# Levocetirizine does not prolong the QT/QTc interval in healthy subjects: results from a thorough QT study 

Réginald Hulhoven • Dominique Rosillon -<br>Michel Letiexhe • Marie-Anne Meeus • Agnès Daoust • Armel Stockis

Received: 15 May 2007 / Accepted: 5 August 2007 / Published online: 21 September 2007
(C) Springer-Verlag 2007


#### Abstract

Objective To conduct a thorough QT study of levocetirizine, a non-sedating antihistamine, in accordance with International Conference on Harmonisation (ICH) E14 guidance. Methods The study was designed as a single-dose, placebo and positive-controlled, four-way crossover, randomised trial in which 52 healthy male and female subjects participated. Levocetirizine ( 5 and 30 mg ) and placebo were administered double-blind, and the positive control, moxifloxacin (400 mg ), was open-label. Electrocardiograms (ECGs) were obtained by continuous Holter monitoring at various time points (three per time point) during a 24-h period at baseline and after each treatment. The ECGs were read centrally in a blinded manner. QT intervals were corrected for heart rate using a gender- and study-specific correction ( $\mathrm{QT}_{\mathrm{cSS}}$ ) and Fridericia's correction $\left(\mathrm{QT}_{\mathrm{cF}}\right)$. The largest QTc timematched and baseline-subtracted difference between each active drug and the placebo (largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ ) was derived from a mixed-effect analysis of variance. Results The one-sided $95 \%$ upper limits of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ for levocetirizine were $5.7 \mathrm{~ms}(5 \mathrm{mg})$ and 3.9 $\mathrm{ms}(30 \mathrm{mg})$, with mean estimates of 2.9 and 1.1 ms , respectively. Similar results were obtained for the $\Delta \Delta \mathrm{QT}_{\mathrm{cF}}$ data. Statistically, moxifloxacin significantly lengthened the $\mathrm{QT}_{\mathrm{cSS}}$, with a one-sided $95 \%$ lower limit of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{css}}$ of 10.5 ms and a mean estimate of 13.4 ms . There

\footnotetext{ R. Hulhoven ( $\triangle$ ) • D. Rosillon $\cdot$ M.-A. Meeus • A. Daoust $\cdot$ A. Stockis

Clinical Pharmacology, UCB Pharma SA, Chemin du Foriest 1420, Braine-l'Alleud, Belgium e-mail: reginald.hulhoven@ucb-group.com }

\section*{M. Letiexhe}

Clinical Pharmacology, University Hospital Centre, Liège, Belgium


#### Abstract

was no relationship between the measured $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and the plasma concentration of levocetirizine, whereas a statistically significant linear relationship was observed with the plasma concentration of moxifloxacin [slope estimate 0.004 $\mathrm{ms} /(\mathrm{ng} / \mathrm{mL}) ; 95 \%$ confidence interval: 0.003-0.005]. Conclusions Overall, the results of this thorough QT study indicate that the methodology of the trial was valid and sensitive enough to demonstrate the absence of effect of levocetirizine at both therapeutic ( 5 mg ) and supratherapeutic ( 30 mg ) doses on cardiac repolarisation.


Keyword ICHE14 guideline • Levocetirizine •
QT/QTc interval • Thorough QT study

## Introduction

Levocetirizine is an antihistamine with a high affinity and selectivity for histamine type $1\left(\mathrm{H}_{1}\right)$ receptors [1, 2]. It is approved in many countries for the treatment of seasonal allergic rhinitis, perennial allergic rhinitis and chronic idiopathic urticaria in adults and children at a daily dose of 2.5 or 5 mg . The pharmacokinetics of levocetirizine is time- and dose-independent, with an elimination half-life of 7.6 h . It is bound to plasma proteins ( $95 \%$ ), and its distribution is restrictive $\left(\mathrm{V}_{\mathrm{z}}=0.4 \mathrm{l} / \mathrm{kg}\right)$ [3]. It is excreted mainly unchanged in urine, with less than $14 \%$ of the dose metabolised in the liver [4], and the metabolism does not exhibit genetic polymorphism. No chiral inversion occurs during absorption and elimination [3, 4].

Levocetirizine is the R-enantiomer of cetirizine. In accordance with pre-clinical data, reports on adverse drug effects compiled in international databases indicate that cetirizine has rarely been associated with cardiac events. Levocetirizine is expected to have a similar safety profile [5]. In confirma-
tion of this expectation, post-marketing surveys of levocetirizine (UCB, data on file) have reported only very rare cases of cardiac disorders, i.e., palpitations.

Regulatory concerns regarding the potential for nonantiarrhythmic drugs to prolong the QT interval, leading to potentially fatal ventricular tachycardias, including torsades de pointes (TdP), have resulted in the adoption, in October 2005, of the International Conference on Harmonisation (ICH) E14 Guidance on the clinical evaluation of the QT/ QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs [6]. Several commentary and review papers discussing the approach have been published, both prior to $[7,8]$ and following the adoption of the ICH E14 guideline [9-12], although there are few published examples of such studies. We report here the results of a thorough QT study carried out for levocetirizine.

## Subjects and methods

The trial was carried out in accordance with both the ICH notes for Guidance on Good Clinical Practice, the principles that have their origin in the Declaration of Helsinki, and applicable laws and regulations. The approval of the Ethics Committees of the University Hospital Centre, Liège was obtained before starting the study.

The study was a single-dose, placebo and positive controlled, four-way crossover, randomised design. Fiftytwo healthy male and female subjects participated in the study. $\mathrm{QT}_{\mathrm{cB}}$ values on the screening electrocardiogram (ECG) had to be $>300 \mathrm{~ms}$ for both genders, and $<450 \mathrm{~ms}$ for males and $<470 \mathrm{~ms}$ for females. After giving their written, informed consent, the subjects were randomly assigned to receive one sequence of four treatments according to a foursequence William's design for a four-way Latin square.

Sample size calculation was based on an intra-subject standard deviation of 10 ms for the primary endpoint and was computed for a $90 \%$ power using $5 \%$ one-sided tests. For the moxifloxacin versus placebo comparison, it was assumed that the real effect on $\mathrm{QT}_{\mathrm{c}}$ is a prolongation of 6 ms . A sample size of 49 subjects was required to show that the result is significantly different from zero. For the comparison of levocetirizine against placebo, it was assumed that there could be a small prolongation of 4 ms . A sample size of 49 subjects was required to show that this effect is significantly smaller than 10 ms . To achieve a balanced design and to allow for a few dropouts, 52 subjects were ask to participate in the study.

Three treatments were double-blind - levocetirizine 5 mg , levocetirizine 30 mg and placebo - and one treatment was open-label - moxifloxacin 400 mg . The supratherapeutic dose was set at 30 mg levocetirizine, which corresponds to sixfold the therapeutic dose. The equivalent
dose of 60 mg cetirizine is also the highest dose ever administered in healthy subjects [13].

Each of the four periods was 3 days in duration (4 days for the first period) and was separated by a 7 -day washout period. On Day -1 , a fixed baseline was recorded for all subsequent post-dose recordings. The dosing of Period 1 occurred on Day 1, the dosing of Period 2, on Day 8, the dosing of Period 3, on Day 15 and the dosing of Period 4, on Day 22. Subjects reported to the clinical unit in the evening before the baseline day and the evening before the dosing day of Periods $2-4$, respectively. During and across the periods, meals, fluid intake and the surrounding environment remained as controlled and consistent as possible.

Continuous Holter monitoring was recorded at baseline (Day -1 from -24 h pre-dose up to pre-dose in Period 1) and from pre-dose up to 24 h post-dose during each treatment period using a 12-Lead digital ECG Holter device (Mortara H12+; Mortara Instruments, Milwaukee, WI). ECG recorder flash cards were sent to the central laboratory (eResearchTechnology, Philadelphia, PA) for a treatmentblinded high-resolution measurement of the cardiac intervals, and morphological assessment was by a central cardiologist blinded to the study treatment. The data files were uploaded in the central ECG laboratory's validated data management system, ExPERT, and interval duration measurements were first obtained by trained analysts using the proprietary validated electronic calliper system applied on a computer screen. The software determined the end of the T wave using the down-slope tangent method for calculating the regression tangent. Standard lead was D2. Another lead was selected if the D2 T wave could not be accurately measured. A cardiologist then verified the interval durations and performed the morphology analysis, being careful to note any $\mathrm{T}-\mathrm{U}$ wave complex that suggested an abnormal form compatible with an effect on cardiac repolarisation.

Three ECGs, each lasting approximately 10 s , were extracted from the Holter data over a $5-\mathrm{min}$ period (the interval between single ECGs was at least 1 min ) and within approximately 5 min of the specified time-point. These readings were made at pre-dose and at 30 min and 1 , 1.5, 2, 4, 6, 9, 12 and 24 h post-dose. All ECGs of one and the same subject were analysed by the same cardiologist. The analyst and the cardiologist were blinded to subject and period identification.

Blood samples for PK analysis were obtained in each study treatment period at pre-dose, 30 min and at $1,1.5,2$, $4,6,9,12$ and 24 h post-dose for the determination of plasma concentrations of levocetirizine and moxifloxacin. The blood samples for pharmacokinetic (PK) analysis were drawn after the three ECG recordings and analysed using validated methods. Subjects rested for at least 15 min before the PK time-points when the individual 10-s
segments of data were extracted from the Holter 12-lead digital ECG continuous recordings. Breakfast was fat-free, and the diet in general was low fat during the confinement period, other than the fasting periods.

Plasma concentrations of levocetirizine were determined by high-performance liquid chromatography (HPLC) with tandem mass spectrometry ( $\mathrm{ms} / \mathrm{ms}$ ). The limit of quantification was $0.2 \mathrm{ng} / \mathrm{ml}$ [14]. Plasma concentrations of moxifloxacin were determined by HPLC-ms $/ \mathrm{ms}$. The limit of quantification was $1 \mathrm{ng} / \mathrm{ml}$ (Parexel International Bioanalytical Laboratories, Poitiers, France). Peak concentration $\mathrm{C}_{\text {max }}$ ), time to peak $\left(\mathrm{t}_{\max }\right)$ and the area under the curve $\left(\operatorname{AUC}_{(0-24} \mathrm{h}\right)$ ) were calculated from the individual plasma concentration versus time profiles using a non-compartmental model for levocetirizine and moxifloxacin with the software winnonlin ver. 4.2 (Pharsight Corp, Cary, NC).

The QT interval was corrected for the heart rate using a gender-specific, study-specific correction: $\mathrm{QT}_{\mathrm{cSS}}=\mathrm{QT} /$ $R R^{\alpha}$. The objective was to establish the QT-RR relationship in the same range of heart rate as that found during the active drug treatment. For this reason, the subjects were requested to rest for at least 5 min when the ECG was being recorded during both baseline and drug treatment periods. For each subject, a non-linear (log/log, using natural logs) regression of QT versus RR was run, using the 60 values corresponding to the individual ECGs from the baseline day and the placebo treatment day. The correction coefficient $(\alpha)$ was derived as the mean of the regression coefficients for all subjects of the same gender. The correction was applied to each of the three single QT measurements for each time-point. In addition, the QT was corrected using Fridericia's correction $\left(\mathrm{QT}_{\mathrm{cF}}=\mathrm{QT} / \mathrm{RR}^{0.33}\right)$. The $\mathrm{QT}_{\mathrm{cSS}}$ and $\mathrm{QT}_{\mathrm{cF}}$ values used for subsequent analyses were computed as the mean of the three $\mathrm{QT}_{\mathrm{cSS}}$ and $\mathrm{QT}_{\mathrm{cF}}$ values for each time-point.

The primary analysis was performed on the timematched, baseline-subtracted $\mathrm{QT}_{\mathrm{cSS}}\left(\Delta \mathrm{QT}_{\mathrm{cSS}}\right)$ using a mixed-model analysis of variance (ANOVA) with repeated measurements. The model included treatment, period, gender and post-dose time as fixed effects, the subject as a random effect nested under gender, the pre-dose value as a covariate and the treatment-by-time and period-by-time as interactions. Model-adjusted means were derived for each treatment at each post-dose time as well as two-sided $90 \%$ confidence intervals (CIs; equivalent to a $95 \%$ one-sided CI) of the differences between each active treatment and placebo at each post-dose time ( $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ ). The absence of effects of levocetirizine on cardiac repolarisation was concluded if the upper limit of the $95 \%$ one-sided CI for maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ between levocetirizine and placebo was less than 10 ms and if the lower limit of the $95 \%$ onesided CI for maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cSs}}$ between moxifloxacin and placebo was greater than 0 ms (sensitivity criterion).

The same approach was adopted for the analysis of $\mathrm{QT}_{\mathrm{cF}}$. The time-matched difference from baseline for $\mathrm{QT}_{\mathrm{cSS}}$ at subject-specific $\mathrm{t}_{\max }$ was also analysed using a similar mixed-effect model ANOVA by study drug ( 5 and 30 mg levocetirizine and 400 mg moxifloxacin).

The relationships between $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and plasma concentration of levocetirizine and moxifloxacin were fitted using a linear regression.

All statistical calculations were performed using the SAS software release 8.2 (SAS Institute, Cary, NC).

## Results

A total of 52 healthy subjects participated in this study (28 men, 24 women; 50 Caucasian, 1 Black and 1 Asian/Pacific islander), with mean (SD; range) age of 31.55 (6.85; 18.446.0) years. One subject had a major protocol deviation in that the Holter recording in Period 3 only started at 6 h post-dose. As a result the subject was excluded from the per-protocol (PP) population but was included in the intention-to-treat (ITT) population.

The correction coefficient, as determined by the nonlinear $(\log / \log )$ regression of QT versus RR, was 0.31 for the women subjects and 0.22 for the men. This is comparable to the correction coefficient for calculating $\mathrm{QT}_{\mathrm{cF}}$ of 0.33 . The $\mathrm{QT}_{\mathrm{cSS}}$ and the $\mathrm{QT}_{\mathrm{cF}}$ did not show any apparent correlation with $R R$, demonstrating an adequate correction with both methods. The average $\mathrm{QT}_{\mathrm{cSs}}$ profile (Fig. 1) was similar following treatment with either levocetirizine dose or placebo, with average values approximately 5 ms higher during the first 2 h after administration as compared to the time-matched baseline. In contrast, the average profile after moxifloxacin administration exhibited


Fig. 1 Mean $\mathrm{QT}_{\mathrm{cSS}}$ interval for the 24-h period at baseline $(\diamond)$ and following treatment with placebo ( $\Delta$ ), 5 mg levocetirizine ( 0 ), 30 mg levocetirizine (ㅁ) and 400 mg moxifloxacin (
a clear increase, with a peak at 4 h post-dose and values close to pre-dose, but they were higher than the baseline and placebo levels during the last 12 h . There was evidence of a postprandial shortening [15] of $\mathrm{QT}_{\mathrm{cSS}}$ at 6 h post-dose for all treatment periods and at baseline. Similar patterns were observed for $\mathrm{QT}_{\mathrm{cF}}$ and QT , although the postprandial shortening was more marked for QT. A lengthening after moxifloxacin was observed for all QT/QTc intervals. The mean $\mathrm{QT}_{\mathrm{cF}}$ and $\mathrm{QT}_{\mathrm{cSS}}$ changes from baseline were virtually identical after both levocetirizine doses and placebo, with an increase during the first 2 h post-dose, reaching a peak value of approximately 5 ms . Corresponding profiles following moxifloxacin administration reached a maximum increase from baseline of 17 ms at 2 h post-dose. Figure 2 shows the mean difference of time-matched changes from baseline between each active treatment and placebo $\left(\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}\right)$ at each time-point. The one-sided $95 \%$ upper limit of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ (Table 1) was lower than 10 ms for both levocetirizine doses ( $5 \mathrm{mg}: 5.7 \mathrm{~ms} ; 30 \mathrm{mg}$ : 3.9 ms ), and the mean estimates were lower than $5 \mathrm{~ms}(2.9$ and 1.1 ms , respectively). Similar results were obtained for the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cF}}$. The one-sided $95 \%$ lower limit of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ of the positive control treatment, 400 mg moxifloxacin, was noticeably higher than $0 \mathrm{~ms}(10.5 \mathrm{~ms})$, and the mean estimate was higher than $5 \mathrm{~ms}(13.4 \mathrm{~ms})$, indicating a statistically significant lengthening of $\mathrm{QT}_{\mathrm{cSs}}$ following moxifloxacin administration (Table 1). Moreover, the moxifloxacin effect appeared as soon as 0.5 h after administration and was maintained until 24 h post-dose. Similar results were obtained for the $\mathrm{QT}_{\mathrm{cF}}$ analyses. As there was a statistically significant interaction ( $p=0.011$ ) between treatment effect and gender, separate exploratory analyses per gender were performed for the $\Delta \mathrm{QT}_{\mathrm{cSs}}$.


Fig. 2 Mean placebo time-matched difference of change from baseline $\left(\Delta \Delta \mathrm{QT}_{\mathrm{cSs}}\right)$ following treatment with 5 mg levocetirizine (○), 30 mg levocetirizine (ㅁ) and 400 mg moxifloxacin (©)

Despite the low number of subjects in each subgroup (PP population 27 males and 24 females), the one-sided $95 \%$ upper limit of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ was lower than 10 ms for both levocetirizine doses and both genders (males 5 mg , $9.21 \mathrm{~ms} ; 30 \mathrm{mg}, 7.48 \mathrm{~ms}$; females $5 \mathrm{mg}, 8.07 \mathrm{~ms} ; 30 \mathrm{mg}$, 5.45 ms ). In addition, the one-sided $95 \%$ lower limit of the largest $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ of moxifloxacin was higher than 0 ms for both genders (males 8.98 ms ; females 9.58 ms ). The timematched difference from baseline for $\mathrm{QT}_{\mathrm{cSS}}$ at the subjectspecific $t_{\text {max }}$ was not statistically significant different between both levocetirizine doses and placebo. The difference between placebo and moxifloxacin was statistically significant, with a mean $(90 \% \mathrm{CI})$ difference of 8.5 (5.3-11.6) ms.

The categorical analysis of QT and QTc did not reveal any value higher than 500 ms for QT, or any value higher than 480 ms for $\mathrm{QT}_{\mathrm{cSS}}$ or $\mathrm{QT}_{\mathrm{cF}}$. There was no case of increase from baseline higher than 60 ms with any treatment. There was one case of tachycardia (change from baseline $>25 \%$ and $\mathrm{HR}>100 \mathrm{bpm}$ ) after 5 mg levocetirizine and four cases of bradycardia (change $<-25 \%$ and HR $<50 \mathrm{bpm}$ ): one after 30 mg LCTZ, one after 400 mg moxifloxacin and two after placebo. One subject exhibited an increased PR interval (change $>25 \%$ and $\mathrm{PR}>200 \mathrm{~ms}$ ) during both levocetirizine treatment periods. All of these events were occasional (observed at a single or two timepoints) and not treatment-related. No case of increased QRS interval was recorded with any treatment, and no consistent morphological changes were observed with any treatment.

The mean concentration versus time profiles of levocetirizine were similar to those reported previously [4]. The levocetirizine mean $( \pm \mathrm{SD}) \mathrm{C}_{\text {max }}(5 \mathrm{mg}: 228 \pm 97 \mathrm{ng} / \mathrm{ml}$, $30 \mathrm{mg}: 1302 \pm 372 \mathrm{ng} / \mathrm{ml}$ ) and $\mathrm{AUC}_{0-24 \mathrm{~h}}(5 \mathrm{mg}: 1.660 \pm$ $340 \mathrm{ng} \cdot \mathrm{h} / \mathrm{ml}, 30 \mathrm{mg}: 9987 \pm 2341 \mathrm{ng} \cdot \mathrm{h} / \mathrm{ml}$ ) were clearly dose-proportional ( $30 / 5 \mathrm{mg}$ ratio of means $=5.7$ and 6.0 , respectively). The median $\mathrm{t}_{\max }$ was identical for both doses, with a value of 1 h . The mean concentration versus time profiles and PK parameters of moxifloxacin were similar to those reported in the literature [16-18]. The range of individual $\mathrm{t}_{\text {max }}$ values was narrow $(0.5-4.0 \mathrm{~h})$, demonstrating the adequacy of the scheduled time-points for ECG recording.

The regression linear model (Fig. 3), $\Delta \mathrm{QT}_{\mathrm{cSS}}=\alpha+\beta \times$ Concentration, gave the following estimates of intercept and slope for levocetirizine: $1.47 \mathrm{~ms}(95 \% \mathrm{CI}:-0.45 ; 3.40)$ and $0.002 \mathrm{~ms} /(\mathrm{ng} / \mathrm{ml})$ ( $95 \% \mathrm{CI}:-0.001 ; 0.006)$. Both coefficients were not statistically different from zero. The predicted $\Delta \mathrm{QT} \mathrm{cSS}_{\mathrm{cS}}$ at the average measured $\mathrm{C}_{\text {max }}$ is 1.9 ms and 4.1 ms for the therapeutic ( 5 mg ) and the supra-therapeutic ( 30 mg ) dose of levocetirizine, respectively, which are below the threshold value of 5 ms . The same linear model for moxifloxacin gave intercept and slope estimates of 2.80 ms ( $95 \%$ CI $0.31 ; 5.28$ ) and $0.004 \mathrm{~ms} /(\mathrm{ng} / \mathrm{ml})(95 \% \mathrm{Cl} 0.003-$

Table 1 Summary statistics of the largest time-matched difference between active treatment and placebo: maximum differences between each active treatment and placebo at each post-dose time $\left(\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}\right)$

| Treatment | Post-dose time (h) | Estimate | Two-sided $90 \%$ confidence intervals ${ }^{\text {a }}$ |  |
| :---: | :---: | :---: | :---: | :---: |
| 5 mg levocetirizine | 24 | 2.86 | 0.02 | 5.70 |
| 30 mg levocetirizine | 24 | 1.06 | -1.78 | 3.90 |
| 400 mg moxifloxacin | 4 | 13.37 | 10.53 | 16.21 |

${ }^{\text {a }}$ Equivalent to a one-sided $95 \%$ confidence limit. The upper limit for levocetirizine and the lower limit for moxifloxacin are given in bold
$0.005)$, respectively. The predicted $\Delta \mathrm{QT}_{\mathrm{cSS}}$ at the average measured $\mathrm{C}_{\text {max }}$ is 15.6 ms , which is clearly above the threshold value of 10 ms and close to the mean observed $\Delta \mathrm{QT}_{\mathrm{cSS}}$ at $\mathrm{t}_{\text {max }}(14.5 \mathrm{~ms})$.

$\Delta \mathrm{QT}_{\mathrm{cSS}}=2.80+0.004$. Conc for moxifloxacin $(\mathrm{p}<0.0001)$
Fig. 3 Plot of individual $\mathrm{QT}_{\mathrm{cSs}}$ change from baseline versus plasma concentrations at corresponding time-points for 5 mg (o) and 30 mg levocetirizine (ㅁ) (a) and for moxifloxacin (b)

## Discussion

The E14 guidance provides recommendations to developers of new drugs with systemic availability or for existing drugs with new indications, new doses, new route of administration or new patient population in terms of the study design, conduct, analysis and interpretation of clinical studies that are conducted to screen for any pro-arrhythmic potential. The guidelines recognise that the degree of QT prolongation is an imperfect biomarker for proarrhythmic risk, but there is a qualitative relationship between QT prolongation and the risk of TdP, especially for drugs that substantially increase the QT interval. The QT interval has an inverse relationship to heart rate and is therefore corrected by means of various formulae to a less heart rate-dependent variable, denoted as the QTc interval. The guideline calls for a "thorough QT study" to be conducted to determine whether the drug has a threshold pharmacologic effect on cardiac repolarisation, as detected by QT/ QTc prolongation. Terfenadine, another non-sedating antihistamine, has been shown to delay ventricular repolarisation and to prolong the QTc [19]. This effect is enhanced when terfenadine is co-administered with cytochrome P450 inhibitors (such as macrolide antibiotics, grapefruit juice), due to the inhibition of terfenadine metabolism and the subsequent increase in plasma levels [20]. Another antihistamine, astemizole, has also been shown to have a proarrhythmic potential and, in particular, to have the ability to induce potentially fatal ventricular arrhythmias, including TdP [21]. As a consequence of these changes, both drugs were withdrawn from the market in several countries.

The study reported here with levocetirizine was based closely on the E14 guideline. As levocetirizine has a short half-life, a crossover design was employed with a baseline day and a positive control. As each subject received placebo treatment, a measure of the variability in heart rate was obtained and, hence, a study-specific, gender-specific correlation coefficient was determined and used to correct the QT values obtained. This correction was used for the primary effect variable, alongside a universally accepted correlation coefficient, as implemented in Fridericia's correction of QT. The adequacy of the correction was assessed by plotting the corrected values against RR values,
the slopes of which were close to zero. Moxifloxacin, a quinolone antibiotic, was selected as the positive control, as it has been demonstrated to prolong cardiac repolarisation. It does have marketing approval, obtained based on a higher benefit-to-risk ratio compared to terfenadine. Again, in accordance with the guidance, all ECGs were read at a central ECG reading laboratory to ensure as accurate interval measurements as possible were made. Increased robustness was achieved by an individual reader providing measurements from all ECGs from a given subject, blinded to both time and treatment. The inter-reader differences (mean $\pm \mathrm{SD} ; 95 \% \mathrm{CI}$ ) for the QT interval readings were $7.2 \pm$ 7.5 ms ; 6.2-8.1 $(n=231)$.

Fifty-two subjects were included in this study. Only one subject was excluded from the PP population due to missing Holter ECG recording during the first 6 h following the administration of placebo. To reduce the intra-subject variability, triplicate ECGs at 1- to 2-min intervals are usually recommended at each time-point [9, 22]; a further reduction in variability is only marginal with more than three replicates [23]). The correction coefficients for the study and gender-specific correction of QT $\left(\mathrm{QT}_{\mathrm{cSS}}\right)$, was 0.31 for females and 0.22 for males. This compares well to the coefficient of 0.33 used to calculate $\mathrm{QT}_{\mathrm{cF}}$. With both methods, no apparent correlation persisted between $\mathrm{QT}_{\mathrm{c}}$ and RR. A single baseline at Day -1 was used for all treatment periods for the categorical analysis of the change to baseline. Baseline was not repeated before each treatment period because of no clear recommendation in the ICH-E14 guidelines and no consensus among the experts at the time of the protocol development [9].

The $\mathrm{QT}_{\mathrm{cSS}}$ change from time-matched baseline ( $\Delta \mathrm{QT}_{\mathrm{cSS}}$ ) did not show statistically significant differences between either of the levocetirizine doses and placebo, whereas moxifloxacin yielded a significantly higher change from baseline than placebo. The main end-point, maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$, derived from the mixed effect ANOVA, yielded point estimates lower than 5 ms for the therapeutic and supra-therapeutic doses of levocetirizine, with the one-sided $95 \%$ upper limit lower than 10 ms . The higher dose of levocetirizine may appear to result in a smaller QT prolongation than the lower dose. However, the respective confidence intervals overlap largely with one another ( $0.02-5.70$ vs. $-1.78-3.90 \mathrm{~ms}$ ), and maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ occurred 24 h after administration (compared to a median $\mathrm{t}_{\max }$ of 1 h ). These findings corroborate the absence of a dose relationship, as further illustrated in Fig. 3a and in contrast with the observations for moxifloxacin (Fig. 3b).

The effect of the positive control was clearly demonstrated; the maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cSS}}$ for moxifloxacin reached 13 ms and the one-sided $95 \%$ lower limit was higher than

0 ms (approximately 10 ms ). The results for maximum $\Delta \Delta \mathrm{QT}_{\mathrm{cF}}$ were comparable. The QTc prolongation observed with moxifloxacin is in the range of those reported in other studies [24-26]. Lengthening of $\mathrm{QT}_{\mathrm{c}}$ after moxifloxacin administration compared to placebo was observed as soon as 0.5 h after dosing and continued until at least 24 h postdose. A peak effect was observed at 4 h post-dose concomitantly with mean peak concentration. Moreover, the residual (intra-subject) variability of both $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and $\Delta \mathrm{QT}_{\mathrm{cF}}$ was markedly low (intra-subject $\mathrm{SD}=8.7$ and 9.2 ms , respectively). All of these findings demonstrate the validity and the sensitivity of the study.

A significant treatment-by-gender interaction was observed, and although the magnitude of the interaction was weak, exploratory separate analyses were performed by gender. These analyses corroborated the overall analysis. Both the sensitivity criterion and the lack of levocetirizine effect were demonstrated for males and females, separately. Secondary end-points also demonstrated the lack of levocetirizine effect on QT lengthening and the effect of 400 mg moxifloxacin. The $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and $\Delta \mathrm{QT}_{\mathrm{cF}}$ at the individual peak concentration of moxifloxacin reached on average 14 ms , whereas the corresponding values observed at the peak concentration of levocetirizine were not significantly different from $t_{\max }$ matched placebo values.

There was neither an apparent nor statistically significant relationship between the measured $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and the levocetirizine plasma concentration, and there was no suggestion of a dose- or concentration-response relationship, whereas this relationship was statistically significant for moxifloxacin. A simple linear regression between $\Delta \mathrm{QT}_{\mathrm{cSS}}$ and moxifloxacin plasma concentration allowed good prediction of the mean $\Delta \mathrm{QT}_{\mathrm{cSS}}$ value for the measured mean $\mathrm{C}_{\text {max }}$ (predicted vs. observed values: 15.6 vs. 14.5 ms ). This finding also demonstrates the sensitivity and the validity of the study.

Overall, the results of this thorough QT study carried out in 52 healthy subjects indicate that the methodology is valid and sensitive enough to demonstrate the absence of effect of levocetirizine on cardiac repolarisation at both therapeutic ( 5 mg ) and supra-therapeutic ( 30 mg ) doses. This is in contrast to the effect of two other antihistamines, terfenadine and astemizole. Additionally, as levocetirizine undergoes only minor hepatic metabolism, co-administration of levocetirizine with hepatic enzyme inhibitors is unlikely to raise plasma levocetirizine concentrations.

Acknowledgements We are grateful to Shikiko Watanabe, Javier Sawchik and Mona Mihaela Troenaru for their aid in reviewing and analysing the results of this study, and to Dr Joel Morganroth for expert advice. We declare that the study reported herein complied with the current laws of the country in which it was performed.

## References

1. Devalia JL, De Vos C, Hanotte F, Baltes E (2001) A randomized, double-blind, crossover comparison among cetirizine, levocetirizine, and UCB 28557 on histamine-induced cutaneous responses in healthy adult volunteers. Allergy 56:50-57
2. Wang DY, Hanotte F, De Vos C, Clement P (2001) Effect of cetirizine, levocetirizine, and dextrocetirizine on histamine-induced nasal response in healthy adult volunteers. Allergy 56:339-343
3. Baltes E, Coupez R, Giezek H, Voss G, Meyerhoff C, et al (2001) Absorption and disposition of levocetirizine, the eutomer of cetirizine, administered alone or as cetirizine to healthy volunteers. Fundam Clin Pharmacol 15:269-277
4. Strolin-Benedetti M, Plisnier M, Kaise J, Maier L, Baltes E, et al (2001) Absorption, distribution, metabolism and excretion of [14C] levocetirizine, the R enantiomer of cetirizine, in healthy volunteers. Eur J Clin Pharmacol 57:571-582
5. Paakkari I (2002) Cardiotoxicity of new antihistamines and cisapride (review article). Toxicol Lett 127:279-284
6. ICH Guidance (2005) Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. E14, Step 4, ICH Steering Committee
7. Morganroth J (2001) Focus on issues in measuring and interpreting changes in the QTc interval duration. Eur Heart J [Suppl 3]: K105-K111
8. Morganroth J (2004) A definitive or thorough Phase 1 QT ECG trial as a requirement for drug safety assessment. J Electrocardiol 37:25-29
9. Darpo B, Nebout T, Sager PT (2006) Clinical evaluation of QT/QTc prolongation and proarrhythmic potential for nonantiarrhythmic drugs. In: Int 9th Conf Harmonization Tech Requirements Registration Pharmaceuticals Human Use E14 Guideline. J Clin Pharmacol 46:498-507
10. Grisanti S, Morganroth J, Shah RR (2005) A practical approach to cardiac safety: Implementing ICH E14 to define cardiac safety in new drug development. Appl Clin Trials Suppl October 2005, pp 10-16
11. Patterson SD (on behalf of Pharmaceutical Research and Manufacturers of America QT Statistics Expert Team) (2005) Investigating drug-induced QT and QTc prolongation in the clinic: A review of statistical design and analysis considerations. Drug Inf J 39:243-266
12. Shah RR (2005) Drugs, QTc interval prolongation and final ICH E14 guideline: An important milestone with challenges ahead. Drug Safety 28:1009-1028
13. Sale ME, Barbey JT, Woosley RL, Edwards D, Yeh J, et al (1994) The electrocardiographic effects of cetirizine in normal subjects. Clin Pharmacol Ther 56:295-301
14. Hussein Z, Pitsiu M, Aarons L et al (2005) Retrospective population pharmacokinetics of levocetirizine in atopic children receiving cetirizine: the ETAC study. Br J Clin Pharmacol 59:28-37
15. Williams GC, Dunnington KM, Hu MY, Zimmerman TR Jr, Wang Z, Hafner KB, et al (2006) The impact of posture on cardiac repolarization: more than heart rate? J Cardiovasc Electrophysiol 17:352-358
16. Stass H, Dalhoff A, Kubitza D, Schuhly U (1998) Pharmacokinetics, safety, and tolerability of ascending single doses of moxifloxacin, a new 8-methoxy quinolone, administered to healthy subjects. Antimicrob Agents and Chemother 42:20602065
17. Stass H, Kubitza D, Schuhly U (2001) Pharmacokinetics, safety and tolerability of moxifloxacin, a novel 8-methoxyfluoroquinolone, after repeated oral administration. Clin Pharmacokinet 40 [Suppl1]:1-9
18. Sullivan JT, Woodruff M, Lettieri J, Agarwal V, Krol GJ, Leese PT, et al (1999) Pharmacokinetics of a once-daily oral dose of moxifloxacin (Bay 12-8039), a new enantiomerically pure 8methoxy quinolone. Antimicrob Agents Chemother 43:2793-2797
19. Monahan BP, Ferguson CL, Killeavy ES, Lloyd BK, Troy J, Cantelina LR (1990) Torsades de pointes occurring in association with terfenadine use. JAMA 264:2788-2790
20. Honig PK, Wortham DC, Zamani K, Conner DP, Mullin JC, Cantelina LR (1993) Terfenadine-ketoconazole interaction: pharmacokinetic and electrocardiographic consequences. JAMA 269:1513-1518
21. DuBuske LM (1999) Second-generation antihistamines: the risk of ventricular arrhythmias. Clin Ther 21:281-295
22. Morganroth J (2007) Cardiac repolarization and the safety of new drugs defined by electrocardiography. Clin Pharmacol Ther 81:108-113
23. Sun H (2007) Pharmacometric methods for assessing druginduced QT and QTc prolongations for non-antiarrhythmic drugs. In: Ette EI, Williams PJ (eds) Pharmacometrics: the science of quantitative pharmacology. John Wileys \& Sons, New York, pp 977-992
24. Skerjanec A, Affrime MB, Milosavljev S, et al (2005) Darifenacin, an M3 selective receptor antagonist (M3 SAR), does not prolong QT/QTc. Clin Pharmacol Ther 79:P10
25. Harris SC, Hoelscher D, Krisensen A, O'Keefe SA, et al (2005) Effect of buprenorphine transdermal system 10 mg and $2 \times 20 \mathrm{mg}$ on QT intervals in healthy subjects. Clin Pharmacol Ther 79:P35
26. Noel GJ, Natarajan J, Chien S, Hunt TL, Goodman DB, Abels R (2003) Effects of three fluoroquinolones on QT interval in healthy adults after single doses. Clin Pharmacol Ther 73:292-303
