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QT prolongation and proarrhythmia by moxifloxacin: concordance of preclinical models in relation to clinical outcome

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1 Moxifloxacin, a fluoroquinolone antibiotic associated with QT prolongation, has been recommended as a positive control by regulatory authorities to evaluate the sensitivity of both clinical and preclinical studies to detect small but significant increases in QT interval measurements.

2 In this study, we investigated effects of moxifloxacin on the hERG current in HEK-293 cells, electrocardiograms in conscious telemetered dogs, and repolarization parameters and arrhythmogenic potentials in the arterially perfused rabbit ventricular wedge model.

3 Moxifloxacin inhibited the hERG current with an IC_{50} of 35.7 μM. In conscious telemetered dogs, moxifloxacin significantly prolonged QTc at 30 and 90 mg kg^{-1}, with mean serum C_{max} of 8.52 and 22.3 μg ml^{-1}, respectively. In the wedge preparation, moxifloxacin produced a concentration-dependent prolongation of the action potential duration, QT interval, and the time between peak and end of the T wave, an indicator for transmural dispersion of repolarization. Phase 2 early after-depolarizations were observed in one of five experiments at 30 μM and five of five experiments at 100 μM. The arrhythmogenic potential was also concentration-dependent, and 100 μM ($\sim$18-fold above the typical unbound C_{max} exposure in clinical usage) appeared to have a high risk of inducing *torsade de pointes* (TdP).

4 Our data indicated a good correlation among the concentration–response relationships in the three preclinical models and with the available clinical data. The lack of TdP report by moxifloxacin in patients without other risk factors might be attributable to its well-behaved pharmacokinetic profile and other dose-limiting effects.

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Abbreviations: AP, action potential; APD, action potential duration; EAD, early after-depolarization; HEK-293, human embryonic kidney cell line 293; hERG, human *ether-a-go-go*-related gene; IACF, individual animal correction factor; ICH, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; PK, pharmacokinetics; QTc, QT interval corrected for heart rate variation; TDR, transmural dispersion of repolarization; TdP, *torsade de pointes*; T_{P-E}, time between peak and end of the *T* wave

Introduction

Drug-induced delay in cardiac repolarization, an effect that can be measured as prolongation of the QT interval on the surface electrocardiogram (ECG), has increasingly drawn attention from regulatory agencies and the pharmaceutical industry and has had a great impact on drug discovery and development (Fermini & Fossa, 2003). The delayed repolarization, mostly due to blockade of the rapidly activating delayed rectifier potassium channel, I_{Kr}, favors the genesis of early after-depolarization (EAD), which can initiate an arrhythmia referred to as triggered activity (Zabel *et al*., 1997). Additionally, the prolongation of QT interval by drugs is often associated with increased heterogeneity of cardiac repolarization (Antzelevitch, 2004), a substrate for a re-entrant mechanism responsible for the maintenance of arrhythmia. One particular type of arrhythmia, *torsade de pointes* (TdP), may cause syncope events and/or degenerate into ventricular fibrillation and death.

While the association between abnormalities of repolarization and life-threatening arrhythmias appears clear, the link between QT interval prolongation and TdP is far more complex. In contrast to relatively common QT interval prolongation, the incidence of TdP is extremely low (De Ponti *et al*., 2000; 2001; Morganroth, 2004). Furthermore, the development of TdP in patients is unpredictable (Yang *et al*., 2002; Witchel *et al*., 2003), despite a readily measurable QT interval prolongation. Moreover, there are some 'safe' drugs that cause I_{Kr} inhibition and QT prolongation without inducing TdP (Fermini & Fossa, 2003; Redfern *et al*., 2003). The value of QT interval prolongation as a predictor of TdP is further limited by a number of cofactors, such as female gender, electrolyte imbalance (e.g. hypokalemia and hypomagnesemia), pathophysiological conditions (existing heart structural abnormalities, diabetes, renal and liver dysfunctions, etc.), and concomitant medications. However, without a clear

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mechanistic understanding of arrhythmogenicity and better methods for TdP prediction, both preclinically and clinically, QT interval will continue to be used as a primary end point required by regulatory agencies (Fermini & Fossa, 2003; Finlayson *et al*., 2004).

The current preclinical guidelines (ICH S7A and S7B, http://www.ich.org) for evaluation of the potential of QT interval prolongation by human pharmaceuticals describe an integrated risk assessment strategy consisting of both *in vitro* and *in vivo* assays. However, reports illustrating concordance of nonclinical studies on a particular drug in relation to its clinical outcome are scarce in the literature. Moxifloxacin, a fluoroquinolone antibiotic known to prolong QT, has been recommended as a positive control by regulatory authorities to evaluate the sensitivity of both clinical and preclinical studies to detect small but significant increases in QT interval measurements. However, reports of its effects in preclinical models are limited and often based on a single model. No reports can be found thus far attempting to explore its arrhythmogenic potential in preclinical models in relation to clinical exposures. In this study, we investigated the effects of moxifloxacin on the hERG potassium current in HEK-293 cells, ECGs in conscious telemetered dogs, and the repolarization parameters and arrhythmogenic potentials in the arterially perfused rabbit ventricular wedge preparation model. Concentration–response relationships and the value of these models are discussed in relation to clinical outcome.

Methods

All animal experiments were conducted in accordance with the regulations of the U.S. National Institutes of Health (NIH Publication No. 8523, revised 1996) and European Guidelines. All surgical procedures were approved by the Pfizer Institutional Animal Care and Use Committee.

Patch-clamp recording

HEK-293 cells stably expressing hERG potassium channels (Zhou *et al*., 1998) were licensed from Wisconsin Alumni Research Foundation or generated in house. Cells were cultured in minimum essential medium (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 0.1 mM nonessential amino acid, 1.0 mM sodium pyruvate, 10% fetal bovine serum, and 0.05% geneticin. On the day of the experiment, cells were dissociated from the flasks using 0.05% trypsin-EDTA and stored at room temperature in M199 medium (Hank's salt). Aliquots of the cells were allowed to settle on the bottom of a recording chamber on an inverted microscope (Olympus Model 1X51, Hachioji, Japan) and continually superfused with Tyrode's solution at a rate of $1.0\,ml\,min^{-1}$. The Tyrode's solution contained (in mM): NaCl, 137; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 10; Hepes, 10; pH 7.4 with NaOH. Experiments were performed at $35\pm1^{\circ}C$ (maintained by TC344B Temperature Controller, Warner Instruments, Hamden, CT, U.S.A.). Whole-cell configuration was formed with a glass pipette of 1–4 MΩ tip resistance when filled with the pipette solution. The pipette solution contained (in mM): KCl, 130; MgATP, 5; $MgCl_2$, 1; Hepes, 10; EGTA, 5; pH 7.2 with KOH. Data acquisition was performed by pClamp (v8.2) software, which controlled the MultiClamp 700A patch-clamp amplifier through a digitizer, DigiData 1322A (Axon Instruments, Union City, CA, U.S.A.). Cell capacitance and series resistance were routinely compensated to reduce the voltage error (limited to 5 mV in an experiment). A giga-ohm (GΩ) seal resistance was achieved in all experiments. Following cell membrane rupture, at least 5 min were allowed for cell dialysis before any recording was initiated. Cells exhibiting significant rundown of the current (e.g. $\geqslant 1\%$ per minute) over the baseline period were discarded. In the presence of the drug, a steady-state response was achieved before a subsequent concentration was applied.

The hERG currents were activated by a voltage step to $+20$ mV for 1 s from a holding potential of -80 mV, followed by a repolarizing ramp back to -80 mV at a rate of $0.5\,mV\,ms^{-1}$ (frequency, 0.25 Hz) (Volberg *et al*., 2002; Cordes *et al*., 2005). At the end of each experiment, 10 μM dofetilide was applied to block any remaining hERG current. The dofetilide-insensitive current, which reflects the possible leakage and endogenous currents, was then subtracted from the experimental data. Peak current amplitudes during the ramp repolarization phase were then measured and the time course was plotted. To quantify the blockade of a compound, a linear fit was applied to the stabilized control data and extrapolated over the period of test compound application to predict the current 'rundown'. Percent block was then computed from the predicted current obtained from the linear extrapolation ($I_{Control}$) to the steady-state response in the presence of the compound (I_{Drug}). Averaged data were fitted with Hill equation to obtain the IC_{50} value.

Dog telemetry study

Beagle dogs (four male and four female) previously implanted with radio-telemetry transmitters (TL11M2-D70-PCT; Data Sciences International, St Paul, MN, U.S.A.) were randomly assigned in a Latin Square design. Each dog received a single dose of moxifloxacin by oral gavage at doses of 10, 30, and $90\,mg\,kg^{-1}$, and vehicle (0.5% methylcellulose and 0.1% polysorbate 80) with a 7-day interdose washout period between each administration. Dogs were fasted overnight prior to dosing and were fed at approximately 6 h postdose. A satellite pharmacokinetic (PK) group of noninstrumented male and female Beagle dogs (four per sex) received the same treatment. All animals were observed at least once daily for clinical signs. Body weights were recorded prior to the administration of each dose for the purpose of dose calculations. On dosing days, telemetered animals were observed once 15–30 min predose and $\sim$6 h postdose for changes in appearance or behavior. Systemic arterial blood pressure and electrocardiographic data were obtained from the telemetry group (15 and 30 min predose and at approximately 1, 3, 6, 8, and 24 h postdose) on each dosing day. Serum moxifloxacin concentrations were measured from the noninstrumented (PK) group just prior to dosing and at approximately 1, 3, 6, 8, and 24 h postdose on each dosing day and from the telemetered animals $\sim$1.5 h prior to dosing and $\sim$6 h postdose.

ECG (lead II configuration) and blood pressure signals were acquired at a sampling rate of 500 and 250 Hz, respectively, *via* a Digital Telemetry Interface and Ponemah® Data Acquisition system (Physiology Platform, Model P3 Plus, V3.322, LDS Life Science, Valley View, OH, U.S.A.). Data were collected continuously for $\geqslant$1 h predose (for baseline), and for $\sim$24 h

following administration of either vehicle or compound. All parameters were measured in the same analysis platform. The QT interval corrected for heart rate variation (QTc) was defined by the expression: $QTc = QT - (\text{heart rate} - 100) \times m$, where the QT interval correction factor m was determined by obtaining the slope of the QT/heart rate regression line for each animal on each treatment day during the predose time frame and served as the individual animal correction factor (IACF). This regression was constructed from values derived from 20-s averages of the automated ECG analysis and included only those averages where at least 95% of the waveforms had all waveform components identified. If the correlation coefficient obtained during this period was <0.6, the slope of -0.535 (an average value obtained from in-house historical data) was used as the IACF value.

The dosage formulations of the test article and the vehicle control formulations (~ 0.250 ml dose level^{-1}) were verified by quantitative nuclear magnetic resonance by the analytical group at Pfizer. All dosing suspensions were confirmed to be within the acceptable range of the intended concentration (90–94%).

Arterially perfused ventricular wedge study

Under anesthesia by 30–35 mg kg^{-1} ketamine HCl (intravenously (i.v.)) following 5 mg kg^{-1} xylazine (intramuscularly (i.m.)), the heart from a female New Zealand White rabbit (2.5–5.5 kg) was removed and placed in cold (4–10°C) 95% O_2–5% CO_2 saturated cardioplegic solution (in mM): 129 NaCl, 24 KCl, 0.9 NaH_2PO_4, 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, and 5.5 glucose. The female gender was chosen to increase the sensitivity of the preparation in proarrhythmic activity based on literature reports (Drici *et al*., 1996; Liu *et al*., 1999; 2005; Spear & Moore, 2000; Lu *et al*., 2001). The left main coronary artery or its major branch (normally circumflex branch) was cannulated and perfused with the cardioplegic solution to washout the intravascular blood. A transmural left ventricular wedge from the anterior wall was dissected with major leaking vessels ligated, placed in a tissue bath and arterially perfused with 36 ± 0.5°C Tyrode's solution (mM): 129 NaCl, 4 KCl, 0.9 NaH_2PO_4, 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, and 5.5 glucose, pH 7.35, when buffered with 95% O_2 and 5% CO_2. The perfusion pressure was maintained at ~ 40 mmHg and monitored through a pressure transducer connected with the PowerLab/8SP Data Acquisition System (ADInstruments, Castle Hill, NSW, Australia). The tissue was paced with ~ 150% suprathreshold stimuli at 1 Hz by a DS8000 Digital Stimulator (World Precision Instruments, Sarasota, FL, U.S.A.) through platinum bipolar electrodes on the endocardial surface. Floating glass electrodes, with a resistance of approximately 10–20 MΩ when filled with 2.7 M KCl, were placed in the epicardial or endocardial myocardium, respectively. Action potentials (APs) from both sites were amplified through an IX2-700 Dual Intracellular Preamp (Dagan Corporation, Minneapolis, MN, U.S.A.). The transmural ECG was recorded by using two Ag/AgCl electrodes placed ~ 1 cm away from epicardial and endocardial surfaces and fed into an EX1 Differential Amplifier (Dagan Corporation, MN, U.S.A.). All the signals were monitored and recorded using Chart 5 software (ADInstruments) through the PowerLab/8SP system. An equilibrium period of at least 1 h was allowed in each experiment before any data collection. Action potential duration (APD) parameters were analyzed using peak parameter extension within the Chart 5 program. QT interval and the time from the peak to the end of the T wave (T_{P-E}) were directly measured on the recording traces. Moxifloxacin at different concentrations was administered in a stepwise manner, and at least 30 min of exposure at a concentration was allowed before data collection.

In an initial validation study, we evaluated basic experimental conditions and parameters collected from the wedge preparation. Owing to tissue contractions, AP recordings were often discontinued within 1–3 min and AP amplitudes often varied. However, recordings from adjacent areas (within 0.5 cm radius) were consistent in APD (<5% variation), which validated APD evaluation by repenetration of the electrode. Furthermore, the APD values were stable over the course of an experiment (up to 7 h of perfusion) when no pharmacological intervention was applied. In a vehicle control study, we observed no significant effect of DMSO at $\leqslant 0.3$% on any of the parameters of interest.

Data analysis

Data were expressed as mean ± s.e.m., unless specified. In the wedge study, the statistical analysis was conducted separately for APD_{30}, APD_{50}, and APD_{90} using an ANOVA model as a blocking factor and the experimental factors included frequency of stimulation (0.5 or 1 Hz), site of measurement (endocardial and epicardial), and concentrations of drug. Pairwise comparisons of each concentration mean to the control mean were made using sequential linear trend tests. QT and T_{P-E} were analyzed using the above model without the factor for site of measurement. In the dog telemetry study, data from each time point were analyzed using an ANOVA model containing the following factors and interactions: Animal, Sex, Dose, Week, Dose × Sex, and Sex × Week. If no Dose × Sex interaction ($P > 0.05$) was seen, then sequential linear trend contrasts were used to compare the means of each treated group (males and females combined) with the control group mean. If the treatment by sex interaction was significant ($P \leqslant 0.05$), then male and female data were analyzed separately using an ANOVA model containing the terms of Animal, Dose, and Week. Differences between treated and control group means were again assessed using sequential linear trend contrasts.

All tests were carried out at the 0.05 level of significance and were two-tailed. Statistics were performed using SAS® software package (6.08, SAS Institute Inc., Cary, NC, U.S.A.), other analysis were performed in Microsoft Excel 2000 (9.0.7616 SP-3, Microsoft Corporation, Redmond, WA, U.S.A.) and Origin v7.0 (OriginLab Cooperation, Northampton, MA, U.S.A.).

Results

Effect of moxifloxacin on the hERG current

The responsiveness of the same batches of HEK-293 cells stably expressing the hERG potassium channels was evaluated in a positive control experiment, in which an 83.0 ± 1.3% ($n = 3$) inhibition of the hERG current was induced by 30 nM dofetilide, a selective I_{Kr} blocker. This result was consistent

with our historical data (81.9±0.6%, $n=64$) and literature reports (Snyders & Chaudhary, 1996; Ficker *et al*., 1998). Moxifloxacin was tested at concentrations of 3, 10, 30, 100, and 300 μM, respectively (Figure 1a and b). Averages of 8.8±1.1, 19.6±2.1, 45.2±5.4, 71.0±3.1, and 86.7±1.8% reduction, respectively, in the hERG current were observed ($n=4$–7 cells at each concentration). As shown in Figure 1c, the concentration–response relationship of moxifloxacin was described by fitting the averaged values with the Hill equation:

$$I_{Drug}/I_{Control} = 1/[1 + (D/IC_{50})^p]$$

where D is the drug concentration, IC_{50} is the concentration for 50% inhibition, and p is the Hill coefficient. The resulting IC_{50} of moxifloxacin in inhibiting the hERG current was 35.7 μM with a Hill coefficient of 1.0, indicating a single binding site of the drug molecule to the hERG channel.

Effect of moxifloxacin in conscious telemetered dogs

Clinical signs Moxifloxacin caused emesis at 30 mg kg^{-1} (1/8) and 90 mg kg^{-1} (8/8) doses. In most cases, several instances of emesis were noted at the ~6 h postdose observation. In the satellite PK study animals, emesis and loose stool were observed at 30 mg kg^{-1}, and emesis, salivation, and partially closed eyes at 90 mg kg^{-1}. Emesis occurred immediately postdose (1/8) or 1–6 h postdose (3/8) in 30 mg kg^{-1} dose group and 5/8 animals at 90 mg kg^{-1} dose group. All other animals were clinically normal.

Cardiovascular observations Following dosing, there was a tendency for RR interval to increase from baseline in all dose groups including vehicle-treated animals. This might be associated with normal circadian changes that are observed over the course of an extended recording period. The increase was smaller in animals treated with 30 and 90 mg kg^{-1} at 1 h postdose (+59 and +24 ms, respectively, vs +187 ms for vehicle treatment, or −7 and −9 vs −16 b.p.m. in heart rate; $P<0.05$) and again in animals treated with 90 mg kg^{-1} at 6 h postdose (+33 vs +190 ms for vehicle, or −7 vs −18 b.p.m. in heart rate; $P<0.05$). There was a tendency for PR interval to decrease in animals treated with 90 mg kg^{-1}. This decrease reached statistical significance at 8 h postdose (−10 vs +0 ms for vehicle treatment, $P<0.05$). The QTc intervals, as shown in Figure 2, were significantly increased in animals treated with 90 mg kg^{-1} (at 1, 6, and 8 h in males only; or at 3 and 24 h when males and females were combined). In general, the response was more robust in the male animals, as demonstrated at the 1-h time point with elevations of 50 and 18 ms for males and females, respectively (average 34 ms when combined). Animals treated with 30 mg kg^{-1} had significant increases in QTc from baseline at 3 h postdose (+20 vs +6 ms for vehicle treatment). There were no statistically significant increases in QTc in animals treated with 10 mg kg^{-1}. No changes in blood pressure were noted at any dose level.

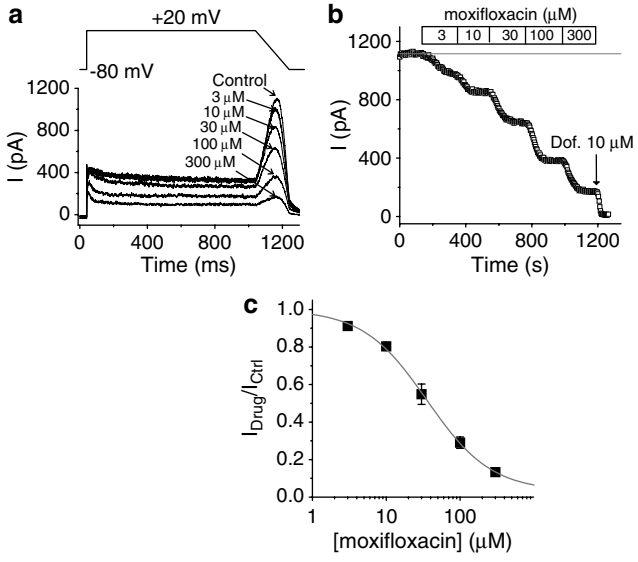

Figure 1 Effect of moxifloxacin on the hERG potassium current stably expressed in HEK-293 cells. (a) Current traces from a representative cell before and after applications of moxifloxacin at various concentrations administrated incrementally. The hERG current was elicited by a voltage protocol shown in the upper panel at 0.25 Hz. (b) Time course of the moxifloxacin effect on the hERG current. Peak tail current amplitudes were measured during the ramp repolarization phase and plotted against time. A steady-state current level was reached before application of next concentration of the drug. A linear fit was conducted in every experiment to the stable control period to account for the rundown of the current, if any. Amplitude of the current at the end of each concentration application was measured and compared with its corresponding control. (c) Concentration–response of the hERG blockade by moxifloxacin. Averaged data were obtained from four to seven experiments and fitted with a Hill equation. The resulting IC_{50} was 35.7 μM with a Hill coefficient of 1.0.

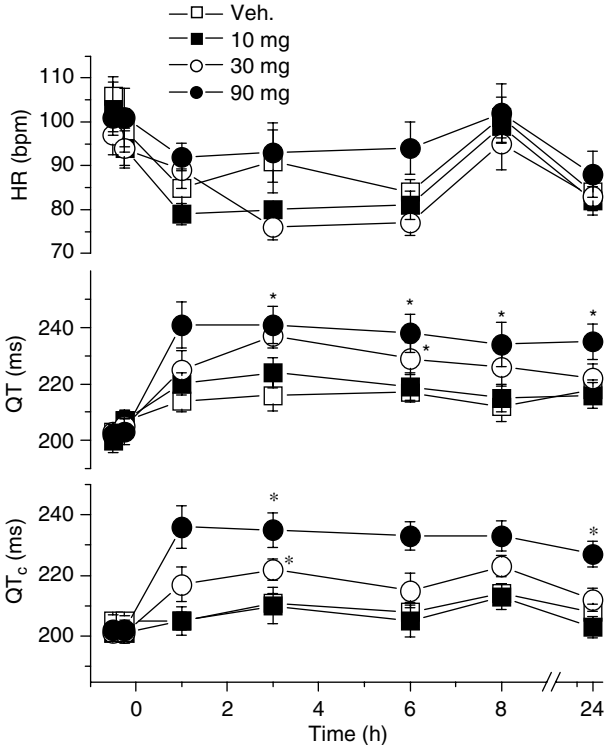

Figure 2 Effect of moxifloxacin on HR, QT, and QTc in conscious telemetered dogs. Data were obtained from eight animals (four male and four female) of a Latin Square design. Each dog received a single dose of moxifloxacin by oral gavage at doses of 10, 30, and 90 mg kg^{-1} and vehicle (0.5% methylcellulose/0.1% polysorbate 80) with a 7-day interdose washout period between each administration. Mean±s.e.m. *$P<0.05$ vs vehicle control.

Serum drug concentrations In the satellite PK-study group of animals, systemic exposure to moxifloxacin as assessed by mean C_{max} and mean $AUC_{0-T_{last}}$ (AUC from time 0 to the last sample collection time) increased with increasing doses from 10 to 90 mg kg^{-1}. Average C_{max} values of 3160±551, 8520±1910, and 22,300±4880 ng ml^{-1} were measured following the 10, 30, and 90 mg kg^{-1}, respectively (Table 1). In the telemetered animals, mean exposures of moxifloxacin collected at ~6 h postdose were 2550±205, 8580±737, and 15,800±1940 ng ml^{-1} at 10, 30, and 90 mg kg^{-1} doses, respectively, which were comparable to the values measured in the PK group at ~6 h postdose (2530±652, 6670±2400, and 16,000±2720 ng ml^{-1} at 10, 30, and 90 mg kg^{-1}, respectively). Individual T_{max} varied between 1 and 8 h postdose, regardless of dosage received; however, the mean T_{max} for each groups was about 3.0 h postdose (Table 1).

Effect of moxifloxacin on AP and transmural ECG parameters in the wedge preparation

Moxifloxacin was tested in a stepwise manner at 3, 10, 30, and 100 μM, respectively, in the rabbit left ventricular wedge. As shown in Figures 3 and 4, moxifloxacin produced a concentration-dependent prolongation in both endocardial and epicardial APD_{90} as well as the QT interval on transmural ECG. At 0.5 Hz, epicardial APD_{90}, endocardial APD_{90}, QT interval and T_{P-E} were increased by 32.3±6.5, 38.1±6.4, 42.6±5.2, and 83.4±25.9%, respectively, by moxifloxacin at 30 μM. Moxifloxacin significantly increased APD_{30} at 30 and 100 μM, but did so on APD_{50} and APD_{90} at 10 μM or higher concentrations ($P<0.05$). The magnitudes of APD_{50} and APD_{90} changes were comparable but apparently larger than that of APD_{30} response, indicating that the delayed repolarization mainly occurred in late Phase 2 and Phase 3 of the AP (Figure 3), a feature shared by selective I_{Kr} blockers. When stimulated at a higher frequency (1 Hz), the increases of the repolarization parameters were reduced (e.g. APD_{50} and APD_{90} at all concentrations, $P<0.05$, Figure 4b vs a), indicating that the effect of moxifloxacin was in a reverse use-dependent manner similar to typical class III antiarrhythmics. Moreover, the prolongation of APD_{50} and APD_{90} in the endocardial region was more prominent than that in the epicardial region ($P<0.05$ at all concentrations and both frequencies), which correlated with a significantly greater increase in the T_{P-E} values as compared with the QT interval prolongation (Figure 4a and b). These results suggested an increased transmural dispersion of cardiac repolarization following application of moxifloxacin. At 30 μM, moxifloxacin induced Phase 2 EAD in one out of five preparations when stimulated at 0.5 Hz. More EADs with R-on-T waveforms were observed at 100 μM (5/5 preparations, Figure 5). Using a

Table 1 PK data from satellite group dogs receiving 10, 30, and 90 mg kg^{-1} moxifloxacin

Dose (mg kg^{-1})	*AUC* (ng h ml^{-1})	C_{max} (ng ml^{-1})	T_{max} (h)
10	36,500±5690	3160±551	3±2
30	11,000±39,900	8520±1910	3±2
90	312,000±46,200	22,300±4880	3±2

Mean±s.d. $n=8$.

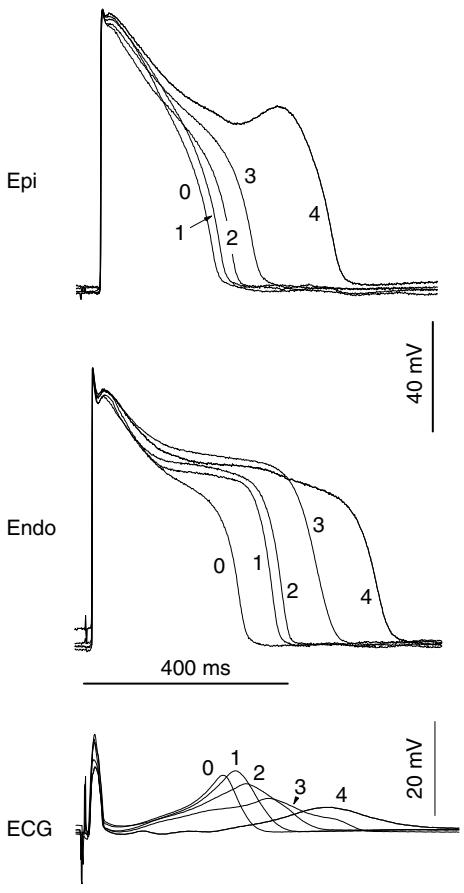

Figure 3 Effects of moxifloxacin on action potentials recorded from epicardial and endocardial regions and transmural ECG in a rabbit wedge preparation. The numbers denote traces obtained in the absence (0) and presence of 3, 10, 30, and 100 μM moxifloxacin (1–4, respectively) from a representative experiment. An EAD was apparent in the epicardial action potential by 100 μM moxifloxacin.

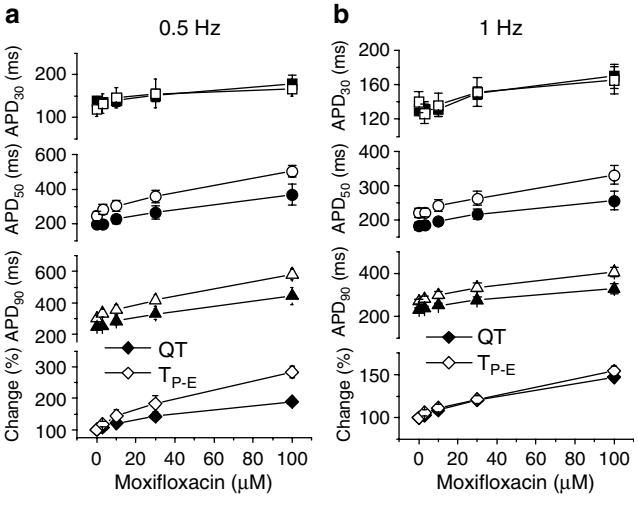

Figure 4 Effects of moxifloxacin on repolarization parameters in the wedge preparation. Summary of the concentration-dependent effect of moxifloxacin on APDs, QT interval, and T_{P-E} at 0.5 Hz (a) and 1 Hz (b). Open and closed symbols in the APD panels represent endocardial and epicardial regions, respectively. For clarity purpose, the statistical significance of differences in stimulation frequency, recording sites, and concentrations are not noted.

modified proarrhythmia scoring system (Table 2) (Yan *et al*., 2001; Joshi *et al*., 2004), 3, 10, 30, and 100 μM moxifloxacin received proarrhythmic scores of 1.2 ± 0.7, 2.0 ± 0.8, 3.2 ± 1.0, and 7.8 ± 0.8, respectively. These scores were obtained by a sum of the individual parameter-associated scores in each experiment, and then averaged at each concentration ($n=5$).

Correlation among preclinical models

Effects of moxifloxacin on the hERG current in HEK-293 cells and the QT interval in arterially perfused rabbit ventricular wedge were plotted against testing concentrations (Figure 6). The QTc interval changes in conscious telemetry dogs were also presented as the maximal averaged effect vs unbound C_{max} assuming a 50% protein binding (Prod Info Avelox®, 2003) (Stass *et al*., 1998). As shown in Figure 6, the extent of QTc prolongation in telemetry dogs appeared to be comparable to that in the rabbit wedge. Similar to many other specific I_{Kr} blockers such as dofetilide and E-4031 tested in our laboratory, moxifloxacin produced roughly 8–10% QT prolongation at a concentration that inhibited 20% of the hERG current (IC_{20}). By using a computer simulation for rabbit ventricular myocytes (Puglisi & Bers, 2001), a similar magnitude of prolongation in APD was observed by inhibiting I_{Kr} channel alone to the same degree (data not shown). Our data indicate that moxifloxacin can produce consistent signals in these preclinical models as a positive control.

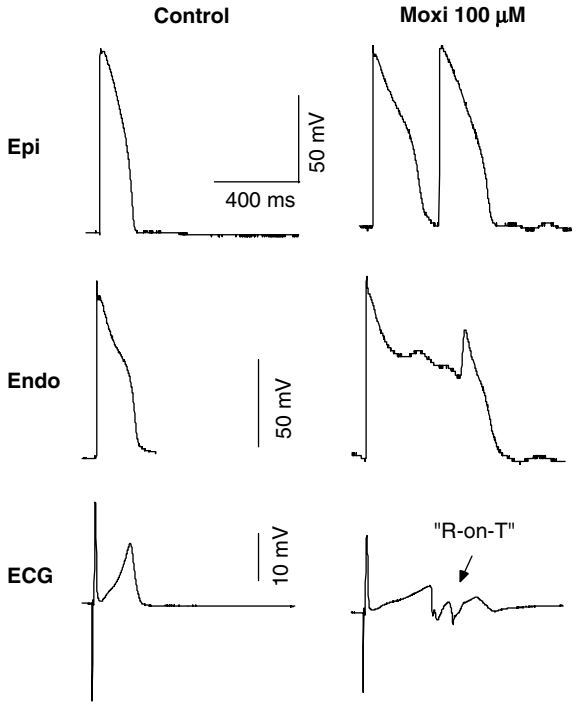

Figure 5 Phase 2 early after-depolization (EAD) induced by 100 μM moxifloxacin. The tissue was stimulated at 0.5 Hz. EADs in action potentials with or without 'R-on-T' in transmural ECGs were seen in all five at 100-μM and one of five at 30-μM experiments.

Table 2 Modified proarrhythmic scoring system to estimate a compound's risk for development of TdP in isolated rabbit left ventricular wedge preparation

Parameter	*Range*	*Score*
QT interval increase (%)	$\leqslant 10$	0
	>10 to $\leqslant 50$	1
	>50 to $\leqslant 100$	2
	>100	3
T_{P-E} (ms)	$\leqslant 75$	0
	>75 to $\leqslant 90$	1
	>90 to $\leqslant 120$	2
	>120	3
Phase 2 EAD	No EAD	0
	EAD without R-on-T	2
	EAD with R-on-T	4
	EAD with TdP	6

QT interval, T_{P-E}, and EADs were scored independently and added up to a final score at a given concentration.

Discussion

Inherent limitations of each preclinical QT assessment model (e.g. species- and tissue-specific responses or lack of, methodological limitations, etc.) and/or the reported interlaboratory variations had once led to a general conception that they could only offer qualitative predictions. As a result, in the draft ICH E14 Guideline (The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs), all investigational drugs were required to go through a 'thorough QT study' regardless of their preclinical testing results. However, recent cumulative evidence indicates that carefully designed studies with rigorous experimental controls can provide results that quantitatively correlate with clinical outcomes. Safety margins can be derived from concentration–response relationships observed in these models that are comparable to effects observed with clinical exposures (Webster *et al*., 2001; 2002; Redfern *et al*., 2003). In

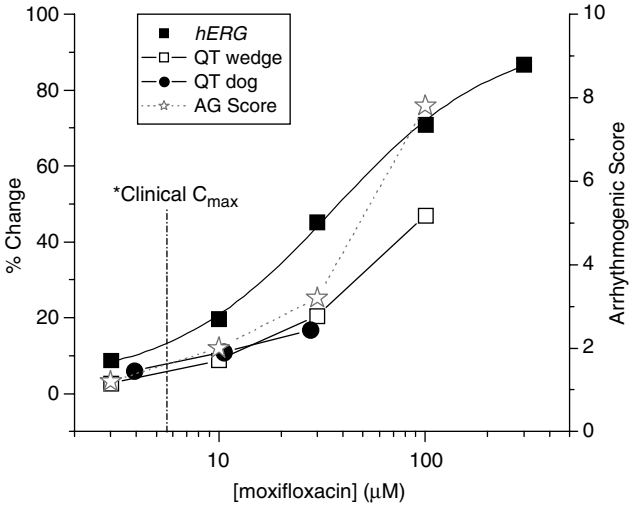

Figure 6 Correlation of preclinical assessment results with the clinical exposures. *Unbound C_{max} following oral treatment of 400 mg, QD for 10 days (Balfour & Wiseman, 1999). For conscious telemetered dogs, mean maximal QTc prolongation was plotted against unbound C_{max} at each dose. The arrhythmogenic (AG) scores in the wedge preparation were obtained by a sum of the individual parameter-associated scores (Table 2) in each experiment, and then averaged at each concentration.

our experience, optimized experimental conditions in *in vitro* and *in vivo* models have consistently yielded data with minimum intralaboratory variations and enabled quantitative correlation studies based on the concentration (exposure)–response relationship.

With a clear clinical signal of QT prolongation and well-behaved PK profile, moxifloxacin has been recommended as one of the few available positive controls for clinical trials assessing QT prolongation potential by the ICH E14 Expert Working Group. In clinical use, peak plasma concentrations of 0.6–4.7 $\mu g\,ml^{-1}$ (0.7–5.9 μM unbound) were obtained in healthy subjects at 2–3 h postdose after oral administration of 100–800 mg moxifloxacin (Stass *et al*., 1998), and a peak level of 4.5 $\mu g\,ml^{-1}$ (5.6 μM unbound) was reported after 400-mg daily doses for 10 days (Balfour & Wiseman, 1999). In 787 patients in Phase 3 clinical trials, daily repeated doses of 400 mg moxifloxacin increased the QT interval 6 ± 26 ms (mean $\pm$ s.d.) (Prod Info Avelox®, 2003). In over 20 specially designed studies in healthy volunteers using standardized approaches and methods, single oral doses of moxifloxacin 400 mg have consistently produced a mean increase in the QTc interval ranging from 5 to 10 ms (Shah, 2005). Our study clearly demonstrated that the concentration-dependent effect of moxifloxacin on various preclinical end points could be directly compared to clinical outcomes. With moxifloxacin, clinically relevant QTc prolongation is seen at a concentration that produces $\sim$10% inhibition of the hERG current (Figure 6), similar to many I_{Kr}/hERG blockers (e.g. dofetilide, E-4031, cisapride, terfenadine, and risperidone) tested in our laboratory. In the telemetry dog study, mean maximum serum concentrations (C_{max}) of 3.9, 10.6, and 27.8 μM (unbound) were obtained following 10, 30, and 90 $mg\,kg^{-1}$ doses, respectively. These concentrations represented a 0.7-, 1.9-, and 4.5-fold exposure, respectively, of that in humans repeatedly dosed with 400 mg. These exposures were associated with mean maximal QTc prolongation of 6, 11, and 17% (vs predose), respectively. Comparable responses in APD and transmural QT interval were observed in the arterially perfused wedge preparation as well.

The isolated arterially perfused rabbit ventricular wedge preparation has demonstrated a high sensitivity and specificity in the assessment of cardiac safety of pharmaceuticals (Yan *et al*., 2001; Joshi *et al*., 2004). The unique benefit of this model is that it allows assessment of the two major mechanisms for TdP: triggered activity (EAD based) and re-entry. Transmural propagation of the EAD under conditions of increased dispersion of repolarization can produce an R-on-T ectopic beat and initiate TdP. In our study, moxifloxacin demonstrated an ability of increasing TDR and inducing EAD. The arrhythmogenic score–concentration relationship shown in Figure 6 indicates that the risk of proarrhythmia by moxifloxacin is concentration dependent. In our preliminary validation study (data not shown), we noticed that T_{P-E} or TDR appeared to consistently show a proportional change with altered QT interval even under physiological conditions (e.g. stimulation frequency or heart rate changes). Therefore, a certain degree of increase in the absolute values of T_{P-E}, while receiving a higher score, does not always translate to an increased arrhythmogenic risk until EADs occur or the increase becomes disproportional. To help identify a 'threshold' of proarrhythmia, we calculated the arrhythmogenic score from 13 experiments with several positive control drugs (i.e. DL-sotalol, dofetilide, cisapride, risperidone, and moxifloxacin) at a concentration starting to induce Phase 2 EADs. A fairly consistent score of $\sim$7.0 was obtained in most of the experiments (7.1 ± 0.2, $n = 13$), indicating a high risk of proarrhythmia. The 'threshold' concentrations for these drugs (DL-sotalol, dofetilide, cisapride, risperidone, respectively) were 2.3-, 1.6-, 16.7-, and 37.5-fold, respectively, their unbound therapeutic concentrations, which appeared to correlate with their clinical outcomes of TdP and individual PK behavior in clinic. In the case of moxifloxacin, the 'threshold' proarrhythmia score of 7.1 ± 0.2 was obtained at 100 μM and, therefore, may represent an exposure of high risk. This concentration represents $\sim$18-fold of C_{max} observed in clinical studies with daily repeated doses of 400 mg.

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. We believe that 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 (Stass & Kubitza, 2001a, b), 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*. Nonetheless, our data and others (Chiba *et al*., 2004) suggested that the potential of causing TdP by moxifloxacin cannot be excluded, and the use of this compound in patients with other risk factors should be cautioned.

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