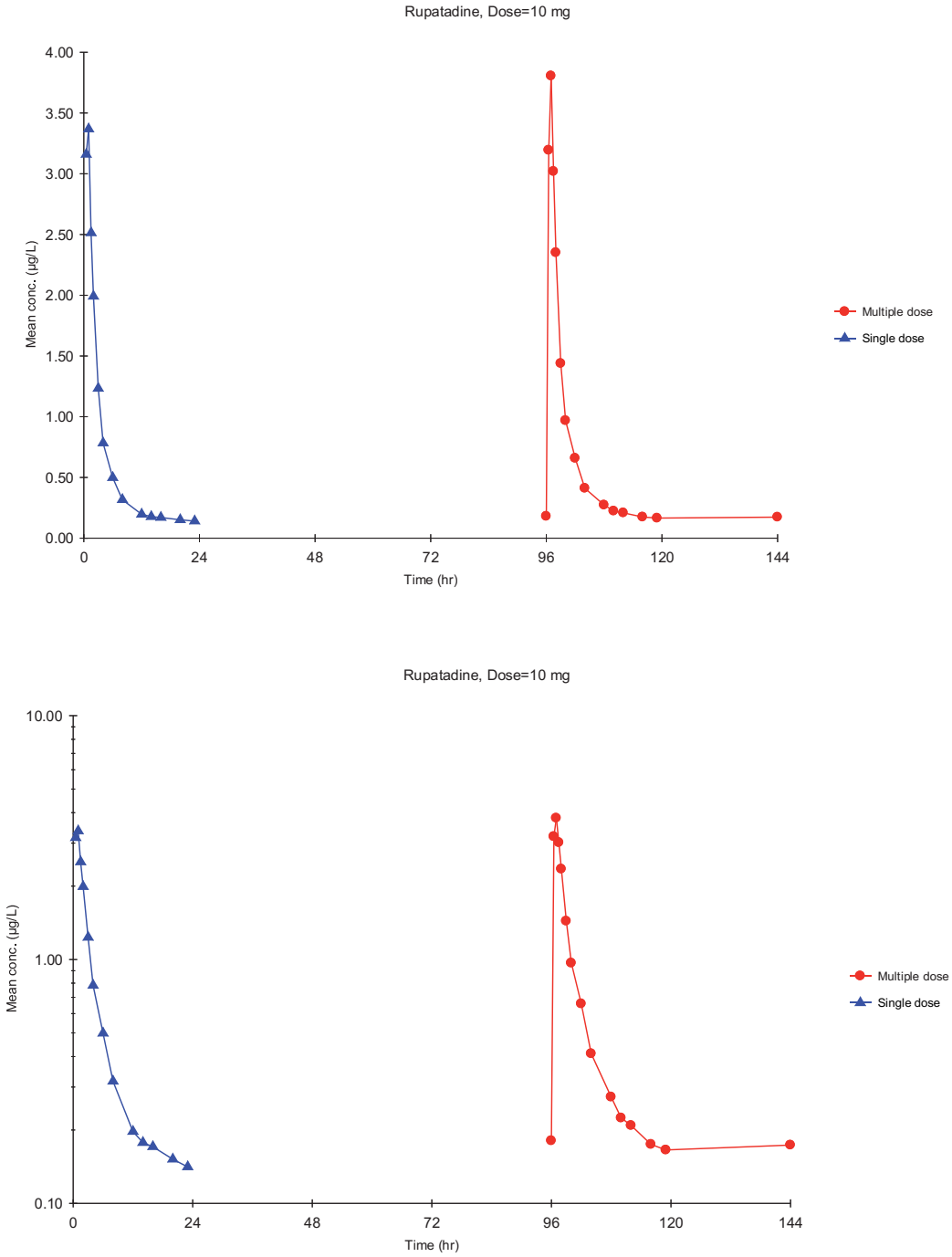

Figure 34: Mean values of the plasma concentrations of rupatadine for single dose and steady state conditions for 10 mg dose

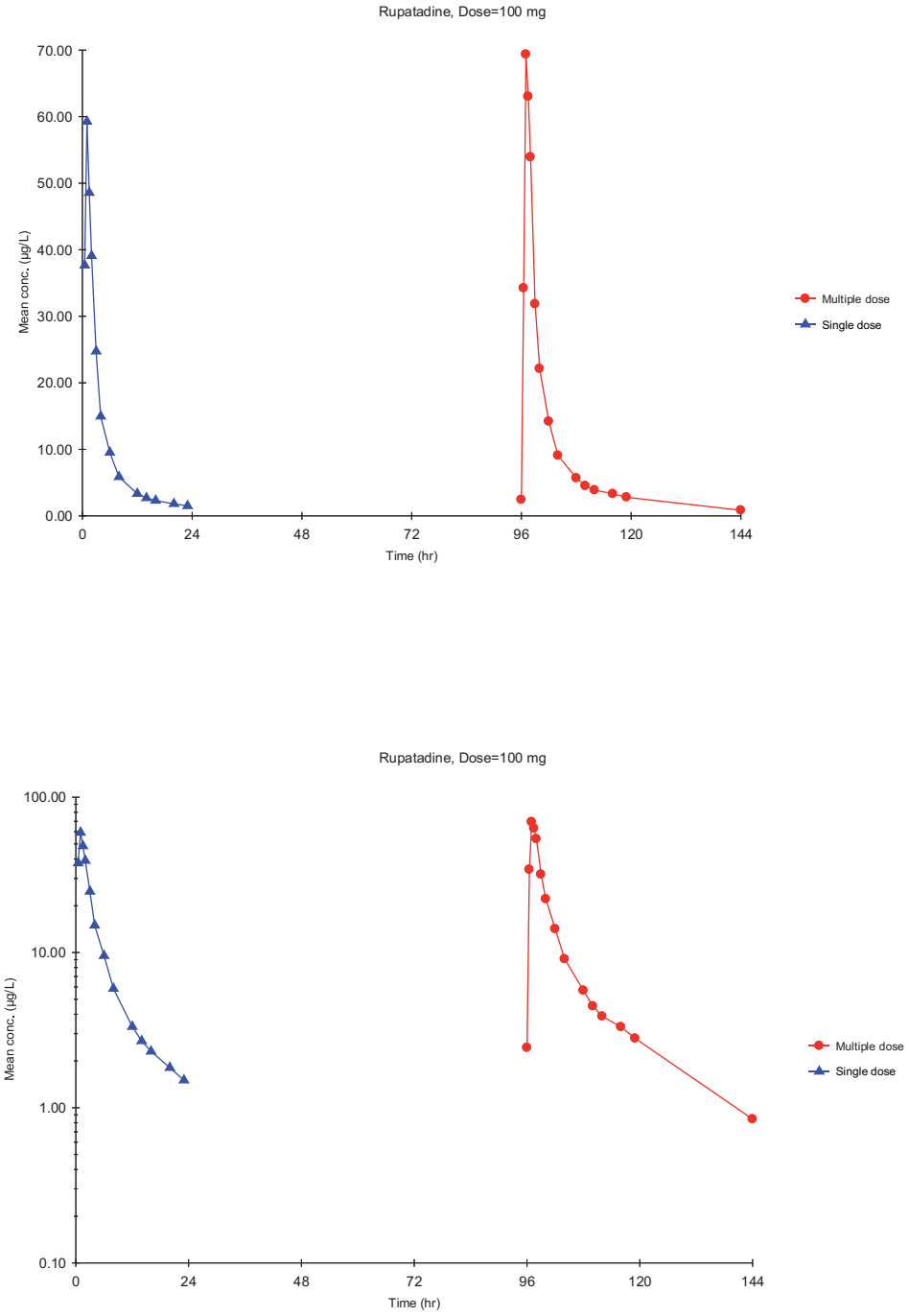

Figure 35: Mean values of the plasma concentrations of rupatadine for single dose and steady state conditions for 100 mg dose

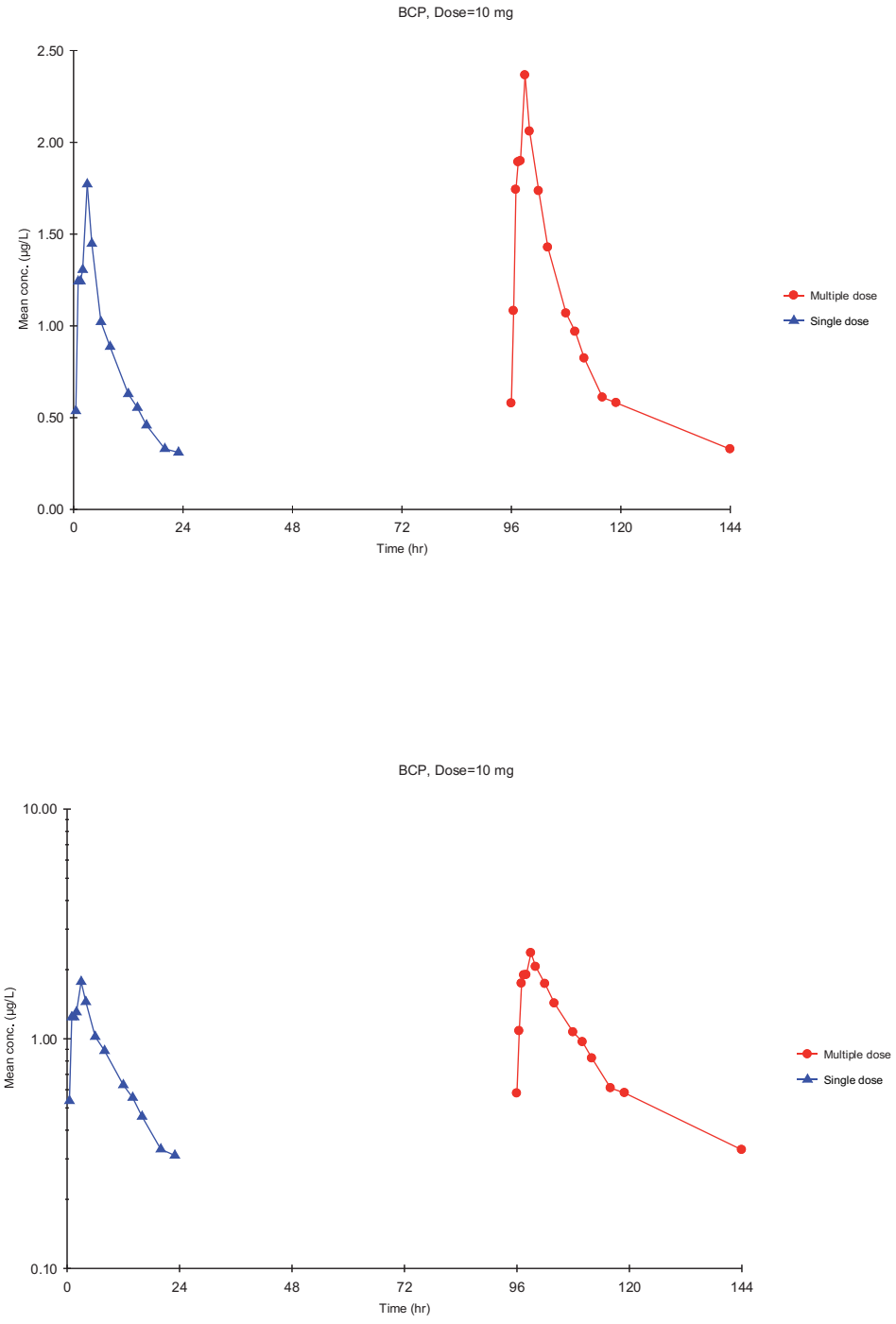

Figure 36: Mean values of the plasma concentrations of BCP for single dose and steady state conditions for 10 mg dose

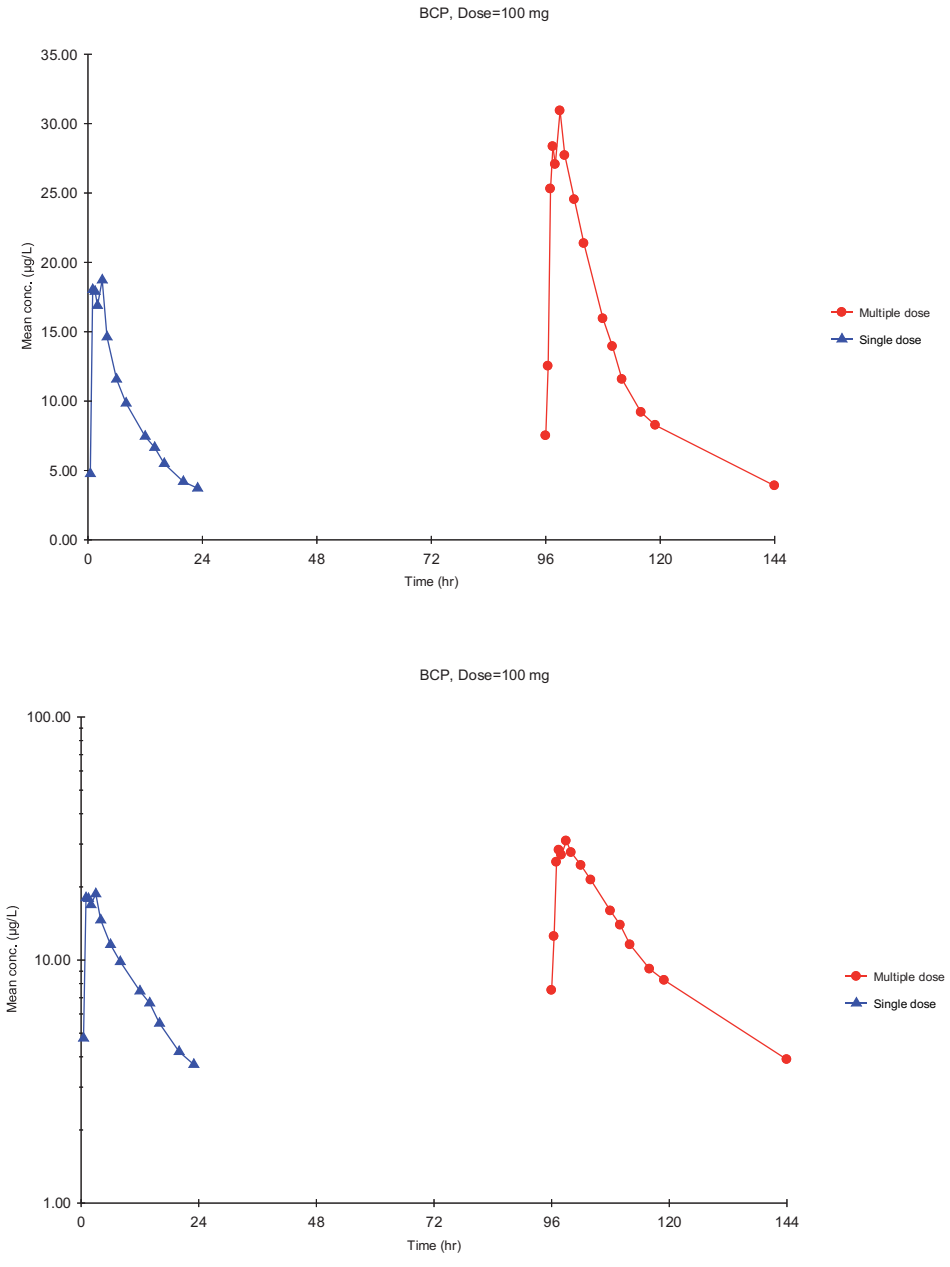

Figure 37: Mean values of the plasma concentrations of BCP for single dose and steady state conditions for 100 mg dose

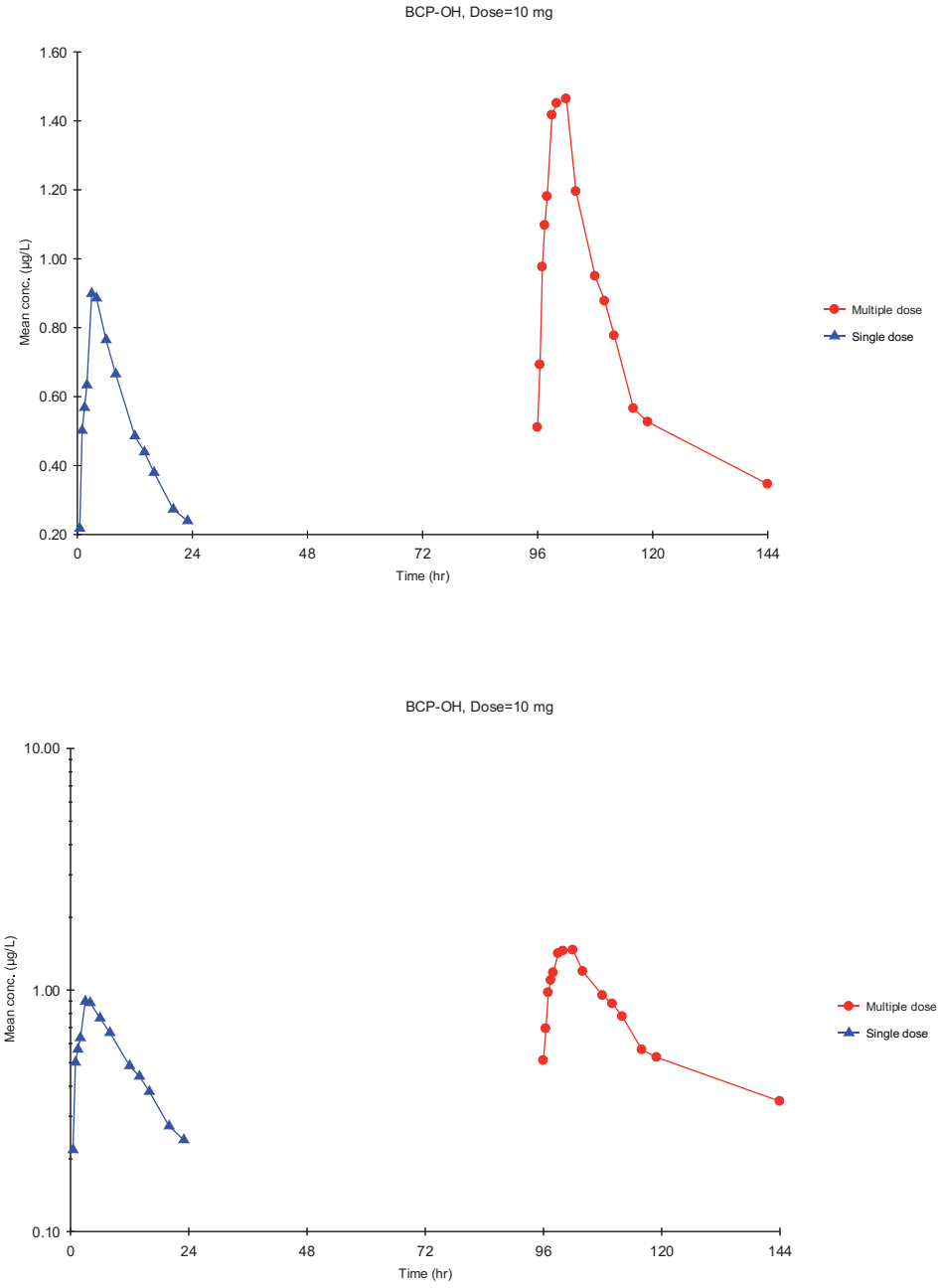

Figure 38: Mean values of the plasma concentrations of BCP-OH for single dose and steady state conditions for 10 mg dose

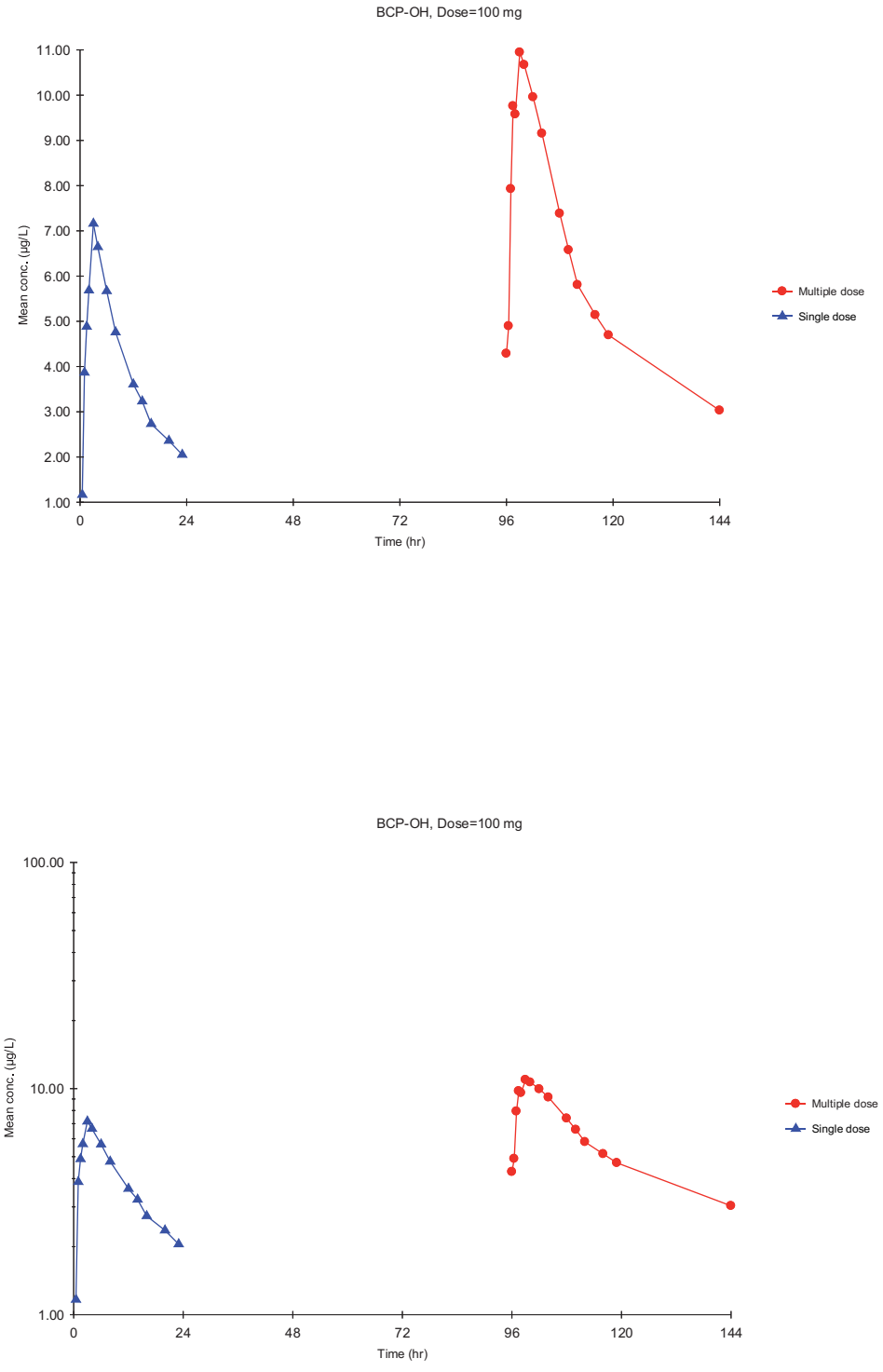

Figure 39: Mean values of the plasma concentrations of BCP-OH for single dose and steady state conditions for 100 mg dose

Description of the plasma curves

Rupatadine 10 mg

After the administration of a single oral dose of rupatadine 10 mg tablets, T_{max} values ranged from 0.50 to 2.00 h with a median value of 1.00 h and a mean C_{max} value of 4.03 ug/L (sd=2.14). Mean AUC_0 value was 11.27 ug.h/L (sd=6.00) and mean AUC_{0^∞} was 12.36 ug.h/L (sd=6.41) being the percentage of extrapolated AUC lower than 20% for all the volunteers, except for the volunteer 93 with an extrapolated AUC of 21.35%.

Mean values of $t_{1/2}$ and MRT for rupatadine were 6.07 h (sd=3.36) and 5.81 h (sd=2.26) whereas Vz/F and CL/F had mean of 7567.27 L (sd=3182.49) and 1035.75 L/h (sd=542.89).

After the administration of multiple doses of rupatadine 10 mg tablets, T_{max} values in steady-state conditions ranged from 0.50 to 2.00 h with a median value of 1.00 h and mean C_{max} value of 4.49 ug/L (sd=2.05). Mean AUC_0 value was 14.55 ug.h/L (sd=7.00) and mean AUC_0 was 16.51 ug.h/L (sd=9.18).

Mean values of $t_{1/2}$ and MRT were 9.16 h (sd=5.47) and 7.82 h (sd=3.18). Vz/F and CLss/F were also calculated for rupatadine, with mean Vz/F values of 9798.76 L (sd=4953.70) and and mean CLss/F values of 864.92 L/h (sd= 488.76).

Rupatadine presented mean values of C_{min}, C_{avg} and Rtheor at steady state of 0.13 ug/L (sd=0.04), 0.61 ug/L (sd=0.29) and 1.22 (sd=0.27) respectively. Mean value of PTF was 750.16% (sd=228.19).

Dose linearity was evaluated comparing AUC_0 of day 1 and AUC_0 of day 5 by means of a t-Student for repeated measures (statistical significance level $p< 0.05$).

Rupatadine 100 mg

After the administration of a single oral dose of rupatadine 100 mg tablets, T_{max} values ranged from 0.50 to 3.00 h with a median value of 1.00 h and a mean C_{max} value of 68.35 ug/L (sd=34.32). Mean AUC_0 value was 214.46 ug.h/L (sd=81.37) and mean AUC_{0^∞} was 238.20 ug.h/L (sd=92.04) being the percentage of extrapolated AUC lower than 20% for all the volunteers, except for the volunteer 33 with an extrapolated AUC of 20.08%. Percentage of extrapolated AUC was not calculated for volunteer 62 since AUC_0 could not be calculated for this volunteer.

Mean values of $t_{1/2}$ and MRT for rupatadine were 9.94 h (sd=3.45) and 8.06 h (sd=2.13) whereas Vz/F and CL/F had means of 6966.83 L (sd=3737.35) and 498.99 L/h (sd=261.25).

After the administration of multiple doses of rupatadine 100 mg tablets, T_{max} values in steady-state conditions ranged from 0.50 to 3.00 h with a median value of 1.00 h and mean C_{max} value of 81.08 ug/L (sd=41.34). Mean AUC_0 value was 296.37 ug.h/L (sd=129.58) and mean AUC_0 was 352.58 ug.h/L (sd=172.10).

Mean values of $t_{1/2}$ and MRT were 13.76 h (sd=2.33) and 10.04 h (sd=2.37). Vz/F and CLss/F were also calculated for rupatadine, with mean Vz/F values of 7904.14 L (sd=3492.06) and and mean CLss/F values of 400.81 L/h (sd= 182.93).

Rupatadine presented mean values of C_{min}, C_{avg} and Rtheor at steady state of 2.36 ug/L (sd=1.52), 12.35 ug/L (sd=5.40) and 1.43 (sd=0.12) respectively. Mean value of PTF was 636.54% (sd=159.26).

Dose linearity was evaluated comparing AUC_0 of day 1 and AUC_0 of day 5 by means of a t-Student for repeated measures (statistical significance level $p< 0.05$).

Dose proportionality was evaluated comparing C_{max} and AUC_0 normalised by the dose (C_{max}/D and AUC_0/D) of day 1 and day 5 between both dose levels by means of a t-Student for independent measures (statistical significance level $p< 0.05$).

Table 17: Summary of pharmacokinetic parameters of rupatadine

PARAMETER	RUP 10MG (S.D) MEAN AND 95 % CI	RUP 100 MG (S.D) MEAN AND 95 % CI	RUP 10MG (S.S) MEAN AND 95 % CI	RUP 100MG (S.S) MEAN AND 95 % CI
C_{max} (µg/L)	4.03 (3.34-4.71)	68.35 (57.38-79.33)	4.49 (3.84-5.15)	81.08 (67.86-94.30)
AUC_0	11.27 (9.35-13.19)	214.46 (188.44-240.48)	14.55 (12.31-16.79)	296.37 (254.93-337.81)
AUC_0 (µg.h/L)	12.36 (10.31-14.41)	238.20 (208.37-268.04)	16.51 (13.58-19.45)	352.58 (297.54-407.62)
Vz/F (L)	7567.27 (6549.47-8585.08)	6966.83 (5755.32-8178.34)	9798.76 (8214.49-11383.03)	7904.14 (6787.33-9020.96)
CL/F (L/h)	1035.75 (862.13-1209.38)	498.99 (414.30-583.68)	------	------
λz (h^{-1})	0.1485 (0.1250-0.1721)	0.0777 (0.0692-0.0861)	0.1015 (0.0831-0.1200)	0.0518 (0.0490-0.0546)
$t_{1/2}$ (h)	6.07 (5.00-7.15)	9.94 (8.82-11.06)	9.16 (7.41-10.91)	13.76 (13.02-14.51)
MRT (h)	5.81 (5.09-6.53)	8.06 (7.37-8.75)	7.82 (6.80-8.84)	10.04 (9.28-10.80)
C_{min} (µg/L)	------	------	0.13 (0.12-0.15)	2.36 (1.88-2.85)
Cavg (µg/L)	------	------	0.61 (0.51-0.70)	12.35 (10.62-14.08)
CLss/F (L/h)	------	------	864.92 (708.61-1021.23)	400.81 (342.31-459.32)
PTF (%)	------	------	750.16 (677.18-823.13)	636.54 (585.61-687.48)
R_{theor}	------	------	1.22 (1.14-1.31)	1.43 (1.39-1.47)

<u>BCP (rupatadine 10 mg)</u>

Following the administration of a single oral dose of rupatadine 10 mg tablets, T_{max} values for BCP ranged from 1 to 4 h with a median value of 3 h and mean C_{max} value of 1.81 ug/L (sd=0.52). Mean AUC_0 value was 17.00 ug.h/L (sd=6.30) and mean AUC_{0^∞} was 21.48 ug.h/L (sd=9.92) being the percentage of

extrapolated AUC lower than 20% for 28 out of 40 volunteers. The percentage of extrapolated AUC for the rest of volunteers ranged from 20.11 to 44.92%. Mean values of $t_{1/2}$ and MRT for BCP were 9.29 h (sd= 2.16) and 13.85 h (sd= 2.88).

When multiple doses of rupatadine 10-mg tablets were administered, Tmax values for BCP in steady-state conditions ranged from 1 to 8 h with a median value of 3 h and mean Cmax value of 2.46 µg/L (sd= 0.95). Mean $AUC_{0-\tau}$ value was 28.21 µg.h/L (sd= 16.29) and mean $AUC_{0-\infty}$ was 42.76 µg.h/L (sd= 22.07).

Mean values of $t_{1/2}$ and MRT were 21.52 h (sd= 6.29) and 22.88 h (sd= 5.17).

BCP presented mean values of Cmin, Cavg and Rtheor at steady state of 0.54 µg/L (sd= 0.46), 1.18 µg/L (sd= 0.68) and 1.87 (sd= 0.35) respectively. Mean value of PTF was 179.65% (sd= 44.01).

Dose linearity was evaluated comparing $AUC_{0-\infty}$ of day 1 and $AUC_{0-\tau}$ of day 5 by means of a t-Student for repeated measures (statistical significance level $p \leq 0.05$).

BCP (Rupatadine 100 mg)

After the administration of a single oral dose of rupatadine 100-mg tablets, Tmax values for BCP ranged from 1 to 4 h with a median value of 1.5 h and mean Cmax value of 23.10 µg/L (sd= 7.63). Mean AUC_{0-t} value was 198.90 µg.h/L (sd= 67.50) and mean $AUC_{0-\infty}$ was 261.42 µg.h/L (sd= 106.69) being the percentage of extrapolated AUC lower than 20 % for 22 out of 40 volunteers. The percentage of extrapolated AUC for the rest of the volunteers ranged from 20.50 to 53.38%. Mean values of $t_{1/2}$ and MRT for BCP were 10.97 h (sd= 5.09) and 15.69 h (sd= 5.70).

After the administration of multiple doses of rupatadine 100-mg tablets, Tmax values for BCP in steady-state conditions ranged from 1 to 6 h with a median value of 3 h and mean Cmax value of 35.52 µg/L (sd= 13.29). Mean $AUC_{0-\tau}$ value was 399.70 µg.h/L (sd= 186.75) and mean $AUC_{0-\infty}$ was 617.86 µg.h/L (sd= 330.97).

Mean values of $t_{1/2}$ and MRT were 21.42 h (sd= 12.17) and 23.72 h (sd= 9.76).

BCP presented mean values of Cmin, Cavg and Rtheor at steady state of 6.96 µg/L (sd= 3.89), 16.65 µg/L (sd= 7.78) and 1.86 (sd= 0.72) respectively. Mean value of PTF was 181.78% (sd= 53.78).

Dose linearity was evaluated comparing $AUC_{0-\infty}$ of day 1 and $AUC_{0-\tau}$ of day 5 by means of a t-Student for repeated measures (statistical significance level $p \leq 0.05$).

Dose proportionality was evaluated comparing Cmax and $AUC_{0-\infty}$ normalised by the dose (Cmax/D and $AUC_{0-\infty}$/D) of day 1 and day 5 between both dose levels by means of a t-Student for independent measures (statistical significance level $p \leq 0.05$).

Table 18: Summary of pharmacokinetic parameters of BCP

PARAMETER	RUP 10MG (S.D) MEAN AND 95 % CI	RUP 100 MG (S.D) MEAN AND 95 % CI	RUP 10MG (S.S) MEAN AND 95 % CI	RUP 100MG (S.S) MEAN AND 95 % CI
C_{max} (µg/L)	1.81 (1.65-1.98)	23.10 (20.66-25.54)	2.46 (2.15-2.76)	35.52 (31.27-39.77)
AUC_{0}	17.00 (14.98-19.02)	198.90 (177.31-220.49)	28.21 (23.00-33.42)	399.70 (339.97-459.42)
AUC_{0} (µg.h/L)	21.48 (18.31-24.66)	261.42 (227.30-295.54)	42.76 (35.61-49.92)	617.86 (509.07-726.65)
λz (h^{-1})	0.0778 (0.0732-0.0823)	0.0723 (0.0654-0.0793)	0.0369 (0.0309-0.0428)	0.0369 (0.0337-0.0400)
$t_{1/2}$ (h)	9.29 (8.60-9.98)	10.97 (9.35-12.60)	21.52 (19.49-23.56)	21.42 (17.42-25.42)
MRT (h)	13.85 (12.93-14.77)	15.69 (13.87-17.52)	22.88 (21.20-24.56)	23.72 (20.51-26.93)
C_{min} (µg/L)	------	------	0.54 (0.39-0.68)	6.96 (5.72-8.20)
Cavg (µg/L)	------	------	1.18 (0.96-1.39)	16.65 (14.17-19.14)
PTF (%)	------	------	179.65 (165.57-193.72)	181.78 (164.58-198.98)
R_{theor}	------	------	1.87 (1.75-1.98)	1.86 (1.62-2.10)

BCP-OH (rupatadine 10 mg)

After the administration of a single oral dose of rupatadine 10-mg tablets, Tmax values for BCP-OH ranged from 2 to 6 h with a median value of 3.5 h and mean Cmax value of 0.96 µg/L (sd= 0.26). Mean AUC_{0-t} value was 11.56 µg.h/L (sd= 2.96) and mean $AUC_{0-\infty}$ was 15.15 µg.h/L (sd= 3.73) being the percentage of extrapolated AUC lower than 20 % for 16 out of 40 volunteers. The percentage of extrapolated AUC for the rest of the volunteers ranged from 20.14 to 46.10%. Mean values of $t_{1/2}$ and MRT for BCP-OH were 10.41 h (sd= 3.33) and 16.60 h (sd= 4.16).

After the administration of multiple doses of rupatadine 10-mg tablets, Tmax values for BCP-OH in steady-state conditions ranged from 1.5 to 8 h with a median value of 4 h and mean Cmax value of 1.58 µg/L (sd= 0.40). Mean $AUC_{0-\tau}$ value was 22.65 µg.h/L (sd= 6.16) and mean $AUC_{0-\infty}$ was 55.13 µg.h/L (sd= 20.57).

Mean values of $t_{1/2}$ and MRT were 41.33 h (sd= 14.64) and 42.88 h (sd= 11.61).

BCP-OH presented mean values of Cmin, Cavg and Rtheor at steady state of 0.48 µg/L (sd= 0.15), 0.94 µg/L (sd= 0.26) and 3.02 (sd= 0.87) respectively. Mean value of PTF was 116.93% (sd= 21.11).

Dose linearity was evaluated comparing $AUC_{0-\infty}$ of day 1 and $AUC_{0-\tau}$ of day 5 by means of a t-Student for repeated measures (statistical significance level $p \leq 0.05$).

BCP-OH (Rupatadine 100 mg)

After the administration of a single oral dose of rupatadine 100-mg tablets, Tmax values for BCP-OH ranged from 1 to 6 h with a median value of 3 h and mean Cmax value of 7.53 µg/L (sd= 2.62). Mean AUC_{0-t} value was 88.65 µg.h/L (sd= 24.34) and mean $AUC_{0-\infty}$ was 131.68 µg.h/L (sd= 43.65) being the percentage of extrapolated AUC lower than 20 % only for 4 out of 40 volunteers. The percentage of extrapolated AUC for the rest of the volunteers ranged from 20.75 to 65.07%. Mean values of $t_{1/2}$ and MRT for BCP-OH were 14.08 h (sd= 6.60) and 21.04 h (sd= 8.56).

After the administration of multiple doses of rupatadine 100-mg tablets, Tmax values for BCP-OH in steady-state conditions ranged from 1 to 8 h with a median value of 3.5 h and mean Cmax value of 12.27 µg/L (sd= 3.98). Mean $AUC_{0-\tau}$ value was 174.95 µg.h/L (sd= 43.46) and mean $AUC_{0-\infty}$ was 445.35 µg.h/L (sd= 158.78).

Mean values of $t_{1/2}$ and MRT were 38.76 h (sd= 15.50) and 46.51 h (sd= 15.08).

BCP-OH presented mean values of Cmin, Cavg and Rtheor at steady state of 3.85 µg/L (sd= 1.24), 7.29 µg/L (sd= 1.81) and 2.87 (sd= 0.92) respectively. Mean value of PTF was 113.93% (sd= 34.01).

Dose linearity was evaluated comparing $AUC_{0-\infty}$ of day 1 and $AUC_{0-\tau}$ of day 5 by means of a t-Student for repeated measures (statistical significance level $p \leq 0.05$).

Dose proportionality was evaluated comparing Cmax and $AUC_{0-\infty}$ normalised by the dose (Cmax/D and $AUC_{0-\infty}/D$) of day 1 and day 5 between both dose levels by means of a t-Student for independent measures (statistical significance level $p \leq 0.05$).

Table 19: Summary of pharmacokinetic parameters of BCP-OH

PARAMETER	RUP 10MG (S.D) MEAN AND 95 % CI	RUP 100 MG (S.D) MEAN AND 95 % CI	RUP 10MG (S.S) MEAN AND 95 % CI	RUP 100MG (S.S) MEAN AND 95 % CI
C_{max} (µg/L)	0.96 (0.88-1.05)	7.53 (6.69-8.36)	1.58 (1.45-1.70)	12.27 (10.99-13.54)
AUC_0	11.56 (10.61-12.51)	88.65 (80.87-96.44)	22.65 (20.69-24.62)	174.95 (161.05-188.85)
AUC_0 (µg.h/L)	15.15 (13.95-16.34)	131.68 (117.72-145.64)	55.13 (48.46-61.80)	445.35 (393.17-497.54)
λz (h^{-1})	0.0716 (0.0662-0.0770)	0.0557 (0.0506-0.0608)	0.0185 (0.0168-0.0202)	0.0203 (0.0180-0.0226)
$t_{1/2}$ (h)	10.41 (9.35-11.48)	14.08 (11.97-16.19)	41.33 (36.58-46.07)	38.76 (33.67-43.86)
MRT (h)	16.60 (15.27-17.93)	21.04 (18.30-23.77)	42.88 (39.11-46.64)	46.51 (41.56-51.47)
C_{min} (µg/L)	------	------	0.48 (0.44-0.53)	3.85 (3.46-4.25)
Cavg (µg/L)	------	------	0.94 (0.86-1.03)	7.29 (6.71-7.87)
PTF (%)	------	------	116.93 (110.18-123.68)	113.93 (103.05-124.81)
R_{theor}	------	------	3.02 (2.74-3.30)	2.87 (2.57-3.17)

Rupatadine exposure showed over-proportionality between the doses of 10 and 100 mg of rupatadine. This fact, along with a decrease in its plasmatic clearance and an increase in the half-life of elimination suggest a non-linear Michealis-Menten kinetics.

Rupatadine was rapidly absorbed (Tmax 1 h), both in single and multiple doses, after the administration of 10-mg or 100-mg tablets of rupatadine.

The metabolism of rupatadine as a rate-limiting process is suggested, producing an over-proportional increase of rupatadine concentrations with the dose of 100 mg at single and multiple dose.

5.4 Safety and tolerability evaluation

5.4.1 Extent of exposure

Each subject included in the study received a placebo dose (day 0), and immediately followed by 5 days of treatment (days 1 to day 5), according the following sequence:

Group Rupatadine 10 mg comp: day 0 (placebo), and on days 1-5 rupatadine 10 mg/day.
Group Rupatadine 100 mg comp: day 0 (placebo), and on days 1-5 rupatadine 100 mg/day.
Group Moxifloxacin 400 mg: day 0 (placebo), days 1 and 5 moxifloxacin 400 mg/day, and on days 2,3 and 4 placebo.
Group Placebo: 6 days placebo treatment.

5.4.2 Adverse events

5.4.2.1 Brief summary of adverse events

Only adverse events starting after randomization were taken into account.
The adverse events presented more frequently were related to the nervous system disorders.

No serious adverse events were recorded during the study.

A detailed description of each adverse event recorded is given in the following sections.

5.4.2.2 Display of adverse events

The following table summarises all adverse events globally reported in any group:

Table 20: Treatment-emergent adverse events

ORGAN CLASS	PREFERENT TERM	RUPATADINE 10 MG N= 45	RUPATADINE 100 MG N=41	MOXIFLOXACIN 400 MG N = 41	PLACEBO N = 41
Blood and limphatic system disorders		1 (2.2%)	1 (2.2%)	0 (0.0%)	0 (0.0%)
	Anaemia	1 (2.2%)	1 (2.2%)	0 (0.0%)	0 (0.0%)
Gastrointestinal disorders		2 (4.4%)	3 (7.3%)	13 (31.7%)	3 (7.3%)
	Vomiting	0 (0.0%)	0 (0.0%)	3 (7.3%)	0 (0.0%)
	Nausea	0 (0.0%)	1 (2.4%)	8 (19.5%)	0 (0.0%)
	Xerostomia	1 (2.2%)	1 (2.4%)	2 (4.9%)	2 (4.9%)
	Diarrhoea	0 (0.0%)	0 (0.0%)	1 (2.4%)	0 (0.0%)
	Odynophagia	0 (0.0%)	1 (2.4%)	1 (2.4%)	1 (2.4%)
	Dysgeusia	1 (2.2%)	0 (0.0%)	2 (4.9%)	0 (0.0%)
General disorders and administration site conditions		3 (6.7%)	1 (2.4%)	2 (4.9%)	0 (0.0%)
	Pirexia	1 (2.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Asthenia	2 (4.4%)	1 (2.4%)	2 (4.9%)	0 (0.0%)
Musculoesqueletal and connective tissue disorders		1 (2.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Arthralgia	1 (2.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Nervous system disorders		23 (51.1%)	35 (85.4%)	18 (43.9%)	12 (29.3%)
	Dizziness	1 (2.2%)	0 (0.0%)	2 (4.9%)	0 (0.0%)
	Somnolence	20 (44.4%)	27 (65.9%)	8 (19.5%)	9 (22.0%)
	Headache	8 (17.8%)	11 (26.8%)	10 (24.4%)	5 (12.2%)
	Syncope	0 (0.0%)	0 (0.0%)	1 (2.4%)	0 (0.0%)
Infections and infestations		0 (0.0%)	0 (0.0%)	1 (2.4%)	3 (7.32%)
	Catarrho	0 (0.0%)	0 (0.0%)	1 (2.4%)	1 (2.4%)
	Influenza	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.4%)
	Tonsillitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.4%)
Reproductive system and breast disorders		1 (2.2%)	1 (2.4%)	1 (2.4%)	1 (2.4%)
	Dysmenorrhoea	1 (2.2%)	1 (2.4%)	1 (2.4%)	1 (2.4%)
Respiratory, thoracic and mediastinal disorders		0 (0.0%)	1 (2.4%)	0 (0.0%)	0 (0.0%)
	Nasal congestion	0 (0.0%)	1 (2.4%)	0 (0.0%)	0 (0.0%)

The following table summarizes those AE related globally reported in any group:

Table 21: Treatment-emergent adverse events related to study drug

ORGAN CLASS	PREFERENT TERM	RUPATADINE 10 MG N=45	RUPATADINE 100 MG N =41	MOXIFLOXACIN 400 MG N =41
Gastrointestinal disorders		2 (4.4%)	1(2.4%)	11(26.8%)
	Vomiting	0 (0.0%)	0 (0.0%)	2(4.9%)
	Nausea	0 (0.0%)	1(2.4%)	8 (19.5%)
	Xerostomia	1(2.2%)	0 (0.0%)	1 (2.4%)
	Diarrhoea	0 (0.0%)	0 (0.0%)	1 (2.4%)
	Dysgeusia	1(2.2%)	0 (0.0%)	2(4.9%)
General disorders and administration site conditions		3 (6.7%)	1(2.4%)	2(4.9%)
	Pirexia	1 (2.2%)	0 (0.0%)	0 (0.0%)
	Asthenia	2 (4.4%)	1(2.4%)	2(4.9%)
Musculoesqueletal and connective tissue disorders		1 (2.2%)	1 (2.2%)	0 (0.0%)
	Arthralgia	1 (2.2%)	1 (2.2%)	0 (0.0%)
Nervous system disorders		17 (37.8%)	28 (68.29%)	12 (29.3%)
	Dizziness	1 (2.2%)	0 (0.0%)	2(4.9%)
	Somnolence	15 (33.3%)	22 (53.7%)	6 (14.6%)
	Headache	1 (2.2%)	5 (12.2%)	4 (9.8%)
	Syncope	0 (0.0%)	1(2.4%)	0 (0.0%)
Blood and lmphatic system disorders		1 (2.2%)	0 (0.0%)	0 (0.0%)
	Anaemia	1 (2.2%)	0 (0.0%)	0 (0.0%)
Respiratory, thoracic and mediastinal disorders		0 (0.0%)	0 (0.0%)	1 (2.4%)
	Nasal congestion	0 (0.0%)	0 (0.0%)	1 (2.4%)

5.4.2.3 Analysis of adverse events

The adverse events incidences were 15.7% for patients taking placebo, 26.5% for rupatadine 10 mg, 35.5% for rupatadine 100 mg and 22.5% for moxifloxacin. The related adverse events incidences were 30.0% for rupatadine 10 mg, 41.1% for rupatadine 100 mg and 28.6% for moxifloxacin.

The most frequently reported adverse events were somnolence (22.0%) and headache (12,2%) after placebo administration; somnolence (44.4%) and headache (17.8%) after rupatadine 10 mg; somnolence (65,9%) and headache (26,8%) after rupatadine 100 mg; and headache (24.4%), nausea (19.5%) after moxifloxacin.

No statistical comparison of the frequency of adverse events onset was performed due to the scarce number of events.

5.4.3 Deaths, other serious adverse events and other significant adverse events

5.4.3.1 Deaths

There was not detected any death during the study.

5.4.3.2 Other Serious Adverse Events

There was not detected any serious adverse events during the study.

5.4.4 Clinical Laboratory Evaluation

5.4.4.1 Laboratory values over time

Blood samples for both haematology (Hemoglobin, Hematocrit, red blood cell, MCV, MCH, MCHC, white blood cell count and differencial (neutrophils, lymphocytes, monocytes, eosinophils and basofils), platelet count) and biochemistry (Urea, Creatinine, glucose, cholesterol, triglycerides, GOT, GPT, CPK, gamma-GT, alkaline phosphatase, total bilirubin, potassium, sodium, calcium, magnesium) were obtained, as well as urinalysis (Acetone, PH, albumin, glucose, red blood cell, biliary pigment, urobilinogen).

5.4.4.2 Individual clinically significant abnormalities

Table 22: Altered laboratory values at the end of study

VOLUNTEER	GENDER	TREATMENT	PARAMETER	RESULT	REPETITION	UNITS
002	Male	Rup 100 mg	Haemoglobin	129	134	g/l
009	Male	Rup 100 mg	AST/ALT/GPT	75/264/129	17/20/42	U/L
033	Male	Rup 100 mg	CK	268	89	U/l
048	Female	Rup 100 mg	Haemoglobin	106	119	g/l
080	Male	Rup 100 mg	Haemoglobin	121	130	g/l
136	Female	Rup 100 mg	ALT	70	39	U/l
006	Female	Rup 100 mg	Total bilirubin	31	24	μmol/l
025	Female	Rup 100 mg	Haemoglobin	111	115	g/l
040	Female	Rup 10 mg	Haemoglobin	112	117	g/l
053	Female	Rup 10 mg	Haemoglobin	109	118	g/l
078	Female	Rup 10 mg	Haemoglobin	108	110	g/l
084	Male	Rup 10 mg	Total bilirubin	35	34	μmol/l
124	Female	Rup 10 mg	Haemoglobin	113	134	g/l
124	Female	Rup 10 mg	Total cholesterol	7.05	6.85	μmol/l
137	Male	Rup 10 mg	ALT	72	27	mmol/l
144	Female	Rup 10 mg	Haemoglobin	110	119	g/l
170	Female	Rup 10 mg	Haemoglobin	107	109	g/l
182	Female	Rup 10 mg	AST/ALT	40/88	25/51	U/l
034	Female	Moxi 400 mg	Total cholesterol	6.57	5.63	mmol/l
037	Female	Moxi 400 mg	Haemoglobin	113	120	g/l
049	Male	Moxi 400 mg	Haemoglobin	127	133	g/l
095	Female	Moxi 400 mg	Haemoglobin	107	110	g/l
095	Female	Moxi 400 mg	Total cholesterol	7.00	6.01	mmol/l
113	Male	Moxi 400 mg	CK	325	115	U/l
020	Female	Placebo	Haemoglobin	113	124	g/l
027	Female	Placebo	Haemoglobin	111	114	g/l
039	Female	Placebo	Total bilirubin	40	34	μmol/l
094	Female	Placebo	CK	920	120	U/l
103	Female	Placebo	Haemoglobin	108	111	g/l
161	Female	Placebo	Haemoglobin	112	125	g/l

No clinically relevant alterations were observed. In comparison to the values obtained before the administration of treatments, the biochemical results

obtained at the end of the study showed slight fluctuations (increases/decreases). All the changes observed were not considered clinically relevant.

5.4.5 Vital signs, physical findings, and other observations related to safety:

Table 23: Mean values (s.d) and median (min-max) of systolic and diastolic blood pressure, heart rate and temperature obtained at the end of the study.

PARAMETER		MEAN (SD)	MIN – MAX	UNITS
Placebo				
Dorsal decubitus	Systolic blood pressure	119.73 (11.39)	94-145	mmHg
	Diastolic blood pressure	61.93 (7.90)	45-80	mmHg
Heart rate (dorsal decubitus)		73 (10)	51-88	bpm
Temperature		36,12 (0,46)	35-36.8	ºC
Rupatadine 10 mg				
Dorsal decubitus	Systolic blood pressure	118.98 (10.90)	100-140	mmHg
	Diastolic blood pressure	60.70 (7.34)	43-78	mmHg
Heart rate (dorsal decubitus)		76 (9)	54-90	bpm
Temperature		36.2 (0.4)	35.5-36.9	ºC
Rupatadine 100 mg				
Dorsal decubitus	Systolic blood pressure	118.90 (10.28)	97-140	mmHg
	Diastolic blood pressure	62.83 (8.42)	46-76	mmHg
Heart rate (dorsal decubitus)		78 (9)	62-95	bpm
Temperature		36.2 (0.4)	35.4-37	ºC
Moxifloxacin 400 mg				
Dorsal decubitus	Systolic blood pressure	116.68 (11.38)	90-145	mmHg
	Diastolic blood pressure	59.13 (6.44)	43-72	mmHg
Heart rate (dorsal decubitus)		76 (9)	54-95	bpm
Temperature		36.1 (0.4)	35.3-36.9	ºC

All the changes observed were not considered clinically relevant.

6. DISCUSSION

The current study confirms the absence of effect of the novel antihistamine, rupatadine, on the prolongation QT/QTc interval, following therapeutic and supratherapeutic doses, as compared with placebo and a positive control (moxifloxacin).

Antihistamines are some of the most widely used drugs in the world today, and they are first-line treatment options in common pathologies such as allergic rhinitis and chronic urticaria (237). As a class, the development of the antihistamines has been a steady evolution based on classical drug-receptor theory. The first products to reach the marketplace included drugs such as chlorphenamine and promethazine. These agents were very effective and potent antihistamines but they were associated with significant adverse CNS effects, most notably sedation. As a result of the detrimental effects on performance and psychomotor activity, the focus turned to the development of "non-sedating" antihistamines. This research led to the introduction of drugs such as astemizole, cetirizine, ebastine, loratadine, mizolastine and terfenadine; drugs which had significantly reduced effects on the CNS. However, as noted earlier, in the 1990s two of the second-generation antihistamines (astemizole and terfenadine) were associated with QTc prolongation and occasional episodes of the life-threatening *torsades de pointes*, what caused the withdrawal of both drugs from the market (234)(285)(286).

Cardiac safety is a mayor concern in the development, approval and prescription of new drugs. A substantial number of drugs have been restricted in their application or have even been withdrawn from the market due to concerns about cardiac safety. It has been estimated that adverse cardiovascular effects are responsible for 45% of post-approval drug withdrawals and around 30% of general drug attrition (25). Drug induced cardiac repolarization prolongation and the associated risk of potentially lethal arrhythmias is among the most serious adverse effects. The inhibition of the rapid delayed-rectifier K+ current (IKr) encoded by the human ether-a-

go-go related gene (hERG), the main repolarizing current in ventricular cardiomyocytes of large mammals, can cause repolarization prolongation, promoting early after depolarizations which may initiate TdP in vivo (25). Delayed cardiac repolarization, measured in the surface electrocardiogram as prolongation of the QT interval, can give a signal of the risk of proarrhytmic events, known as torsade de pointes (TdP). Its reporting rate in association with non-cardiac drugs increased exponentially from the early 1990s and was associated with an increasing number of new non-cardiac drugs whose proarrhythmic liability was not appreciated in clinical development. This epidemic provoked a comprehensive global response from drug regulators, drug developers and academia (26). In 2005, the international Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) released Guidelines S7B (165) and E14 (176) that currently govern the cardiac safety landscape. The S7B guideline provides a nonclinical testing strategy to evaluate the potential for human pharmaceuticals to affect cardiac repolarization. The highlighted biomarkers of particular interest are the repolarizing ionic current Ikr and the QTc interval. The S7B is narrowly focused on hERG current block and QTc prolongation, both of which are surrogates for proarrhytmia. The core testing sytems recommended are an in-vitro assay of the rapid component of the delayed rectifier potassium current (IKr) and an in-vivo study in dog or other non-rodent laboratory animals (10)(165). Since its adoption and with evolving nonclinical and clinical data, it is now appreciated that the effects of hERG current block may be modulated by multiple cardiac ion currents during repolarization and that hERG current block sometimes does not provide a meaningful indicator of proarrhytmic risk. Because of this, it's need to complement the research with more clinical data. The ICH E14 Guidance addresses clinical evaluation of QTc prolongation and the proarrhythmic potential of nonantiarrhytmic drugs. The document describes a dedicated clinical trial, the thorough QT (TQT) study, designed to assess the degree to which a drug compound affects the QT duration (25).

The cardiac safety of rupatadine has been extensively assessed as part of its preclinical and clinical evaluation programme. In preclinical development, it

can be highlighted the fact that it has no clinically relevant effects on the cardiovascular system as shown by the following findings: doses of >100 times that recommended in humans had no effect on ECG parameters (QTc, PR or QRS intervals), mean blood pressure and heart rate in rats, guinea pigs and dogs; it was not associated with arrhythmias or an increased rate of cardiovascular mortality in these animal models; neither rupatadine nor its main metabolites affected the cardiac action potential in isolated dog Purkinje fibers at concentrations at least 2000 times greater than the C_{max} reached after administration of a 10 mg dose in humans; and, i*n vitro,* rupatadine concentrations required to block the HERG potassium channel or the human cloned hKv1.5 potassium channel expressed in a mouse L-cell line, were almost 2,´000-fold greater than serum concentrations determined after administering rupatadine 10 mg to human volunteers. The integrated evaluation of the preclinical assessment of the ventricular repolarization risk for rupatadine is negative regarding two key levels of non clinical approaches from ICH S7B Guidance: hERG current and Purkinje fibres in vitro studies and in dog and guinea pigs in vivo studies (269)(287).

Clinical evaluation of the cardiovascular effects of rupatadine has been widely reviewed elsewhere. A total of 6,450 ECGs from 4,000 healthy volunteers and 2,450 adult patients with allergy have been evaluated as part of this analysis (276). Rupatadine dosages in these studies ranged from 2.5 mg to 100 mg either as single doses or once daily for 2-4 weeks and were tested under a wide range of conditions: with or without food; administered alone or concomitantly with alcohol, erythromycin, or ketoconazole; and in young and elderly healthy volunteers of both sexes. In these evaluations, rupatadine doesn't produced clinically relevant changes in QT/QTc intervals despite the fact that drugs which increase the systemic exposure of the antihistamine (erythromycin and ketoconazole are potent cytochrome P450 3A4 isoenzyme inhibitors) were co-administered (269)(276).

The clinical phase of rupatadine coincides temporarily with the development of TQT study guideline E14 (176). As commented, this guidance requires the majority of new chemical entities with systemic availability to be tested in a

study specifically designed to exclude small drug-induced QTc effects. In order to complete the request documentation for the marketing authorization, a TQT study was planned and conducted for rupatadine.

After a comprehensive lecture of the draft guideline, the first challenge we afford, it was the fact to define the **supratherapeutic dose** of rupatadine to be used. As the guide recommends (176), two doses of the new drug should be studied - the therapeutic dose, and a supra-therapeutic dose which would simulate levels seen in hepatic or renal failure or with metabolic inhibitors.
There are several advantages of the incorporation of a supratherapeutic dose. First, use of a supratherapeutic dose can help define the magnitude of risk using the steepness of the dose-QTc relationship. Second, the use of such a high dose in healthy participants may mimic the risk of therapeutic dose in a target of population of patients with concurrent diseases, or mimic the consequences of an inappropriately high dose being prescribed or administered to a patient; for example, a patient with impaired metabolic capacity would not metabolize the drug as quickly as expected when prescribed the usual dose, and so the patient is likely to experience a higher plasma concentration of the drug, one that may be similar to that produced by a supratherapeutic dose in healthy volunteers without hepatic impairment. Third, consider a scenario where the eventual therapeutic dose turns out to be much higher than the originally planned therapeutic dose. Without the inclusion of a supratherapeutic dose, we would not have any data that could be used to estimate the QT liability in these circunstances (288).
The selection of the supratherapeutic dose is one of the most critical features in defining whether the "thorough QT" study was adequately designed, and the most common reason for discussions between regulators and sponsors. The selection of a supratherapeutic dose should be selected based on the known pharmacologic properties of the drug and how the extent of exposure will change when it is taken by a patient who has effect modifiers. Once this modeling has identified the maximum extent of exposure, then the dose that provides the equivalent exposure is the best definition of the supratherapeutic dose. Alternatively, the magnitude of this supratherapeutic

dose can be just below the maximum tolerated dose that has been defined in the typical ascending phase I dose trial, if, in fact, the intolerability was clinically significant and that a maximum toleration was properly obtained.

In recent literature the most of clinical trials assure a correct selection of the supratherapeutic dose based in the drug's metabolism (289)(290)(291)(292), or extensively justify the no inclusion of the high dose based mainly for tolerability (293)(294), as in the case of antidiabetic drugs; or safety reasons (295)(296) in case of oncologic drugs. In these cases, warning recommendations are included in the summary of product characteristics when the product is marketed.

A failure to study a sufficiently high dose has occasionally led to a requirement for a repeat TQT study; this is therefore an important part of the dialogue between sponsors and regulators before the TQT study is initiated. It should also be borne in mind that formulation changes that result in substantially higher plasma levels of a drug may require conduct of an additional TQT study (297). A recent example is exenatide, a GLP-1 agonist intended for the treatment of type 2 diabetes mellitus. Two separate TQT studies had to be conducted for two different exenatide formulations (Byetta® and Bydureon®), both of which were negative (298)(299). The first TQT study was conducted with the daily subcutaneous formulation (Byetta®) at the approved therapeutic dose (10 µg twice daily) (298). On chronic administration with a therapeutic dose of the later developed once-weekly formulation (Bydureon®, 2 mg once weekly), exenatide plasma levels were at least twofold higher than those with the daily formulation and even higher in patients with impaired renal function. Accordingly, the FDA required to conduct a second TQT study, in which substantially higher plasma levels were achieved through an IV infusion of the drug (299).

In the clinical development phase, metabolic drug–drug interactions of rupatadine were studied: administration of 20 mg rupatadine plus known CYP3A4 inhibitors, ketoconazole (200 mg/day) or erythromycin (500 mg t.i.d.) in steady state conditions(272). Ketoconazole inhibited both the presystemic and systemic metabolism of rupatadine, increasing the exposure of unchanged drug by nearly 8-fold and decreasing the exposure of metabolites.

Erythromycin showed lower inhibition of the presystemic metabolism than ketoconazole, leading to a 3-fold increase of the systemic exposure to unchanged rupatadine, without significant increases in rupatadine elimination half-life and systemic exposure to the assayed metabolites. Due to this potential interaction, it is not recommended to use rupatadine in combination with ketoconazole, macrolides or any potential inhibitors of CYP3A4. For a more extensive knowledge of rupatadine potential interactions, there were two more studies with azithromycin and fluoxetine, because fluoxetine has been described as a powerful inhibitor of some cytochromes of the P450 family, such as CYP2D6 and CYP3A426. No clinically relevant modifications in mean pharmacokinetic parameters of rupatadine and metabolites were observed when azithromycin or fluoxetine were coadministered (270)(272)(276).

As a reference for our current project, in the prior published evidence from clinical trials with antihistamines, we found a clinical trial (300) conducted with ebastine, that evaluated the electrocardiographic effects of three and five times the maximum recommended dose of ebastine (60 mg and 100 mg once daily), but this magnitude seemed not enough for rupatadine due to its metabolism.

Taking account that in previous clinical trials with rupatadine the maximum therapeutic levels achieved were 8-fold higher and, moreover, some authors recommends that in the case of antihistamines, the supratherapeutic dose may over 10x apart(25); we considered that for going aligned with recommendations and assure a confident safety margin for the clinical use, the supratherapeutic dose tested should be 10-fold higher than therapeutic dose, this means a dose of 100 mg of rupatadine. Additionally, investigating supratherapeutic dosages is, in this case, of particular interest considering the pharmacokinetic profile of rupatadine. This dose should generate plasma levels of rupatadine and its metabolites, which clearly exceed those that can be observed in patients with impaired clearance of the drug, based on intrinsic (e.g., age and hepatic impairment) or extrinsic factors, specifically drug interactions, especially because rupatadine is a CYP3A4 substrate.

As highlighted in the ICH publication (176), there is the possibility to use a drug–drug interaction study for obtaining supratherapeutic plasma levels, but this includes the underlying assumption that the inhibitor has no effect on heart rate or QT interval. Ketoconazole, which inhibits cytochromes oxidase enzymes that metabolize many drugs, is the most interaction agent use (301)(302)(303)(304), but some authors consider that its should be avoided as ketoconazole itself can prolong the QT interval (206), as we can observe in bilastine thorough study, where the interaction of bilastine and ketoconazole gives a positive result (303). Then to minimize bias and avoid confounders, we prefer the administration of a supratherapeutic dose of rupatadine (100 mg) instead use an interaction drug.

Due to this, meanwhile other design aspects of the protocol were discussed; a tolerability dose escalating study, from 10 mg to 100mg, was conducted in order to confirm that 100 mg dose was not a safety concern for the volunteers. This dose was also a challenge from a galenic point of view. As we commented previously, for pharmaco-technical reasons, the 100 mg dose was administered as the sum of three tablets (40 mg + 40 mg + 20 mg). Obtain a clear tolerability result of the 100 mg dose was a milestone to achieve our TQT study.

In most TQT studies to date, both a supratherapeutic and **a therapeutic dose** of the new compound have been studied. There are, however, a few studies in which only a supratherapeutic dose was included (305)(306)(307)(308)(309). Krishnaswami et al (307) took the decision not to include a therapeutic dose based in part on the findings from nonclinical assays and PK/PD analysis of early clinical data indicating that tasocitinib, the study drug, is unlikely to cause QTc prolongation. More important, since the purpose of including a supratherapeutic dose was to evaluate risk at the extremes of therapeutic exposures, they considered that the effect of the supratherapeutic dose on QTc interval would be sufficient to determine labeling/monitoring requirements. In addition, the supratherapeutic dose was expected to provide plasma concentrations that span the range of Cmax values for the dose range under investigation in efficacy/safety studies for tasocitinib. Thus, in the

event of a positive result with the supratherapeutic dose, concentration-QT analysis could be used to estimate the risk of QTc prolongation at lower doses. This was also the approach used by Suico(305), Iwamoto(306) and Vourhavis(308) et al in that a single supratherapeutic dose was used to assess the risk of QT prolongation, whereas Zhang et al (309) used a dose escalation approach to achieve supratherapeutic concentrations prior to performing ECG assessments.

This approach is obviously sufficient if results are clearly negative. Most sponsors tend however to also include the therapeutic dose, in case the high dose is "slightly positive." It can be argued that the effect of the therapeutic dose can be projected, and it would therefore be sufficient to include only a supratherapeutic dose, but this has so far not gained widespread acceptance. We prefered include the therapeutic dose of rupatadine (10 mg) in order to have a more robust result.

There is no requirement *per se* on **dosing to steady state** of the new drug. Single doses can be used in the TQT study, provided that a sufficiently high exposure of both parent and major metabolites can be achieved. If there are slowly appearing metabolites, which require many days of dosing to achieve sufficiently high exposure, multiple dosing is warranted. Since the objective of the TQT trial is to define the effect of a treatment on ECG parameters that treatment must be given in a manner that reflects the extent of exposure of parent compound and its metabolites in the target population when the drug is in clinical use. If the pharmacokinetics of a drug, following a single dose and at steady state, following multiple doses, are essentially identical, then a single dose trial can be conducted. If there is any evidence of accumulation of parent or if the metabolites are not well characterized and the drug is to be used in a multiple dose manner for therapy, it seems logical that only a multiple dose study designed to steady state would be appropriate. At the moment, it's generally accepted that any drug that it's used for chronic therapy or at least for multiple days is likely to need a multiple dose trial design, as in the case of antihistamines (300), unless the sponsor has definitive data to show that there is no difference in exposure of

parent/metabolites after such multiple dose vs single dose. The only clear advantages of a single dose TQT study is to reduce the duration of the trials (decreases also any time effects on ECG intervals) and the cost of conducting the trial (25).

Rupatadine is extensively metabolized mainly by P450 (CYP3A4) system to two active metabolites UR-12790 and UR-12788, that seem to contribute to rupatadine activity. In previous studies the steady state conditions were achieved on day 4 after dose of 10 mg once daily, and elimination half-life was approximately 6-8 hours for rupatadine; and 27-33 hours and 35-41 hours, respectively, for the active metabolites UR-12790 and UR-12788. Taking account these pharmacokinetic data, the decision of conduct the study in steady state conditions seemed more appropriate.

In a TQT study, we are trying to demonstrate the lack of an effect of a drug on cardiac repolarization by showing that there is no prolongation of QT and, at the same time, prove that this test is sufficiently sensitive to detect an effect if there was one. The guidance recommends that both a concurrent placebo negative control arm and a concurrent positive control arm should be considered in the study.

The purpose of comparing the investigational drug to placebo is to exclude a QTc effect due to the drug. All the reviewed published TQT studies include this approach. As the guideline recommends (176), a **placebo arm** was included to define the real effect and to deal with potential bias, mainly spontaneous diurnal and day-to-day variation in QT interval (310). The collection of drug-free ECGs on two or three different days can also help document inter-day variability in the baseline (297)(311). Moreover, we include a complete predose day in all groups of treatment for minimize the known variability in QT interval, as we are going to set forth later.

The reason for a **positive control arm** is that "The confidence in the ability of the study to detect QT/QTc prolongation can be greatly enhanced by the use

of a concurrent positive control group to establish assay sensitivity." Detecting the positive control's effect will establish the ability of the study to detect such an effect of the study drug. To establish assay sensitivity, there needs to be enough evidence to show that at least one 90% lower bound is above 5 ms, as afterwards we are going to comment (179).

As the guidance didn't recommend specifically any compound as use as positive control, we had to perform a literature search to select the more appropriate drug to be used. At the moment of the design development of our trial, few published studies had employed a positive control for check the assay sensitivity (300)(312)(313). The first of them (300) was the trial comparing the effects of high doses of ebastine on QTc comparing with placebo and as positive control terfenadine. This was an investigator-blinded, four-way crossover study to compare the ectrocardiographic effects of 3 and 5 times the maximum recommended dose of ebastine (60 mg and 100 mg once daily) with three times the recommended dose of terfenadine (180 mg twice daily) and placebo administered for 7 days. The second of them (312) evaluated the effects of vardenafil and sildenafil on QT duration trough a placebo-controlled and positive-controlled, 6-way crossover study evaluated therapeutic and supratherapeutic oral doses of vardenafil (10 and 80 mg, respectively) and sildenafil (50 and 400 mg, respectively), therapeutic doses of moxifloxacin (400 mg - positive control), and a placebo, with dosing every 3 days. Finally, the third study (313) evaluated telavancin at a dose of 7.5 mg/kg or 15 mg/kg, placebo or moxifloxacin (400mg - positive control) administered once daily for 3 days as 60-minute iv infusions.

When we discussed the positive control selection for our study, the first step was select the chemical entity to be used. According the literature review, two options were evaluated: terfenadine and moxifloxacin. Terfenadine was quickly discarded because of the difficult to obtain it and the higher historical risk of TdP than moxifloxacin.

In most studies, moxifloxacin has caused a larger peak effect than 5 ms, more in the range of 8–15 ms(314). The differences in peak response across studies is however quite striking and ranges between 7.5±2.2 ms (315) and 19.0±3.9 ms (316) in the published studies that used a single oral dose of 400 mg

moxifloxacin. It is therefore that, some authors hypothesize that may not provide a convincing demonstration of the assay sensitivity. Indeed, the large increase observed in QTc with moxifloxacin (mean increase of 10 ms) may make it relatively easy to confirm the validity of the QT/QTc study, but it is likely to compromise the assay sensitivity. Therefore, moxifloxacin could be too strong as a positive comparator in QT/QTc studies. In contrast, positive comparators that are known to produce mean changes close to the regulatory guidelines of 5 ms, and which can be detected by the assay in use for a particular study, would provide a more rigorous analysis of the study drug and examine its potential to prolong QTc in a more effective manner. As example, data from a levofloxacin study showed that both levofloxacin and moxifloxacin can fulfil the criteria for a positive comparator as defined by the ICH E14 regulatory guidelines. By comparing these two drugs directly on the same subjects, it was shown that levofloxacin could have the potential to provide a more rigorous evaluation of the assay conditions used to detect clinically significant changes in QTc when evaluating new chemical entities.

Nonetheless, so far, the most common drug used as positive control is moxifloxacin (317), and for all these reasons, it was the selected drug to be used as positive control in our study.

Second step was to define the dose and administration route to be used. Clearly the administration route selected was oral route, like rupatadine; but the selection of single or multiple dose of moxifloxacin required a more detailed discussion. The similar peer studies reviewed had used a multiple dose approach, probably for achieving plasma concentrations of moxifloxacin which ensure a positive outcome. We consider that this approach could be related to a higher risk of QTc prolongation, althought, some authors consider it as conceivable that QT interval prolongation may be less pronounced during repeated administration because with other QT prolonging drugs it has been shown that the extent of QT prolongation at a given plasma concentration decreases during repeated administrations (318) (319).

It has been widely demonstrated that a single dose of 400 mg of moxifloxacin is enough to produce a QT prolongation as the guidance requests. Demolis et al (318) published a study that specifically assessed the effect of single oral

doses (400 mg and 800 mg) of moxifloxacin on the duration of QT intervals in healthy subjects. This study was a placebo-controlled, randomized, double-blind, crossover study (for the first 3 study periods), with an open fourth study period in which subjects received a single intravenous administration of 400 mg moxifloxacin over 60 minutes for pharmacokinetic assessment. Moxifloxacin induced a significant but modest increase in QT interval duration. The increase in QT interval duration relative to placebo remained between 2.3% ± 2.8% and 4.5% ± 3.8% across the range of RR intervals tested. In the tested doses, this lengthening was not related to dose but was related to plasma moxifloxacin concentrations. Pharmacokinetic parameters of moxifloxacin were comparable to those of previous studies in healthy male volunteers (320)(321). The significant correlation that was found between QT interval prolongation with moxifloxacin relative to placebo and plasma concentration indicates that moxifloxacin-induced QT prolongation is predictable and the risk of moxifloxacin-induced torsades de pointes is expected to be minimal when the drug is administered at the recommended dose of 400 mg/d.

For most of the posterior TQT studies, moxifloxacin is used as the positive control: 68 of 84 reviewed studies used a single oral dose of 400 mg (see Annex 2: List of TQT studies), 5 used multiple dosing, mainly studies designed during the very early period after guidance publication (303)(322)(323)(324)(325). Also some authors used moxifloxacin in an IV infusion (326). Three trials selected other drugs to obtain a positive result (300)(327)(328): ketoconazole in 2 of them and quetiapine in other. It worth mentioning that in one of these studies, quetiapine was selected as the positive control for assay sensitivity because quetiapine is an antipsychotic that known for mild QTc prolongation with no clinically significant TdP consequences and it is more ethically suitable to use in the stable schizophrenic population included in the study, than moxifloxacin (328).

These data support the use of a single dose of moxifloxacin to produce the expected QT prolongation. Then, for minimize adverse events risks to the volunteers, the single dose schedule was selected for administration only in

the key study days (day 1-single dose of rupatadine and day 5-steady state of rupatadine).

The criteria for demonstrating assay sensitivity with moxifloxacin were finally addressed in the E14 Q&A document (329). The criteria include:

- The lower bound (LB) of the 90 % CI of ΔΔQTc should be above 5 ms for at least one prespecified post-dose timepoint.
- The peak ΔΔQTc should be within the range of responses seen in similar studies, i.e., about 8–16 ms, even though the exact cutoff points are less clear.
- The mean peak ΔΔQTc should be observed between 1 and 4 h post-dose and thereafter declines. Note that lately, it has also asked for a timepoint earlier than the peak effect.

Althought the probability of a volunteer would suffer a torsade de pointes with moxifloxacin administration is very low, we included in study protocol the procedures to implement in case of the event. All personnel were training on it. We considered appropriate to perform baseline ECGs and blood tests before the drug administration, including potassium, magnesium and calcium concentrations determination. ECG recorders and an electric cardioversor were also available in the unit during all the study conduction.

In case of an event, as for isolated QT prolongation, responsible agent should be discontinued and modifiable risk factors such as hypokalaemia, hypomagnesaemia and hypoxia should be addressed. Several sources recommend that serum potassium is maintained in the high normal range (4.5-5.0 mmol/L), nevertheless evidence is limited (330)(331). The protocol included the administration of an inmediate bolus of 2 gr MgSO4 iv in 2 min. At incomplete response within 15 minutes, repeat bolus of MgSO4 in infusion at a rate of 3 to 20 mg per minute. In case of bradycardia, atropine 0,25 mg shoul also be administered.

Administration of magnesium sulphate is currently recommended as immediate first line treatment for TdP (331). The mechanism for benefit is uncertain(330), but magnesium may reduce the amplitude of early abnormal

depolarizations (EAD) by inhibiting the late calcium influx via L-type calcium channels that is associated with delayed ventricular repolarisation. As a result, EADs are less likely to reach threshold potential and provoke or sustain TdP (332). As a co-factor for the sodium potassium ATPase, magnesium may stabilise the membrane potential by facilitating potassium influx, correcting dispersed repolarisation without shortening the action potential duration. Efficacy has not been demonstrated in a randomised controlled trial, but in a case series of 12 adult patients with TdP (in 9 induced by antiarrhythmic drugs) a single 2g (8 mmol) dose of magnesium sulphate administered intravenously over 1-2 minutes caused resolution in 9 patients, with a second dose being effective in the remaining 3, without reducing the QT interval (333). Similar results were reported from a French study, where TdP was completely abolished after administration of intravenous magnesium 1-3 g in 4 of 6 patients. In the remaining 2 patients, TdP improved but recurred in one and was suppressed only partially in the other (334). Magnesium therapy is simple and relatively safe to administer. The most prominent adverse effect is flushing, but nausea and vomiting, hypotension, and drowsiness can occur, especially with higher doses (335)(336).

Initially, the guidance recommended **blinding of the positive control**, but in some case with over-encapsulated moxifloxacin, the QTc effect was, however, unexpectedly small, which likely was caused by altered pharmacokinetics of the drug due to the encapsulation. Thereafter it was accepted the use of open-label moxifloxacin in "thorough QT/QTc" studies and the same recommendation was later made in the Q&A document by the ICH E14 Implementation Working Group (IWG) (329): "The use of a double-blinded positive control does not appear to be essential, provided that the reading of ECGs is performed in a blinded manner, and the study is carefully designed to ensure that specified study procedures are followed uniformly. This means that the same protocol for administering the test drug and placebo, taking blood samples and collecting the ECG data should also be used when giving the positive control. This does not mean that other aspects of the

study, such as the duration of treatment with the positive control and the other treatment groups, would be identical."

We evaluated the risk-benefit ratio of not use a strictly blinded positive control, and finally decided not to encapsulate moxifloxacin: it was very difficult, almost impossible to meet that criterion, because we didn't have a validated encapsulation method that gave us the sure would not interfere in plasmatic concentrations of moxifloxacin; but taking all other measures to assure the minimal risk of bias. The main reasons to take this decision was the fact that we consider the QT interval measure as quite objective, only the personnel on charge of drug administration wouldn't blinded, but neither investigators nor ECG evaluators would known the treatment assigments. Previous ebastine trials had tested this approach: the study treatments were dosed by a third party in order to blind the investigator and all other study personnel; with no biased results (300)(327). Moreover, to minimize the subjective perception of the participants, we made all study procedures identical between treatment arms/groups, as for example blood draws in the placebo and moxifloxacin groups. Other authors use another design(305), named Williams design, in which the sponsor, investigators, and subjects were blinded to the placebo and test drug but were unblinded to the positive control treatment, moxifloxacin.

As an illustration of the analyses that FDA can perform across ''thorough QT/QTc'' studies, data from these studies that had used blinded or open-label moxifloxacin were presented at the April 2008 DIA meeting (209). The conclusion from this analysis was that assay sensitivity using moxifloxacin could be achieved with either approach; that is, the QTc effect was very similar (180).

Therefore, the use of a double-blinded positive control is deemed not essential according to the current regulatory guidance provided that the personnel responsible for overreading the ECGs are blinded to identify all the treatments, time points, and subjects; as was the case in this study.

In order to maintain the blinded conditions, all the groups of treatment took three tablets (active treatment + corresponding placebo tablets). Drug was administered in fasting conditions, to avoid interferences in moxifloxacin

absortion. Food had no significant effect on the extent of rupatadine absorption although delayed the time to peak plasma concentration in approximately one hour, but previous studies with moxifloxacin showed that coadministration of moxifloxacin with meal has been shown to modestly affect the absorption of moxifloxacin (337). In a recent study evaluating the effect of pomaglumetad methionil on the QTc interval in subjects with schizophrenia, the results showed a lack of assay sensitivity. The authors comment that the lower moxifloxacin plasma concentration observed was likely due to food effect, because all study drugs were administered within 30 min of a meal to reduce gastrointestinal AEs (292).

With regards to **study population**, it is typically carried out in healthy volunteers (as opposed to individuals at increased risk of arrhythmias). From a clinical point of view, it seems more meaningful to conduct the thorough study in the population that is the target of the drug's use; however, healthy volunteers are the subject population to use because trying to use the target population will add marked heterogeneity to the study in terms of disease magnitude, concomintant drugs, and comorbidities. Obviously, a TQT study will be followed by ECG data in the target population in phases II and III. This was the approach that we followed in our TQT study and it was also followed for the vast majority of TQT studies performed. However, there are occasional examples of TQT studies conducted in a relatively limited patient population, such as Parkinson's disease, with stringently controlled experimental conditions (297)(338). In addition, some drugs are not suitable for study in healthy volunteers because of issues related to tolerability (e.g., neuroleptic agents, chemotherapeutics), then some examples of TQT studies in these patient populations have been also conducted (292)(328). Nevertheless, in the case of chemotherapeutics with a proven safety profile a study in healthy volunteers can be conducted. This is the case of a study of lenvatinib, a multityrosine kinase inhibitor with antitumor activity in patients with melanoma and renal cell carcinoma. Lenvatinib is not genotoxic; therefore, this TQT study followed the design in the E14 guidance. Moreover, this avoid a limitation observed with other oncologic drug TQT investigations:

when a oncologic population is included in a TQT study, the use of placebo and moxifloxacin as a positive control can be difficult to ethically justify. Many studies with oncology agents are therefore uncontrolled (297).

In the firsts steps of the guideline, there were no requirements on gender considered in the conduct of the TQT study. It is known that women have a somewhat longer QTc interval than men, and it has been shown that the degree of drug-induced QTc prolongation may vary in different phases of the menstrual cycle. It is also well documented that women are at higher risk for the development of proarrhytmias caused by drugs with an effect on cardiac repolarization (339).

Since the adoption of the E14 guidance, gender has been discussed and, it would seem reasonable to assume that women also react with a large degree of QTc prolongation than men at the same plasma exposure of a drug. There are a few documented examples thereof. For drugs with only mild effect on the QT interval, has been difficult to demonstrate a gender difference in sensitivity for the drug-induced QTc prolongation. In a pooled analysis of data from 2 studies in healthy volunteers who where dosed with levofloxacin, age and gender did not have an effect on the level of QT prolongation when analyzed with a linear exposure-response (ER) model (340). Sex differences in QTc prolongation for moxifloxacin were investigated in a pooled analysis of 20 TQT studies that used moxifloxacin as a positive control (341). Women had approximately 40 % higher moxifloxacin peak plasma levels than men and a statistically significant larger peak QTcF effect with a placebo-corrected ΔQTcF of 12.4 ms (confidence interval (CI): 11.1–13.7 ms) compared to 9.1 ms (CI: 8.1–10.1 ms) in men. There was however no difference in the slope estimated for the exposure-response relationship, which means that the observed difference in QTcF prolongation can be explained by the differences in plasma levels. In line with these considerations, the latest version of the Q&A document states Question 8 (329): “The thorough QT study is primarily intended to act as a clinical pharmacology study in a healthy population using a conservative primary objective defining the drug’s effect on QT. It is unlikely that any of a variety of baseline demographic parameters would

introduce a large difference in QT response to a drug in subpopulations defined by factors such as age, co-morbidity, and gender that is not explained by exposure. It is encouraged, but not mandatory, to include both men and women in the thorough QT study. Analyses of concentration response relationship by sex can be helpful for studying the effect of the drug on QT/QTc interval in cases where there is evidence or mechanistic theory for a gender difference. However, the primary analysis of a thorough QT study should be powered and conducted on the pooled population. If the primary analysis is negative and if there is no other evidence suggesting gender differences, subgroup analysis by sex is not expected."

We consider relevant to include women in our study in order to reflect the real life conditions of the drug's use and to avoid any bias related to gender interaction. According the guideline, the primary analysis was conducted in the global population and it was planned that if a statistically significant treatment by gender interaction would be detected, results would be presented also by gender.

Among the 84 TQT studies reviewed, the majority (n=69) were conducted in both male and female healthy volunteers, whereas nine studies were conducted only in males (300)(312)(327)(342)(343)(344)(345)(346)(347) and only three in females (316)(348)(349). Two of the studies justified the inclusion of only males due that the drug indication was erectile disfunction (312)(347) and other two justified the inclusion of only women due to drug indications in anticonception and menstrual bleeding (348)(349). This contrasts with the fact of other two studies: one with retosiban (291), an oxytocin receptor antagonist for treatment of spontaneous preterm labor, which included both gender; and other with naloxegol (344), a pegylated derivative of naloxone for the treatment opioid-induced constipation, mainly used in women, which only included male subjects. No clear explanation of the selection criteria is given in these articles.

At last, other factor related with the selection of study population is the ethnic origin. Although data are limited, it is not expected that the results of

the "thorough QT/QTc study" would be affected by ethnic factors (297). Because of this, no selection criteria were specified with regards to this point.

Another relevant decision to take was the selection of a **parallel or crossover design**. Crossover or parallel group study designs can be suitable for trials assessing the potential of a drug to cause QT/QTc interval prolongation. Crossover studies at least have two potential advantages: They usually call for smaller numbers of subjects than parallel group studies, as the subjects serve as their own controls and hence reduce variability of differences related to inter-subject variability and they might facilitate heart rate correction approaches based on individual subject data. Parallel group studies might be preferred under certain circumstances: For drugs with long elimination half-lives for which lengthy time intervals would be required to achieve steady-state or complete washout, if carryover effects are prominent for other reasons, such as irreversible receptor binding or long-lived active metabolites and if multiple doses or treatment groups are to be compared (297).

When considering the clinical study trial design, it is critical to know the variability that exists between study types. The "within-subject" variability is lower than the "between-subject" variability, and a crossover-designed TQT study is therefore more efficient than a parallel-designed study and as such requires a slightly reduced sample size and, obviously, fewer subjects. This was pointed out in the E14 guideline (176) and has been emphasized by some authors on several occasions (25)(297). When the drug needs to be dosed for more than approximately a week to reach sufficiently high steady-state plasma levels due to accumulation, or needs to be titrated based on tolerability, a parallel-designed study is preferable (288).
Based on the need to administer rupatadine during at least 5 days to obtain the steady state conditions, selection of a parallel design makes sense. Selection of a parallel study design also eliminated any concern about carryover effects of the parent durg of any metabolite. Moreover, and more relevant for us, with a parallel design, the study duration, i.e., from first-subject-in to last-subject-out, can also be shorter, provided the clinical site

can handle sufficiently large cohorts concurrently. Duration of study was a critical issue for us due to the planned deadlines to present the CTD document to regulatory authorities. Following all these considerations, a parallel design was selected for our TQT study.

This approach was not the most common design selected in the literature review: 54 of 84 clinical trials reviewed were conducted with a crossover design, a more efficient design, as commented, when its possible, mainly for the less sample size required (see Annex 2).

Nevertheless, a parallel-designed study can be made more effective as compared to the standard design with four treatment groups (i.e., placebo, moxifloxacin, and a therapeutic and supratherapeutic dose of the drug), by using a nested crossover comparison within a combined placebo/moxifloxacin group, as suggested by Dr. Joanne Zhang (350). In this nested design, half of the subjects in the combined placebo/moxifloxacin group are dosed with the positive control on the first day of treatment, and in the other half of subjects, dosing occurs on the day after the last treatment day. Zheng et al (351) tested this design in a randomized, double blind, positive and placebo-controlled, parallel and nested-crossover groups to evaluate the effect of a novel antidepressant. The nested-crossover treatments consisting in two groups of a positive control of 400 mg of moxifloxacin and placebo, one group received moxifloxacin on day 1 and the rest of days placebo, and the other group received placebo until the last day of treatment in which they received moxifloxacin. This design allowed reducing the number of placebo and positive control subjects by half. Another novel design it's used by Ring and colleagues (352) in a TQT study with empagliflozin. The five-period crossover design employed in this TQT study showed to be more efficient than the usual four-period design for TQT trials. The design is based on the objective that all comparisons are performed between active drugs and placebo, and no comparisons between active drug groups are necessary. Furthermore, the use of two placebo periods increases the number of measurements taken while on placebo, thus reducing the variability of placebo estimates and of placebo-corrected values for active treatment groups. As a consequence, a 25% smaller sample size is required to achieve the same power as the

corresponding four-period trial design. The sample size required to maintain an overall power of 90% for this five-period TQT trial was 30 subjects (including three potential drop-outs), compared with 40 subjects (including four potential drop-outs) in a conventional four-period design. Moreover, the number of ECG recording sessions was reduced by 7% (150 compared with 160), leading to a reduction in cost and effort of a similar magnitude (353)(354).

We didn't evaluate this approach, and therefore a classical parallel design was selected with four groups of treatment administered: rupatadine 10 mg during 5 days; rupatadine 100 mg during 5 days; placebo during 5 days; and moxifloxacin on day 1 and 5 and placebo on days 2-4.

One of the most relevant decisions in the design of our TQT study was the **selection of ECG recording procedures**. Inadequate acquisition or measurement of ECG data may lead to an incorrect assessment of the drug's ECG effects and lead to approval of a drug with unacceptable risk–benefit ratio or to inappropriate discontinuation of a promising drug during development*(25)*. To provide for a more standardized approach to ECG acquisition and measurement, the FDA–Health Canada Concept paper (179) notes that:

- The clinical ECG database should be derived primarily from the collection of standard 12-lead ECGs.
- The ECGs should be recorded and stored as a digital signal, but the assessment of intervals and the overall interpretation may be made from the digital record or from a printed record. If the analysis will be based on a paper record and the resolution for QT/QTc interval verification is within the desired range of < 5.0 ms, a paper speed of 25 ms is prefered, as higher speeds (e.g., 50 ms) may lead to distortion of low amplitude waves such as U waves.
- The QT/QTc interval should be determined as a mean value derived from at least three to five cardiac cycles (heart beats), preferably—from lead II. *"Historically, lead II has been preferred for QT/QTc interval measurements,*

as the end of the T wave is usually most clearly discerned in this lead. […] While a description of morphological changes in the T–U complex is important, a discrete U wave of small amplitude should be excluded from the QT/QTc interval measurement. If the size of the U wave and the extent of T–U overlap are such that the end of the T wave cannot be determined, inclusion of the U wave in the QT/QTc interval measurement may be necessary and should be discussed with the regulatory authority.[…] All ECG readings should be performed by a few designated cardiologists operating from a Centralized (Core) ECG laboratory who are blinded to time, treatment and patient identity" (329).

In addition to standard resting supine ECGs, new Holter technology allows for continuous ambulatory recordings of 12-lead digital ECGs for 24 h at a time. This approach should prove useful for the assessment of ECG intervals, and especially cardiac repolarization at numerous discrete time points following drug administration or at baseline, and it also has additional applications for continuous beat-to-beat analysis, as well as for detecting cardiac arrhythmias. Validation that the 12-lead ECGs recorded by Holter technology rather than a standard ECG recorder has been required to be certain that the new technology is comparable to the historic standard(25). Such a study has been conducted as has a technical validation in accordance with the 21CFR11 regulations. Title 21 CFR Part 11 is the part of the Code of Federal Regulations that establishes the FDA regulations on electronic records and electronic signatures (ERES). Part 11, defines the criteria under which electronic records and electronic signatures are considered trustworthy, reliable, and equivalent to paper record.

The study (355) compared the utility for QTc risk assessment of ECGs recorded by standard or digital 12-lead Holter devices, as well as the precision of QT and RR interval measurement by the manual digitized systems (digitizing board and digital onscreen calipers) on standard and Holter-derived ECGs. This was the first study to compare the standard 12-lead ECGs to discrete ECGs derived from continuous digital 12-lead Holter recorder for their utility in the assessment of drug induced changes in QT/QTc and RR intervals, measured on a very large number of serial ECGs (1600 simultaneously

recorded pairs of ECGs). It was also the first study to compare the precision of QT/QTc and RR interval measurement by the two manual digitized methods (digipad and onscreen calipers). The results of the study showed that the QT, QTcF, and RR data produced by digipad on standard ECGs were essentially equivalent to those from digital 12-lead Holter ECGs. Overall, despite somewhat differences in lead positions (12-lead Holter ECG has electrodes only on the torso, whereas the standard ECG also uses the limb leads) and differences in the sampling frequency of ECG recordings, this study validated the utility of digital 12-lead Holter for the assessment of QT prolongation in clinical drug research. A very relevant advantage of the 12-lead ECG Holter is that also permits ECG recordings available for retrospective analysis at critical time points after dosing that are difficult or impossible to capture by advance scheduling (maximum plasma concentration of the parent drug and active metabolites, clinical adverse events possibly related to QT prolongation). Numerous QT and RR intervals derived from continuous Holter ECGs allow for easier employment of subject-specific QT correction formulae that are more accurate and preferred versus the Fridericia's in evaluating the true drug-induced QT effect at different heart rates(25). Additionally, a standard format for the submission of annotated ECG waveforms has been developed in cooperation with the HL7 standards organization.

Thus, the standard for 12-lead ECGs as recommended should employ a validated central ECG laboratory using digital acquisition, manual digitized analysis, and processing. This eliminates the site-to-site variability and provides for a more "definitive" thorough ECG Trial.

Due to the complexity of the procedures, we asked for the expertise knowledge of eResearch Technology, Inc (eRT) and Mortara Instrument company. ERT is a private company that has been conducting intense ECG collection in Phase I since the year 2000, proven solutions for centralized cardiac safety trials. Mortara, another private company, was selected for providing us with Holters resources. It has a long-standing relationship with the FDA, providing ECG algorithm tools and data warehousing solutions to support the Agency's regulatory review of cardiac safety data. This knowledge was considered crutial due to annotated ECG datasets, supporting new drug

applications to FDA, must to be uploaded by sponsors and central laboratories into the ECG Warehouse to facilitate regulatory review. Since 2006, the FDA has been using a ECG Warehouse to review all thorough QT Trials submitted to them. The ECG Warehouse, provides tools for annotated ECG review, scoring and warehousing.

The selected device for ECG recording in our study was the H12+ Mortara Holter recorder, provides 12-lead data recorded beat-by-beat on a compact flash memory card for up to 24 hours. The time limiting memory card was the reason for the last recording time to 23 h instead 24 h usually performed in PK studies, because the recording of data began 1 hour before the drug intake, to assure the predose evaluation and then only 23 h more could be recorded.

One of the major advantages of using the 12-lead ECG Holter recorder in a thorough ECG Trial is that the clinical site is not involved in viewing the ECGs because they are recorded on a flash memory card, as commented. Thus, the cost of an investigator review and processing is eliminated. But this advantage implied also a serious concern for us. We wouldn't have current information about the ECG conditions of the volunteers during the study days. In order to avoid this lack of information, some regularly recorded "safety" ECGs were added for patient safety and were done at screening, baseline, and certain days on-treatment. These safety ECGs were not used in the formal analysis, but data are in the trial listings and available for regulatory safety review.

The procedure to record and select ECGs was the recommended by the guideline (176): from a continuous recording, select 3 replicate ECGs at each time point within a ± 5 minutes period. Replicate ECGs are much of the variability observed under stable conditions over several minutes is due to natural biological variability and how much is due to measurement error is an open question. Using the mean from several ECGs recorded over a few minutes try to reduce potential measurement error and obtain a more precise estimate of the subject's true response at a nominal time. In addition to the variability of the interval measurements as such(356), several components of the study conduct have an impact on the variability of the data(180). Experimental conditions must be strictly standardized with regard to meal

intake and composition and physical activity. To minimize heart rate fluctuations, subjects should be supinely resting for at least 10 min in an undisturbed environment at the prespecified timepoints for ECG recordings.

As commented, the use of continuous 12-lead ECG recordings (Holter's) is preferred as it allows extraction of replicate ECGs around prespecified timepoints with optimal signal-to-noise ratio. In line with this assumption, blood draws should always be done immediately after the ECG recording to avoid confounding stress and should be performed in all treatment groups even though the samples from the placebo and positive control may not be analyzed, in order to avoid awareness of treatment, which may introduce a confounding effect on the QT interval. The pharmacokinetic properties of the drug must be well characterized before the TQT study is initiated, and ECG acquisition and blood samples should encompass the anticipated Tmax of the drug and major metabolites (and of moxifloxacin) and at least one timepoint before and several timepoints after Tmax. Often, this can be achieved with 6 to 8 timepoints. This range gives a reasonable risk benefit balance, because it has to be taken into account that a high number of timepoints increase the likelihood of false-positive results. To avoid alterations of autonomic tone, which also has an impact the QTc interval, it is important to avoid timepoints at which subjects may be sleeping, i.e., nighttime.

Even so, due to pharmacokinetics of rupatadine, we consider relevant to also include some late timepoints, during the night and until almost 24 h after dosing, to capture possible delayed effects including hERG trafficking, as other authors reveal (357)(358)(359)(360).

Averaging replicates of ECG recordings from each timepoint is now standard as it reduces the variability of the QT measurement and therefore increases the power to exclude small QTc effects. With semiautomated methods of ECG measurement, where the computer-based measurements are "overread," i.e., adjusted manually, there are several data sets that demonstrate that the reduction of variability is pronounced when averaging up to triplicates and then levels off (361). Further reduction in variability with >3 replications is marginal (362). Most ECG laboratories today use triplicate ECG recordings at each timepoint (67 studies used 3 replicates (triplicates), 5 studies used 4

replicates, 5 used 5 replicates, and 2 used 10 replicates, respectively) (see Annex 2).

To date, there are still differences between ECG laboratories in measurement of the QT interval in thorough QTc studies. Our study utilized a semi-automatic method to evaluate the length of the QT interval in subjects, whereby a skilled analyst makes manual adjustments to points placed by an automatic algorithm. A recent study by Tyl et al (363) compared the data generated using the semi-automated method, and found that the semi-automated method was associated with less QT variability than the fully automated method; thus providing further support for use of the semi-automatic method to measure QT as applied in this study.

To maintain consistency in QT measurement, and to prevent reader bias, as the guide recommends, a centralized ECG reading lab (eRT) was used to read the ECGs with interpretation by a high-resolution manual on-screen caliper method with annotations to minimize inter-reader variability, all perfomed with stringent quality control processes to maintain high standards of ECG interpretation. The central ECG laboratory was blinded to subject treatment. The intervals to measure were RR, PR QRS and QT, 3 beats each one using the II lead as primary lecture and V5 lead as a supportive lecture. As standard derived values heart rate, QTcB and QTcF were also reported. It was used a patented method for the analysis of 12 lead ECG for clinical trials called CalECG, an application designed for the on-screen measurement of ECG signals in the context of pharmaceutical trials. It is capable to handle digital ECGs, seamlessly interfaced with several types of formats. If an interval duration measurement failed into any of the prespecified range, then the ECG was automatically forwarded to the cardiologist for review and evaluation. This approach is aligned with ICH recommendations about the use of fully manual or manual adjudication approaches for clinical trials in which the assessment of ECG safety is an important objective, such as the TQT study (329).

Moreover, we included another methodology to assure a accurate evaluation of the QT interval: the use of an individually corrected formulae to measure the QT interval in order to avoid bias induced by heart rate variations that

other calculations, Bazzet or Fridericia don't allow(364). We consider this approach necessary because histamine exerts a series of actions upon the cardiovascular system that could modify heart rate with the consequent repercussion in QTc calculations. Thus, through mediation of the H1 and H2 receptors, histamine increases vascular permeability and induces hypotension, with reflex tachycardia. Although there was no consistent evidence that rupatadine modifies heart rate, we selected as measurement method the individual corrected QT calculation, that potentially avoid the bias of heart rate in the calculations. The measures for minimizing heart rate variations included other additional procedures as obtaining the blood sample 5 min after the ECG recording.

Hence, the individual correction formulas are preferred by the regulatory authorities (176).There are, at least two correction individualized methods: individual corrected QT (QTcI), corrected via an exponential function, and QTcIL, that uses a linear function. QTcI was chosen by us as the primary clinical endpoint over other correction methods not only because it uses individualized values but also because it corrects for heart rate changes via an exponential function and will provide corrected QT values that can exhibit a normal distribution.

The formulae selected by this calculation, QTcI, was determined by taking each individual and calculating that individual's exponent that best eliminates the influence of heart rate on QT duration. This requires at least 35 to 50 or more ECGs on placebo/baseline in which these ECGs encompass a range (usually 50-80 beats per minute) of spontaneous heart rate changes to have enough power to accomplish this task. The correction of QT interval for HR used the following formulae: $QTcI = QT/RR^b$, where RR is the interval between adjacent QRS complexes and b is the patient-specific correction factor. All the individual QT and RR interval pairs collected on day -1 were used to calculate the correction factor b for QTcI. QTcI was thus selected to be the primary endpoint in the clinical trial protocol, and therefore we report QTcI data as the primary endpoint data herein. QTcF and QTcB data were provided as secondary endpoints and also for historical reasons. The limitations of

Bazett's QT correction (QTcB) are widely acknowledged, since this algorithm overcorrects the QT interval with increasing heart rate, thereby producing a false-positive QTc prolongation, as we commented previously. Consequently, it is no longer a requirement to report this interval for TQT studies (329). For drugs without clear effect on the heart rate, it has been the experience of many sponsors, that QTcF works well (351). For these drugs, there does not seem to be much of an advantage to use a subject-specific QTcI derived from supinely resting drug-free data only, which is the standard way of generating QTcI and often results in a correction factor near 0.33 (i.e., very similar to Fridericia, QTcF). Furthermore, the derivation of QTcI is sometimes used to justify an additional full baseline day in crossover-designed studies, which is difficult to defend when there is no added value of using this correction method. Drugs with an inherent, substantial (e.g., more than 8 bpm peak effect) heart rate effect supose much more of a challenge, and there is no firm guidance. The Cardiac Safety Research Consortium (http://www.cardiac-safety.org/) recently issued a white paper on this topic, which discussed five alternative ways for QT assessment of drugs with a heart rate effect. Methods include "Holter-bin" (338)(365)(366), QTcI derived from a broad range of QT/RR pairs through continuous Holter recordings at baseline, beat-to-beat analysis (367)(368), PK/PD modeling with heart rate as a covariate (369), and assessment of the QT interval at a fixed heart rate through, e.g., submaximal exercise (198)(370). The advantages and disadvantages of the methods are discussed, but there is a lack of comparative data across methods. A shared feature of all methods is that baseline QT/RR pairs must be collected from a sufficiently broad range of heart rates, which covers the ranges seen post-dosing with the drug (329). It was also recently suggested by Dr. Joanne Zhang, that the variability around the correction factor for the slope estimate should also be taken into account when analyzing the change from baseline placebo corrected QTc (350). Obviously, there would be a penalty in terms of wider CIs if the slope is derived from a limited data set according to standard practice and no penalty at all if the choice was to use QTcF, which uses a fixed correction factor of 0.33. Since QTcF is regarded as not fully reliable for drugs with a heart rate effect (314), the bottom line is however to use much

richer data sets with a broad range of heart rates for the calculation of an "optimized" QTcI. The accuracy of obtaining the best point estimate of an individual's ECG interval at baseline (pre-treatment) is a factor that critically influences the observed variability in the QTc interval effects in a trial. The recommendation is that baseline ECGs should be computed as the mean of multiple ECGs to enhance the precision of the measurement in light of the large degree of spontaneous variability in QTc duration. Regulatory guidance is to collect drug-free ECGs on two or three different days to help document inter-day variability in the baseline. Baseline ECGs should be collected at similar times of the day to minimize the possible effects of diurnal fluctuation and food. In addition, posture and activity levels at the time of the ECGs should be standardized to the extent possible for all recording periods. When these concepts are applied to the TQT study, especially with the desirability to define individual correction formulae for QT to QTc, careful attention to the number of ECGs used to define the baseline and the treatment effects is critical. The best time points for ECG selection to best characterize the *baseline* is constructed to be very similar to a standard set of time points used to define the pharmacokinetic profile of the new drug.

For parallel-designed TQT studies, a full baseline day is the most widely used approach and change from baseline QTc (ΔQTc) is then calculated by comparing the QTc value for each timepoint at baseline and post-dosing ("time-matched") or the averaging of all values from a full baseline day versus mean and/or maximum values post-dosing ("time-averaged"). There are some data suggesting that results would be the same and the variability lower if baseline was generated through averaging of all values from a full baseline day (371).

It is recognized that drugs that prolong the mean QT/QTc interval by about 5 ms do not appear to cause TdP. Data on drugs that prolong the mean QT/QTc interval by >5 and <20 ms are inconclusive. In such cases, the clinical relevance of these modest changes may be best determined by careful examination of the clinical trial and postmarketing adverse events possibly related to QT/QTc interval prolongation, such as TdP, cardiac arrest, sudden cardiac death, and ventricular arrhythmias (e.g., ventricular tachycardia and

ventricular fibrillation). Drugs that have an average QT/QTc interval prolongation of >20 ms have an increased likelihood of being proarrhythmic. A basis of these interpretation guidelines is the survey of mean peak QTc prolongation by several drugs(372). Because of this, the firsts steps of the guideline specified that: "a negative thorough study is one where the largest difference between the drug and placebo (baseline substracted) for the QTc interval is around 5 ms or less, with a one-sided 95% confidence interval that excludes an effect >8 ms"

But, in the last version of CHMP/ICH/2/04(176), it was established that the main analysis must be based on the one side 95% CI should exclude a 10 ms effect. This amendment introduced more related changes as the modification in the evaluation of QTc effect using the time average method which was the historically accepted before the finalization of E14 in May 2005 when the time matched analysis was defined as the primary method (172). The publication of the final guidance version during the clinical trial conduction implied a major amendment to the original protocol design. This amendment was refered to the update of the ICHE14 guideline on May 2005. When the protocol was designed, the selected QT analysis was based on the previous bibliography and on the drafts of the guideline (172).

Then, the initial analysis was based on 5-milisecond QTc effect with a one side 95%CI that excludes an 8 ms effect at the maximum QT/QTc increase from baseline. It means for each subject, determine the largest time-averaged increase from baseline, defined as substracting time-averaged baseline (one single value as mean of all baseline measurements) from the mean of maximum measurements on treatment (one single value). This definition yields an assessment of the worst potential for adverse outcome when assessing the effect of a drug on QT/QTc intervals. However, this is also a disadvantage as it may yield upwardly biased results.

Due to this, and according with the last guidance recommendations, a relevant amendment was submmited in order to select the time matched method as measurement for the analysis. The time matched method is defined as substracting hour X at baseline from hour X on treatment day to

obtain a change from baseline for each time point for each volunteer. Then, the primary analysis of QTc has been based on individually corrected QT in each timepoint (QTcIX). Treatment effects on QTcIX have been assessed using the largest time-matched difference between the drug and placebo (baseline substracted) for the QTc interval. A negative TQT study is considered if the largest time-matched difference between the drug and placebo (baseline-substracted) is around 5 ms or less, with a one-sided 95% confidence interval that excludes an effect >10 ms. In the prospective analysis based on the draft ICH guidelines (172) the greatest and the mean changes from baseline of QTc were considered.The final ICH guideline(176) uses the wording 'largest time-matched mean effect', which may be interpreted as analyzing all time points separately; it would have to be ruled out at each of these time points.The target here is the greatest of several time-matched treatment differences, rather than the treatment difference of the greatest change from timematched baseline.

Some other studies were also affected by the new guidance publication. Vandemeulebroecke et al based the study analysis upon the then current draft ICH guideline (373). During the planning and conduct of this study the new version was issued. For this reason, a hierarchical evaluation was chosen, with thresholds of clinical significance set at 7.5 and 5 ms. In a study designed today, only the latter value would be considered relevant. Poordad et al designed a trial according to the ICH E14 draft Step 2 guidance (178), where a limit of 8 ms was specified for the upper1-sided 95% CI. Dall the same, data analyses were carried out to reflect an upper limit of 10 ms, as specified in ICH E14 Step 5(176). While this study was ICH E14 compliant, additional evaluations outside the ICH E14 requirements but recommended by the ICH E14 Questions & Answers were also performed to further assess study sensitivity and risk associated with QTc decrease(199).

The final guideline additionally recommends one-sided testing instead of the two-sided testing implemented in this study; however, the two-sided test is more rigorous, and therefore its use does not compromise the conclusion of the study.

These modifications didn't affect to the estimated sample size, or the logistics of study performance. The sample size of the TQT study is defined by the requirement of having enough power to detect a 5 ms QT effect (change from baseline) with a power of 80% and an α of 0,05. A key determination of sample size is the variance of the QTc that can be assumed to be >8 ms if >36 ECGs are obtained at baseline and on-treatment. Sample size calculation would thus be in the range of 30-40 subjects per arm. More over, we included that half of which should be women, because they could to have increased sensitivity to drug-induced QTc effects, as commented previously (25).

We collaborate closely to experts in order to define the adequate timepoints for **pharmacokinetic analysis**, taking account not only the parent compound but also its main active metabolites. Generally, to adequately characterize a new drug its dose–response or concentration response relationship for QTc prolongation should be provided using a sufficient number of ECGs, paired with the highest levels of drug concentration that may occur in the target population under the potential maximum extent of exposure. For all clinical trial phases, collection of plasma samples around the time of the ECG measurement is encouraged so as to permit an exploration of these relationships. The TQT study provides an opportunity to further characterize this relationship because the use of the supratherapeutic dose will at least add to or often provide the highest concentration of drug by which to evaluate ECG interval changes. Important considerations in characterizing the dose– or concentration–response relationship include: the maximal degree of the QTc prolongation; the steepness of the slope between QTc prolongation and dose–concentration; and the relationship between the threshold dose for QTc prolongation and the therapeutic dose range, linearity or nonlinearity of the dose–concentration-effect dependency, and the time course of QTc prolongation in relation to plasma levels. For selection of these timepoints the pharmacokinetics of moxifloxacin and rupatadine were taking accunt. According previous data, moxifloxacin presents its Tmax aprox 4 hours post dose, rupatadine 0,75 - 1 h post dose, and its active metabolites BCP (UR-12790) at 1,5h and BCP-OH (UR-12788) at 5 h. Elimination half-life following

multiple daily rupatadine dosage at 10 mg/day was approximately 6-8 hours for rupatadine; and 27-33 hours and 35-41 hours, respectively, for the active metabolites UR-12790 and UR-12788.
All treatment arms in the TQT study were subjected to the same experimental conditions. If a QTc effect of the agent would be identified, then analyzing all paired ECG-plasma samples of drug could be used to identify perhaps the lowest drug concentration associated with a QTc effect (25).

The primary **pharmacokinetic-pharmacodynamic (PK/PD) analysis** explored the relationship between changes from baseline in QTcIX and plasma concentrations of rupatadine. The concentration response to modeling QTc data is an important component of a totality of evidence assessment of the risk of QT prolongation. Understantding the concentration QT relationship may also assist the interpretation of equivocal data. If the positive control does not have its usual amplitude or time course, exposure response analysis of the data can provide reassurance that the effects seen are similar to other studies, after correcting for any confounding factors. If the results for the study drug would be ambiguous (e.g, QT prolongation at lower dose, but not prolongation at higher dose or QTc at a single isolated point), exposure response analysis could be helpful to interpret the data.

The **outlier or categorical analysis** is defined as determining what percentage of the patients on each treatment show a change from baseline in QTc that is of particular magnitude that identify them to be at potencial risk because of the QTc effect. This is done by taking the largest positive change from baseline value for each ECG interval at whatever time point on-treatment that this occurs (take the mean of the 3 ECGs used around that time point to establish that point's ECG interval value). A specific clinical criterion is a new >500 ms QTc duration or the observation on drug of an abnormal T-U wave often thought to represent an early after-depolarization that may be a presage of TdP. Statistically, a specific change in QTc of >60 ms from baseline in an individua is considered as a specific outlier criterion. An often too-sensitive (too many subjects on placebo will show this effect) criterion is a

30- to 60- ms change from baseline. The literature review shows that if the mean shows no effect on cardiac repolarization in a definitive QT trial, no signal is showed in the outlier analysis (25)(225). Nevertheless, the mean change from baseline placebo corrected values “hides” outliers, and thus, a categorical or outlier analysis is critical. Moreover, some authors suggest that an approach similar to a time average analysis could be also valid to look for outliers. This is done by looking at the largest positive change from baseline on-treatment at any time point (average the ECGs around that time point for better time point precision) in each subject and then calculates the mean maximum change for all subjects in each treatment arm. This process would select the time points on treatment and define the change from baseline for each of these time points by sustracting the ECG interval data obtained at the matched time point at baseline. Each resultant treatment time point change from baseline would be placebo corrected and a 95% confidence interval established. If any time point demonstrated a >8 ms change in QTc duration at the upper CI, the drug under investigation would be declared as having an effect on cardiac repolarization.

As specified in the protocol, the primary analysis was the time average method, which was the historically accepted method before the finalization of E14 in May 2005 when the time matched analysis was finalized as the primary method. The protocol was powered using the previous literature based on the time-averaged method at 40 subjects per group with 3 ECGs around each time point. Using this method there was no signal of any change in QTcI from baseline when placebo corrected for either the clinical dose or the supratherapeutic dose of rupatadine. The upper confidence interval for QTcI was 0 and 0 ms for the clinical dose and -1 and -1 ms for the 100 mg supratherapeutic dose of rupatadine after one dose and steady state respectively.

To be certain that this negative result was not a false negative the use of a concomitant positive control group (studied in exactly the same manner as the other treatment arms) demonstrated the expected placebo corrected

change from baseline at single dose of 6 ms and 5 ms on day 5 (upper confidence intervals were 6 and 4 ms respectively).

To explore the time matched analysis as now recommended by E14, each of the 13 baseline compared to single dose and then baseline compared to steady state dose time points were examined. Moxifloxacin again showed the expected result on day 1 and again on day 5 (each a single dose) with a 90% upper confidence interval (see tables) peaking about 1-4 hours after drug administration at a level of 6-17 ms. The graphs show that the mean response was as expected around 10 ms. Hence, the positive control performed as expected except that at hours 16-23 when no QTc change should have been noted the QTcI increased back to around a 10 ms mean with an upper confidence limit of 14 ms at hour 16. This change on the last 3 time points probably represents a spurious finding due to small sample size for this kind of analysis, change in subject activity, or unknown factors.

In most studies, moxifloxacin has caused a larger peak effect than 5 ms, more in the range of 8-15 ms. The differences in peak response across studies is however quite striking and ranges between 7.5 ± 2.2 ms (315) and 19.0 ± 3.9 ms (316) in studies that used a single oral dose of 400 mg moxifloxacin. Even, there are some examples of relatively recently published studies in which the criteria for moxifloxacin assay sensitivity that were not met are shown. In example, a study which, the peak effect of ΔΔQTc after moxifloxacin (a single oral dose of 400 mg) was comparable with other studies, but the precision of the ΔΔQTc estimate was poor, which resulted in very wide limits of the 90% CI with the lower bound below 5 ms at all timepoints. It's estimated than the assay sensitivity test with moxifloxacin has failed in about 5% of cases, but for majority of the studies, the confidence in the data is very high.

Using the placebo controlled change from baseline upper confidence intervals, the first dose for Rupatadine at 10 or 100 mg showed an effect which was always <9 ms and hence a negative response as shown in the time averaged analysis. For day 5, likewise all time points up to 14 hours had a response <10 ms for the 10 mg dose and <6 ms for the 100 mg dose of rupatadine except that at hour 16 an 11 ms change was noted for the 10 mg

and a 11 upper bound change for the 100 mg dose. At only 1 time point (hour 16) rupatadine at day 5 had a 11 ms upper bound for the 10 mg dose and a 11 ms upper bound for the 100 mg supratherapeutic dose group with a 4 and 4 ms upper bound at hour 12 respectively and 4 and 4 at hour 20 suggesting a spurious hour 16 set of data (1 hour spurious out of 52 assays, 13 time points looked at four times-single dose, steady state, 10 and 100 mg doses). The cause for this spurious effect may well be that the maximum negative change on placebo occurred at this hour since the QTc change from baseline on Rupatadine was still a reduction from baseline. This single time point of 13 time points for single and again 13 time points for multiple doses to steady state showing a >10 ms effect is assuredly spurious. It may be speculated that a difference in the subjects' autonomous balance (eg nervousness) may have been a contributing factor towards higher mean QTc values on the night. It has been reported that anxiety is associated with imbalance of the autonomic system (326). Placebo spontaneous variation in QTc is common and may be accounted for simply by environmental conditions such as food or activity or emotional levels.

The validity of the trial was demonstrated by the fact that the moxifloxacin positive control group demonstrated the expected small change in QTc duration and that the placebo group's change from baseline was within 1-2 ms for QTcI demonstrating that the spontaneous factors for QTc change were controlled. Nevertheless the moxifloxacin response using time matched analysis was exactly as expected except for the day 5 hours 14 to 23 (4 of 13 time points) during which moxifloxacin had an increase in QTc change vs. a continuing decrease as expected.

There was no effect on heart rate at the clinical dose of Rupatadine but at the supratherapeutic dose of 100 mg (10x) a 5-10 bpm increase was observed. Hence, this heart rate increase for the supratherapeutic does of rupatadine requires that to define the cardiac repolarization effect of this agent the best correction method that eliminates the marked influence of heart rate on QT duration be employed (QtcI).

As recommended by the ICH E14 guideline (176) information on changes in T and U wave morphology were provided. Using the specific criterion of a new >60 ms change in baseline of QTcI duration or a new >500 ms or a new abnormal U wave, no outliers were identified. The nonspecific criterion of a 30-60 ms change from baseline in QTcI duration revealed no evidence of any outliers in addition compared to placebo whereas there were more outliers as expected on moxifloxacin (the positive control) compared to placebo. There were no abnormalities in U-wave morphology or other treatment-emergent ECG abnormalities or ECG morphology findings associated with the administration of rupatadine at a therapeutic or supratherapeutic dose.
The results of this thorough ECG Trial showed no signal of any effect on AV conduction, depolarization or cardiac repolarization as measured by the PR, QRS or QTc interval durations. No changes in cardiac morphology were identified of any significance.

The TQT study is formally powered to exclude a small (around 5 ms) QTc prolongation. The variability of other ECG parameters (such as the PR and QRS intervals) are in fact lower than for the QTc interval, and it has become increasingly apparent that these studies also can and should be used for assessment of other ECG effects. These data, unfortunately, are not always given in publications on TQT studies, which makes it difficult to independently evaluate the QTc effect, or lack thereof. As an example, there is no mention of effects on heart rate, PR, or QRS interval in the publication on the TQT study with liraglutide (294) and no such effects are mentioned in the US prescribing information. In contrast, in the Health Canada Summary Basis of Decision, it is described that liraglutide at therapeutic doses causes a sustained increase in heart rate and prolongation of the PR interval. The incidence of subjects with heart rate values greater than 90 bpm was 20 % for 1.2 mg and 24 % for 1.8 mg liraglutide, as compared to 8 % and 4 % on the respective day for placebo. A peak placebo- and baseline-adjusted PR prolongation of 9–10 ms was seen. A PR prolongation of 7 % ms (maximum increase) was also observed in the TQT with the subcutaneous (SC) formulation of exenatide (299). The clinical relevance of these small increases

in the PR interval can be debated but warrants further evaluation in terms of the incidence of high-degree AV block in late phase studies in the targeted patient population.

The Cmax and AUC values observed for the supratherapeutic dose of rupatadine in this study were approximately greater, than the values reported by coadministration with ketoconazole and azhitromycin. The supratherapeutic dose of rupatadine used in the present study thus provided greater systemic concentration of rupatadine for QTc evaluation than those likely to be observed during routine clinical use of rupatadine.

The relationship between the change in QTcI from baseline to plasma concentration of parent and metabolites of rupatadine demonstrated no signal of any positive effect to suggest that this antihistamine effects cardiac repolarization. The pharmacokinetic profile of the major metabolites was taking account in the study design. Because the timing of the ECG monitoring was consistent with the t½ value of both rupatadine and the metabolites, it can be assumed that any contribution of the metabolite to QTc prolongation was adequately captured in this study. The E–R analysis has become more widely utilized and has been adopted as an essential component in the approval and labeling decision of new drug candidates (329)(374). This modeling and simulation approach can quantitatively describe the drug-related effects on the QT interval for all types of QT studies and can predict the potential risk of QT prolongation under various treatment conditions or unexpected exposures (eg, drug overdoses)(329). E–R analysis was conducted based on rupatadine data from days 1 and 5 in this study.

No major safety concerns were identified with the therapeutic or supratherapeutic dose of rupatadine used in this study. Clinically significant findings on ECGs observed by the investigator were reported as adverse events, whereas those observed post-hoc by the cardiologist at the central laboratory were included in the statistical analysis but not reported as adverse events.

The AE profile observed in the current trial is consistent with the known antihistaminic pharmacology of rupatadine on HR, BP and sedation. The use of supra-therapeutic doses of rupatadine in addition to the study conditions likely contributed to the high incidence of some AEs observed, especially sedation. As expected, an increase in adverse events was observed with the supratherapeutic dose (100 mg) of rupatadine; however, most AEs were mild in severity and resolved without treatment. Very few subjects exhibited an SBP, DBP, or pulse rate that met outlier criteria, even at supra-therapeutic doses of rupatadine. The incidence of morphological changes in the current study was consistent with the reported values seen in healthy subjects without apparent heart disease, suggesting that they may not have been due to study drug. In addition, no degree atrioventricular (AV) block was observed across different treatment groups..

The current study, based on the most up-to-date requirements of the regulatory authorities, shows that rupatadine at therapeutic and supratherapeutic dosages did not demonstrate any effects on QTc duration which would indicate that it has proarrhythmic potential. In the updated ICH E14 guideline (176) a negative result "is one in which the upper bound of the 95% one-sided CI for the largest time-matched mean effect of the drug on QTc interval excludes 10 ms". In this clinical trial 25 of 26 timepoints for both rupatadine 10 mg/day and 100 mg/day after either a single dose or following 5 days treatment, met this criteria while 18 of 26 timepoints for moxifloxacin (a positive control known to increase QTc duration) failed this test. Furthermore the one timepoint on which both doses of rupatadine increased the upper bound 95% CI for the change in QTcI by more than 10 ms was almost certainly spurious since it coincided with the largest negative change in QTcI value for placebo group, while the rupatadine QTcI value *per se* was lower than the recorded at baseline (day 0). Placebo spontaneous variation in QTc is common and may be accounted for simply by environmental conditions such as food or activity or emotional levels. Moreover, at hour 20 when the QTc value for placebo returned to usual levels, the upper bound 90% CI for rupatadine was 4 for both the therapeutic and supratherapeutic doses.

Then, we consider this result as a misleading finding. Firstly, for time-matched data in the therapeutic rupatadine group only 1 time-point exceeded the 5ms mean and 10 ms 90% CI limit, and neither time-point corresponded to the tmax for rupatadine. Secondly, rupatadine had no effect on QTcF interval at the supratherapeutic dose of 100 mg (the mean and 90% CI limits for placebo-adjusted change from baseline in QTcF were not exceeded at any time-point). As we will be discussed in detail later categorical analysis also showed that treatment did not prolong PR or QRS intervals, and there was also a lack of drug exposure-response.

In addition to relatively small changes in placebo-corrected QTcI interval values and a negative "Thorough QT/QTc Study" results, no pharmacokinetic-pharmacodynamic relationship with either rupatadine or its main metabolites was observed, no gender effects occurred, and there was no clinically relevant imbalance of ECG waveform outliers during the study.
There was no effect on AV conduction, depolarization or cardiac repolarization as measured by the PR, QRS or heart rate at the clinical dose of rupatadine but at the supratherapeutic dose of 100 mg (10x) a 5-10 bpm increase was observed. No changes in cardiac morphology were identified of any significance.

This study demonstrates the lack of effect of rupatadine on prolongation of the QTc interval and was conducted in accordance with current regulatory guidelines for evaluating the potential of new drugs to prolong the QTc interval; however, several limitations of this study should be noted. The sample size for this trial was 40 subjects per treatment which was defined by the time average analysis plan during the formulation of the trial's design. This is somewhat small for the time matched analysis approach, a statistical method that was not contemplated until after the trial was underway (Protocol final version, September 2004). Nevertheless the moxifloxacin response using time matched analysis was exactly as expected except for the day 5 hours 14 to 23 (4 of 13 time points) during which moxifloxacin had an increase in QTc change vs. a continuing decrease as expected. Another possible weakness of our study

is that the healthy subject pool was restricted to adults between 18 and 45 years of age. This is recommended by the guidance due to safety reasons and because the use of healthy subjects in a TQT study, such as this one, reduces variablitiy in the measurement of QTc intervals. But this inclusion criterion implies a restrictive labelling in the technical information of the drug and doesn't give a contundent answer for patients with comorbidities associated to proarrhytmic risk. Besides, a closer monitoring of the rest conditions during the timepoints would be also desirable in order to avoid a higher variability in the ECG adquisition. Another controversial point, could be the inclusion of an arm receiving a therapeutic dose, taking account that some authors consider it unethical because healthy volunteers are included in an arm of treatment that not provides additional information to the group of the supratherapeutic dose.

A literature search was conducted to assess whether there was any evidence of repolarization-related cardiac events with rupatadine in the wider patient population. Terms used in the search included rupatadine, cardiac conduction, arrhythmia, and torsades de pointes. There are two reports on the cardiac effects of rupatadine (375)(376). In the first, the authors state that data from Spain show a statistical association between rupatadine use and heart rhythm disturbances(375). This assertion seems confusing since the lower limit of the 95% CI for rupatadine Reporting Odds Ratio (ROR) contains the value 1. Besides, we have recalculated the 95% CI for rupatadine ROR and it yielded 0.97-10.48. The ROR for levocetirizine, desloratadine, loratadine and ebastine are indeed statistically significant, the first two being higher than that for rupatadine. Also, the ROR, as a disproportionality measure, is simply one way of selecting drug-ADR combinations that may be interesting for clinical review and, more importantly, ROR does not confer a causal relationship.

The second one, is a brief letter to the editor in with the authors referred a case of torsade de pointes and associate with rupatadine (376). However, it was answered by means a letter of editor in the same journal (377), where it was specified important clarifications regarding his letter. The patient had been receiving sertraline therapy for 6 previous months before and he was studied

by a syncope episode previously. Furthermore, the patient had been shown some ECGs with prolonged QTc, in 2001 and 2003.

We believe that the possible relationship of cardiac effects of rupatadine has not been accurately demonstrated in both papers. The analysis of the cases it is partial and incomplete, given that in these cases other aspects were involved, such as concomitant treatment, previous familiar history of two prolonged QTC, etc. Nevertheless, a continual and rigorous post-marketing pharmacovigilance system is mandatory for all authorized drugs due to the relevance of the data obtained in the clinical practice.

Similar negative TQT results were reported for other second generation antihistamines like levocetirizine, in a trial designed to comply with a "Thorough QT/QTc Study" criteria (378). This trial differed from the present one in that it employed a crossover design with 52 healthy volunteers and only assessed single doses of the antihistamine being the supratherapeutic dose only six times higher than the therapeutic one.

Another TQT study with bilastine has been also recently published showing some similarities with our design (303). The effect of bilastine on cardiac repolarization was studied in 30 healthy participants during a multiple-dose, triple dummy, crossover, thorough QT study that included 5 arms: placebo, active control (400 mg moxifloxacin), bilastine at therapeutic and supratherapeutic doses (20 mg and 100 mg once daily, respectively), and bilastine 20 mg administered with ketoconazole 400 mg. Time-matched, triplicate electrocardiograms (ECGs) were recorded with 13 time points extracted predose and 16 extracted over 72 hours post day 4 dosing. Four QT/RR corrections were implemented: QTcB; QTcF; a linear individual correction (QTcNi), the primary correction; and a nonlinear one (QTcNnl). In this study, moxifloxacin was associated with a significant increase in QTcNi at all time points between 1 and 12 hours, inclusively. Bilastine administration at 20 mg and 100 mg had no clinically significant impact on QTc (maximum increase in QTcNi, 5.02 ms; upper confidence limit [UCL] of the 1-sided, 95% confidence interval, 7.87 ms). Concomitant administration of ketoconazole and bilastine 20 mg induced a clinically relevant increase in QTc (maximum increase in QTcNi,

9.3 ms; UCL, 12.16 ms). Authors conclude that this result was most likely related to the cardiac effect of ketoconazole because for all time points, bilastine plasma concentrations were lower than those observed following the supratherapeutic dose.

In any case, there is a consistency in the clinical data and the 3 studies which provide a level of comfort regarding the lack of cardiotoxicity with these newer second-generation antihistamines. A clinical trial with ebastine was also conducted but before the implementation of the ICH E14 guidance, and the analysis was therefore not performed in the same way as in the current study(300). Chaikin et al (327) describes two drug interaction studies using the same design with antihistamines, ebastine and loratadine, both CYP 3A4 substrates, and ketoconazole. Both studies were of parallel design and one treatment group (n = 26 and n = 30) received ketoconazole 400 mg daily plus placebo for 8 days. On the last day of dosing, the mean change-from-baseline QTcI was 6.96 ms (95% CI: 3.31–10.62) and 7.52 ms (95% CI: 4.15–10.89) in the ebastine and loratadine study, respectively.

Therefore, the accurate clinical evaluation on the arrhythmogenic potential has become mandatory before marketing authorisation (i.e., Thorough QT study—TQT) since 2005; but, so far, though, these studies have only been conducted for three antihistamines, namely bilastine, levocetirizine and rupatadine, and in all cases provided negative results (297). In addition, for rupatadine, the pooled analysis of patients with allergic rhinitis or crhonical idiopatic urticarial doesn't show any concern about QT interval prolongation or torsade de pointes. Pharmacokinetics and safety findings were consistent with previous studies of rupatadine in healthy volunteers and patients with allergic rhinitis and chronic idiopathic urticaria.

Thus, the findings of the "Thorough QT/QTc Study" with rupatadine at therapeutic and supratherapeutic dosages administered for 5 days are in accordance with its reported extensive preclinical and clinical experience, and indicate that it has no proarrhythmic potential and hence no cardiac safety concerns.

Existing ICH E14 regulatory guidance has been very successful. No drugs introduced into the marketplace have been removed from market for reasons of TdP induction since the implementation of E14 ten years ago. Nevertheless, regulatory approaches to evaluation of the drugs that prolong QT have been criticized on the basis of the undeniably inadequate correlation between QT interval and induction of TdP(297)(379).

It is true that not all drugs that induce TdP prolong QT to an equivalent degree, and drugs that do prolong QT interval to an equivalent degree do not always carry equivalent risk of TdP; the fact remains, however, that TdP, the ultimate clinical outcome that matters, is invariably preceded by QT prolongation.

Determining the parameters of success of regulatory decisions for withdrawing problem drugs is a difficult task. It could include any one or more of the following: expected lives saved due to market withdrawal of the drug; increased reporting of QT-prolonging medicines due to better awareness of this unique problem in clinical practice; pharmacovigilance system better able to catch more related cases and elucidate the mechanisms of action; decrease in the time interval from approval to detection of increased QT length and then to market withdrawal.

Whilst these are highly desirable parameters, they are often impractical or not feasible. Therefore, the most obvious and simplest approach would be to examine how many non-cardiac drugs have been withdrawn from the market in relation to the measure enacted. No new non-cardiac drug approved after the implementation of the CPMP 'Points to Consider' strategy in 1998 has been withdrawn from the market.

A slightly different approach to evaluating the success of the strategies implemented might be to consider the number of spontaneous reports of TdP received by the regulatory authorities. Although one cannot deny the limitations of the systems of spontaneous reporting, the trends identified by the FDA AERS database are perhaps less encouraging. Although TdP continues

to be reported, the rate of reporting seems to have stabilized after about 2001 – just about the time when cisapride was withdrawn from the market. This residual reporting probably reflects the continued use of other non-cardiac QT-prolonging drugs that remain on the market due to their favorable benefit-risk profile. Moreover, this may also reflect better awareness of drug-induced TdP.

On the other hand, some drugs appear positive in TQT studies, but present little proarrhythmic risk either because the true effect is small or because there are stabilizing effects on inward ionic currents. Unnecessarily restrictive labelling can result in denial of the drug to potential beneficiaries. For obvious reasons, there is no information on this aspect of the response.

The response, if successful, can only be considered partially successful when one considers that a number of older torsadogens continue to remain available when safer alternatives have been developed. Perhaps the most worrying aspect that emerges from analysing the success of the aforementioned regulatory strategies is how many otherwise valuable drugs may have been terminated from development because of the sponsors' concerns regarding the drugs' perceived proarrhythmic risk, regulatory approaches to their evaluation, approvability and prescribing restrictions. In the aftermath of the implementation of the regulatory response, the findings communicated by one major pharmaceutical company were most worrying (379). The company revealed that resulting directly from the implementation of the strategy in the CPMP 'Points to Consider' document, 11 new chemical entities were found, over the 18-month period to November 1999, to have an effect on QT – representing an attrition rate of 10%. None of these compounds were intended to have an effect on ion channels. These 11 were a range of therapeutic and chemical classes. Hanson and Bass et al. (380) have also reported similar concerns – owing to fears that a drug will have a QT effect and that this will result in significant drug development challenges and regulatory hurdles, many companies are stopping at earlier stages the

development of new chemical entities that have a non-clinical signal suggesting QT liability.

Thus, while it is true that no new drug will induce TdP if the regulatory thresholds of QT concern are set at a conservatively very low level, there are legitimate questions concerning the impact of these guidelines on the promotion of public health, an equally important goal of regulatory authorities.

The TQT study is also resource intensive(379). Given the sample sizes dictated by the above requirements coupled with the known intrinsic and extrinsic variability of QTc, it is not surprising that the cost of a TQT study ranges from US$ 2 to 5 million(319). Bouvy et al (381)analyzed the cost-effectiveness of the ICH E14, comparing two pharmacoeconomic scenarios: the health effects and costs resulting form implementing ICH E14 vs not implementing. The incremental cost-efectiveness ratios of regulation vs no regulation were aprox €2,4 million per sudden death prevented and aprox €187,000 per quality-adjusted life year (QALY) gained in users of antipsychotic drugs. The main driver of cost was the requirement fro electrocardiogram (ECG) monitoring of users of QTc-prolonging drugs. Even when several of the assumptions in the model were varied, there were no results in favor or regulation.

Almost 10 years have now elapsed since the implementation of the ICH E14 guidance in May 2005, and several hundreds of TQT studies that basically follow the E14 guidance have been performed and submitted to regulatory authorities. As of October 2012, the FDA's IRT had evaluated 288 TQT studies.

Based on the high confidence in data derived from the TQT study, it will be challenging to replace it with "early QT assessment," and the process, if successful, will likely include several steps. Generation of more prospective data to demonstrate that "early QT assessment" can provide results concordant to the results of TQT studies will be required, and alternative methods for demonstrating assay sensitivity will have to be successfully tested. It is the E14 "threshold of concern" (<10 ms) on which the confidence

that a drug with a negative TQT study is truly devoid of proarrhythmic liability in patients is based. However difficult to prove, it is generally accepted that the TQT study has been very effective in terms of protecting patients by identifying "QT liability" for new drugs (382), with consequent regulatory actions (precautionary statements, black box warnings, restricted access and withdrawals). It seems highly unlikely that a different threshold will be widely accepted across regions without substantial further advancement of our knowledge of the relationship between mild QT prolongation and its consequences in large populations. The same threshold should therefore be used for "early QT assessment" based on ER analysis, i.e., the upper bound of the 2-sided 90% CI of the QTc estimate should be lower than 10 ms at concentrations that are relevantly high for the targeted patient population. The TQT study will not be replaced with "early QT assessment" overnight, and it seems unlikely that ICH E14 will be revised until a sufficient amount of data have convinced all participating parties that alternative approaches can provide data at the same level of confidence as the TQT study. Replacing the TQT study will therefore probably be a stepwise, staggered approach, in which the request for a TQT study may be waived for some compounds with certain characteristics, while others will have to undergo a TQT study. Examples of the former may include compounds from a pharmacological class known to have no members with QT liability, a clean nonclinical safety pharmacology package, and robustly negative ER analysis of SAD/MAD data with the upper bound of the 2-sided 90 % CI of the projected QTc effect below 10 ms at concentrations that are relevant for the targeted patient population. Other drugs, such as those with a small underlying effect or where "early QT assessment" has not provided a sufficiently precise estimate of the QT effect, would still require an E14-compliant TQT study(297).

7. CONCLUSIONS

The results of this Thorough ECG Trial showed no signal of any relevant effect of rupatadine on QTc interval duration.

At only 1 time point rupatadine at day 5 had a 11 ms upper bound for the 10 mg dose and a 11 ms upper bound for the 100 mg supratherapeutic dose group with a 4 and 4 ms upper bound at hour 12 respectively and 4 and 4 at hour 20 suggesting a spurious hour 16 set of data. This result is considered spurious due to placebo spontaneous variations in QTc.

The lack of any PK/ PD relationship signal, gender effect, or changes in outliers also supports the conclusion that there is no real signal that rupatadine changed QTc duration.

There was not detected any serious or unexpected adverse event during the study. No patients had clinically abnormal physical examination at final visit.

The validity of the trial was demonstrated by the fact that the moxifloxacin positive control group demonstrated the expected small change in QTc duration and that the placebo group's change from baseline was within 1-2 ms for QTcI demonstrating that the spontaneous factors for QTc change were controlled.

The preclinical and clinical cardiac safety data of rupatadine demonstrated no signal of a likely effect of this drug on cardiac repolarization. This Thorough ECG Trial confirms that rupatadine does not have any ECG effects and hence raises no cardiac safety concerns.

8. EPILOGUE

The thorough QT (TQT) study has been a key component of the clinical evaluation of the propensity of new drugs to cause QTc prolongation since the adoption of the International Conference of Harmonisation (ICH) E14 clinical guidance document in May 2005. The request to study each new drug in a specifically designed study in healthy subjects if justifiable from a tolerability and safety perspective, and otherwise in the target patient population, was triggered by a number of drug withdrawals in the 1990s for arrhythmias associated with QT prolongation (297).

The TQT study has been successful in terms of detecting drugs with a QT effect and thereby avoiding the introduction of new drugs with an unknown QT liability to the market. However, this has had its price; based on a conservatively chosen threshold (10 ms) and the requirement that the QT effect is evaluated separately at each post-dosing timepoint, without consideration of the pharmacology of the drug, the study is overly sensitive and has therefore resulted in a number of 'false' positives, i.e., drugs are labeled as QT prolongers without a demonstrated underlying proarrhythmic risk(211). If data could be generated with the same level of confidence from other studies routinely performed as part of clinical development, this would represent a more efficient approach, with other potential advantages, such as improved understanding of any liabilities early in clinical development. Furthermore, the cost of this security has been high. Although estimates differ, usual TQT studies are expensive (177)(379)(383).

Another major weakness of using QTc prolongation to assess risk of TdP is that it does not directly address the most critical issue: is the drug actually proarrhythmic? A significant increase in the QTc is sensitive, but not highly specific for the development of TdP. Given this issue, the Cardiac Safety Research Consortium (CSRC), in conjunction with the Health and Environmental Sciences Institute (HESI) and the FDA held a Think Tank on July 23, 2013 at the FDA to critically discuss a new paradigm to directly evaluate the potential for a drug to be proarrhythmic. The focus would be on

nonclinical proarrhythmic assays and the goal would be to reduce the premature termination of drugs that effect hERG or increase the QTc but do not appear to be proarrhythmic. This would effectively move the bulk of proarrhythmia signal detection to the discovery phase, where the assays could potentially play a role in candidate selection, and obviate the TQT. Specific efforts are concentrated on assessing the effect of a drug on a platform of ion channels using in silico techniques10 to assess the proarrhythmic potential: the Comprehensive In Vitro Proarrhytmia Assay (CiPA), in which the proclivity to develop early after depolarizations and enhanced susceptibility of ventricular depolarization during the repolarization phase are being studied. To asses overall proarrhytmic risk, CiPA relies upon (a) characterization of electrophysiological effects of evolving or existing drugs on multiple human cardiac currents measured in heterologous expression systems, whose electrophysiological effects will then be integrated in silico by computer models reconstructing human cellular ventricular electrophysiology, and (b) confirmation of the electrophysiological effects in a myocyte assay such as human induced pluripotent stem cell–derived cardiomyocytes (382)(383).

Evaluations of hemodynamic and electrocardiographic (ECG) effects from standard nonclinical cardiovascular in vivo studies (as described in ICH S7A (162) and S7B (165)) will remain part of the new paradigm, along with careful ECG assessment in phase 1 studies to evaluate a drug's effects on ECG intervals (QTc, PR, and QRS durations), atrioventricular conduction, and heart rate. These later studies would confirm that there were no unanticipated clinical ECG changes as compared with the nonclinical testing; if unanticipated changes are found, the reasons for the discrepancy would need to be understood. With this new paradigm in place, the ICH S7B guideline defining hERG as the primary ion channel of focus for proarrhythmia would need to be revised, and the Thorough QT (TQT) study described in ICH E14 guidelines (176).

It will be critical to determine whether there are findings in humans that were not anticipated based on the nonclinical assays (and thus the mechanism would need to be understood) as well as the effects of a drug on other important ECG variables such as atrioventricular nodal conduction, ventricular

conduction, heart rate, and possibly T-wave morphology. Other approach is based on the exposure-QT (ER) relationship. The 'first-in-human' studies [single ascending dose (SAD) and multiple ascending doses (MAD)] seem well suited for this purpose because achieved plasma levels of the parent compound and abundant metabolites often substantially exceed therapeutic levels later observed in patients. Provided serial ECG assessment and pharmacokinetic sampling are incorporated into the design, SAD and MAD studies represent an opportunity to generate ECG data with the same high quality as the TQT study. Several doses of the investigational drug are typically administered to small cohorts with only six to eight subjects receiving active drug (and often only two per cohort receiving placebo), and the power to exclude small effects in a 'by timepoint' analysis for each dose group as in the TQT study is therefore unacceptably low (222). If, on the other hand, exposure-response (ER) analysis is employed, all data across a wide range of plasma concentrations of the drug are used, and the power to detect and exclude small QT effects would be substantially improved (181).

The experience with ER analysis of ECG data has increased over the last decade, among both regulators and sponsors. The US FDA Interdisciplinary Review Team (IRT) for QT studies was formed shortly after the adoption of the ICH E14 document and has since provided sponsors with consistent advice on the design and analysis of TQT studies and has independently reviewed and analyzed close to 400 TQT studies to date(383). ER analysis has become an integral part of the IRT review of data from QT assessment studies and has proven invaluable in terms of enhancing the confidence in characterizing drug induced QTc prolongation (181)(384). ER analysis is now routinely used to predict the QT effect in the targeted patient population, including clinical scenarios with doses and formulations not directly evaluated in the TQT study and QT effects in specific populations and under certain conditions (e.g., drug interactions) with increased exposure of the drug. Extensive experience with QT-prolonging drugs demonstrate that the effect on the QT interval is directly related to plasma levels of the drug or main metabolites, with few exceptions (e.g., QT prolongation inhibition of hERG protein trafficking, which is delayed in relation to peak plasma levels). In our view, it therefore makes sense to

focus on QT effects in relation to plasma concentration of the drug, rather than by timepoint without consideration of the pharmacology of the drug, and a wider role for ER analysis in the assessment of drug-induced ECG effects seems justified (382)(383).

Even though the experience of many pharmaceutical sponsors from the application of ER analysis on data from first-in-human studies is favorable, publicly available reports are relatively scarce. Based on discussions with the FDA, research collaboration was therefore initiated between the Clinical Pharmacology Leadership Group of the Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) and the Cardiac Safety Research Consortium (CSRC) with the intention of conducting a prospective study to evaluate whether ER analysis applied to ECG data from a small SAD-like clinical pharmacology study could serve as an alternative to the TQT study (385).

Six marketed drugs with well-characterized QTc effects were identified in discussions with FDA; five have caused QT prolongation above the threshold of regulatory concern ondansetron, quinine, dolasetron, moxifloxacin, and dofetilide. The 5 QT-positive drugs were chosen in discussions with FDA and selection criteria include the toxicity profile allowing administration to healthy subjects, lack of substantial heart rate effect and the degree of QTc prolongation. The sixth drug selected was a negative drug, levocetirizine. The study was a 3-period, third-party blinded, randomized, placebo–controlled study in 20 healthy volunteers conducted in a design similar to a single ascending dose (SAD) Phase 1 study with the primary objective to estimate the effect of the drugs on the QTc interval using ER analysis. Two doses (low and high) of each drug were given on separate, consecutive days to 9 subjects. Six subjects received placebo. Data were analyzed using linear mixed-effects ER models. The primary analysis will be based on an ER analysis of the relationship between drug plasma concentrations and QTcF. The primary variable was change-from-baseline QTcF, and adjustment for placebo and circadian variability will be done within the ER model. Criteria for QT-positive drugs was the demonstration of an upper bound (UB) of the 2–sided 90% confidence interval (CI) of the projected QTc effect at the peak plasma

level of the lower dose above the threshold of regulatory concern (currently 10 ms) and a positive slope of ER relationship. The criterion for QT-negative drug was an UB of the CI of the projected QTc effect of the higher dose <10 ms. Replicate 12-lead ECGs were extracted from continuous recordings pre-dose and serially after dosing and paired with drug concentration determinations. The ER criteria for the identification of a QT effect, a statistically significant positive ER slope and an effect above 10 ms, were met with all five positive drugs, and an effect exceeding 10 ms could be excluded at the supratherapeutic dose of the negative drug, levocetirizine. The study results thereby provided evidence to support that careful QT assessment in early phase clinical studies can be used as an alternative to the thorough QT study (382)(383)(385).

Initiatives such as ER assessments and the new preclinical paradigm could conceivably move the bulk of proarrhythmia assessment to the discovery phase. It has the potential to make drug development more efficient and significantly reduce the number of cases in which there is a need for the TQT study.

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