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Original article

Cardiovascular monkey telemetry: Sensitivity to detect QT interval prolongation

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Abstract

Introduction: Preclinical evaluation of delayed ventricular repolarization manifests electrocardiographically as QT interval prolongation and is routinely used as an indicator of potential risk for pro-arrhythmia (potential to cause Torsades de Pointes) of novel human pharmaceuticals. In accordance with ICH S7A and S7B guidelines we evaluated the sensitivity and validity of the monkey telemetry model as a preclinical predictor of QT interval prolongation in humans. **Methods:** Cardiovascular monitoring was conducted for 2h pre-dose and 24h post-dosing with Moxifloxacin (MOX), with a toxicokinetic (TK) evaluation in a separate group of monkeys. In both studies, MOX was administered orally by gavage in 0.5% methylcellulose at 0, 10, 30, 100, 175 mg/kg. Each monkey received all 5 doses using a dose-escalation paradigm. Inherent variability of the model was assessed with administration of vehicle alone for 4 days in all 4 monkeys (0.5% methylcellulose in deionized water). **Results:** MOX had no significant effect on mean arterial pressure, heart rate, PR or QRS intervals. MOX produced significant dose-related increases in QTc at doses of 30 ($C_{\max}=5.5\pm 0.6\mu\text{M}$), 100 ($C_{\max}=16.5\pm 1.6\mu\text{M}$), and 175 ($C_{\max}=17.3\pm 0.7\mu\text{M}$) mg/kg with peak increases of 22 (8%), 27 (10%), and 47 (18%) ms, respectively ($p\leq 0.05$; compared to vehicle). **Discussion:** In conclusion, we have developed a reproducible, sensitive and reliable primate telemetry model in rhesus monkeys, which exhibits low inherent intra-animal variability and high sensitivity to detect small but significant increases in QT/QTc interval (~4%) with MOX in the same range of therapeutic plasma concentrations attained in humans. Therefore, the primate telemetry model should be considered an important preclinical predictor of QT prolongation of novel human pharmaceuticals.

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Keywords: Rhesus monkeys; Telemetry; Methods; Moxifloxacin; QT interval; Sensitivity

1. Introduction

In the mid-1990s, the use of several cardiac and non-cardiac drugs including antihistamines, antipsychotics, antibiotics, and antiarrhythmics was associated with QT interval prolongation and a potentially fatal polymorphic tachyarrhythmia Torsades de Pointes (TdP) (Fermini & Fossa, 2003; Redfern et al., 2003). This serious cardiac side-effect potential of these drugs compromised their therapeutic value and often led to limited use or withdrawal from the market (Fermini & Fossa, 2003). QT interval prolongation (an electrocardiographic manifestation of

delayed ventricular repolarization) could result from drug effects on a number of cardiac ion channels, including inhibition of the rapidly activating delayed rectifier K^+ current, I_{Kr} , but also can arise from inhibition of the slowly activating delayed rectifier K^+ current, I_{Ks} or by slowing inactivation or increased activation of Na^+ or Ca^{2+} currents (Antzelevitch & Shimizu, 2002; Salata et al., 1996). Most drugs that increase the QT interval prolong action potential duration by inhibiting I_{Kr} encoded by the human ether a-go-go related gene (hERG) (Redfern et al., 2003; Sanguinetti & Zou, 1997). The propensity of a drug to produce TdP in humans can be difficult to predict in preclinical studies, because the human condition is often complicated by genetics, pre-existing diseases, and other medications (Hoffmann & Warner, 2006). Thus, preclinical

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electrocardiographic evaluation of delayed ventricular repolarization (QT interval prolongation) is used as an indicator of potential pro-arrhythmic properties of novel human pharmaceuticals (ICH, 2001, 2005).

According to the S7A and S7B guidelines provided by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the potential for QT interval prolongation by human pharmaceuticals may be evaluated in well characterized in vitro (cell, tissue or isolated organs to evaluate ionic mechanisms of repolarization) and in vivo (conscious or anesthetized dogs, monkeys, rabbits, swine, guinea pig, and ferrets) model systems (ICH, 2001, 2005). In vivo assessments in anesthetized and conscious animals along with in vitro hERG inhibition studies are the most widely used preclinical industrial safety paradigms to evaluate the potential pro-arrhythmic potential of novel pharmaceuticals in humans (Friedrichs, Patmore, & Bass, 2005). Several published reports have outlined the value of anesthetized cardiovascular studies in dogs and monkeys, but the literature on conscious unrestrained cardiovascular studies is primarily composed of telemetry studies in dogs (Bennett & DePetrillo, 2005; Ollerstam et al., 2005; Singh, Chakravarti, Chhuttani, & Wahi, 1970; Thomsen et al., 2004). Monkeys are one of the most commonly used preclinical safety species because of their genetic, cardiovascular and metabolic similarities to humans, but there are only a few published reports illustrating the usefulness of conscious telemetry in monkeys (Benardeau, Weissenburger, Hondeghem, & Ertel, 2000; Gauvin, Tilley, Smith, & Baird, 2006; Hassimoto & Harada, 2004; Kaufman & Detweiler, 1999). Additionally, there are no published investigations to date that have evaluated the sensitivity of the monkey telemetry model for its ability to predict QT interval changes in humans. Therefore, in accordance with the guidelines of ICH S7A and S7B, there is need to characterize the monkey telemetry model as a preclinical predictor of pro-arrhythmic potential of human pharmaceuticals (ICH, 2005).

Moxifloxacin is a therapeutically effective fluoroquinolone antibiotic, which has been complicated by its potential to produce small but significant QT prolongation in humans (Demolis, Kubitzka, Tenneze, & Funck-Brentano, 2000; Noel et al., 2003), mediated most likely by its known inhibitory activity on hERG (Bischoff, Schmidt, Netzer, & Pongs, 2000; Kang, Wang, Chen, Triggle, & Rampe, 2001). Because very moderate but reproducible increases in the QT interval are observed with administration of moxifloxacin in normal healthy human volunteers with little to no risk of TdP, it has been employed as a positive control agent to evaluate the sensitivity for detection of QT changes in human Thorough Clinical QT studies (TCQT) (Camm, 2005). In this study, we have developed a telemetry model in rhesus monkeys and have used moxifloxacin to assess the sensitivity and efficiency of this preclinical model to mimic and/or predict QT interval prolongation in humans. The objectives of this study were to evaluate the electrocardiographic effects of escalating oral doses of moxifloxacin in rhesus monkeys, and to evaluate further the normal variability of the model/study design with repeated

administration of vehicle (0.5% methylcellulose in deionized water).

2. Methods

2.1. Cardiovascular studies

2.1.1. Chemical information

Moxifloxacin Hydrochloride (Chempacific, Baltimore MD) was formulated as a suspension in 0.5% Methylcellulose in Deionized Water (Dow Chemical Company/Callahan Chemical Company Inc., Grove City OH USA) using an AR-250 Defoaming Conditioning Mixer. Moxifloxacin was administered orally by nasogastric gavage in 0.5% methylcellulose at 0, 10, 30, 100, 175 mg/kg (5 mL/kg). Each monkey received all 5 doses using a dose escalation paradigm. In a separate study, four doses of 5 mL/kg vehicle (0.5% Methylcellulose in Deionized Water) were administered to monkeys using the same paradigm as in the moxifloxacin study.

2.1.2. Animal model/study design

All aspects of the animal use were in accordance with guidelines provided by the USDA and approved by the Merck Institutional Animal Care and Use Committee.

2.1.2.1. Surgical procedures. Monkeys were instrumented with a radiotelemetry device (Konigsberg implant (Model # T27F-4), Integrated Telemetry Services, Pinckney, Michigan), during one major survival surgery. All procedures were conducted according to standard aseptic surgical techniques. Ketamine (10–30 mg/kg, IM) was administered initially to sedate the monkeys, which were then intubated and maintained under inhaled isoflurane (1–3%) anesthesia with mechanical ventilation. Analgesia was maintained pre-induction by intramuscular buprenorphine (0.01 to 0.05 mg/kg) and post-induction by epidural morphine (0.1 mg/kg). Monkeys were also administered either Cefazolin (20 mg/kg IV) or Enrofloxacin (2.5–5 mg/kg, IM) pre-surgery. The battery module was placed intraperitoneally and the electronics were placed in an intermuscular abdominal subcutaneous pocket on the left flank. The associated sensor wires were tunneled subcutaneously to the incision made on the left thorax (over the 5th intercostal space). In general, the ECG lead was implanted in a base apex configuration. The pressure probe, along with the negative ECG electrode, was placed and secured (with appropriate sutures) in the lumen of the thoracic aorta distal to the aortic arch via a small incision. The positive end of the ECG spur electrode wire was secured to the region on the 7th rib close to sternum, and this position was optimized and finalized based on the T wave amplitude (as evident by the ECG waveform observed during surgery). Monkeys were administered buprenorphine (0.01 to 0.05 mg/kg) for 2 to 3 days (6 to 12h intervals) post surgery and thereafter as required. Monkeys were closely monitored daily for approximately 10 days. Monkeys were allowed to recover for at least one month post surgery before being used on study.

Four male *Macaca mulatta* (Rhesus) monkeys instrumented with a Konigsberg cardiovascular implant were used in the

moxifloxacin study and 2 males and 2 females were used in the vehicle study (age: 4 to 6 years; weight: 5 to 9 kg). Monkeys were supplied from the following sources: Mannheimer Foundation (Homestead, FL), University of Texas MD Anderson Cancer Center (Bastrop, TX), Covance Research Products (Alice, TX), and University of Miami (Miami, FL).

Animals received approximately 10 to 15 biscuits of PMI Certified Primate Diet and one mineral and vitamin supplement (PRIMA-TREAT™, Bioserve, Frenchtown, New Jersey, U.S.A.) twice daily. They also received one piece of fresh fruit or vegetable daily. The animals were fed approximately 2 to 3 h pre- and post-dose. Water was available ad libitum. Monkeys were individually housed in stainless-steel cages in environmentally controlled HEPA-filtered rooms with a 12 h light cycle. For the duration of the study, the monkeys were transferred to a separate monkey room equipped for telemetry recording. Monkeys were placed into restraint chairs for dosing only.

Data, transmitted via radiotelemetry, was recorded by CA Recorder™ Systems (D.I.S.S. LLC., Pinckney, Michigan U.S.A.). Arterial blood pressure, Heart rate, PR interval, QRS interval, and QT interval were recorded. QT interval was corrected for heart rate using methods described by Miyazaki and Tagawa (2002). The log(QT) was expressed as a function of the log(HR) for all of the vehicle values for each individual monkey and fit with a linear regression (Miyazaki & Tagawa, 2002). Shown in Eq. (1) is the association of QT with heart rate was analyzed by linear regression on a logarithmic scale. Shown in Eq. (2) is the slope estimate used in an analysis of covariance (ANCOVA) model to correct QT (Eq. (2)), HRref=reference/mean heart rate for each monkey).

$$\log(\text{QT}) = \alpha + \beta \cdot \log(\text{HR}) \quad (1)$$

$$\log(\text{QT}_{\text{ca}}) = \log(\text{QT}) - \beta \cdot [\log(\text{HR}) - \log(\text{HR}_{\text{ref}})] \quad (2)$$

Data was collected for ≥ 24 h prior to the vehicle dose, ≥ 2 h pre-dose and ≥ 24 h post-dose with moxifloxacin or vehicle. Data were collected as 1-min mean values and reported as mean 15 min values \pm standard error of the mean.

2.1.3. Statistical analysis

In order to account for inherent differences in baseline cardiovascular values between monkeys, drug-induced changes in each parameter (variable) was normalized as the difference from its baseline for each individual animal. The baseline for

each animal was defined as the median measurement of each parameter collected 2 h prior to dosing. A binning of the data from the post dose period was achieved by dividing the data into 21 time intervals or bins: twelve 15-min intervals for the first three hours, three 60-min intervals for hours 3–6, three 120-min intervals for hours 6–12 and three 240-min intervals for the hours 12–24 after dosing. Each monkey at each time interval post dose contributed one observation to the analysis. The NOSTASOT (NO STATistical Significance Of Trend) method (Tukey, Ciminera, & Heyse, 1985) was used to test for an increase or decrease in response at each time interval over the dose range, as follows: Each response variable (difference from baseline) was analyzed using contrasts in an analysis of variance. Contrasts were constructed using an arithmetic dose scale. The NOSTASOT dose was defined to be the highest dose level if no statistically significant ($p > 0.05$) trend was found. If the trend was statistically significant at the highest dose tested, ($p \leq 0.10$ after multiplicity adjustment) then the NOSTASOT analysis was repeated with the highest dose group deleted. Dose groups were deleted by adjusting the vector of contrast coefficients, so all analyses used the same error term. Successive analyses were performed in this way until $p > 0.05$ was obtained. The NOSTASOT dose was defined to be the highest dose that yielded $p > 0.05$, and no additional p -values were computed. If the lowest active dose group yielded $p \leq 0.05$, the NOSTASOT dose was specified only as below the lowest dose in the experiment. All p -values were one-sided and adjusted for multiplicity of statistical tests using a permutation method to control the family-wise error rate.

2.2. Pharmacokinetic study

In a separate study, moxifloxacin was administered orally by nasogastric gavage in 0.5% methylcellulose at 10, 30, 100, 175 mg/kg to 1 female and 3 male non-instrumented monkeys. Each monkey received all 5 doses using a dose escalation paradigm identical to the cardiovascular study design. Samples of blood were collected from the monkeys dosed with moxifloxacin at the following time points: Pre-dose, 0.5, 2, 4, 8 and 24 h post-dose. Approximately 0.5 mL of blood was collected into chilled EDTA blood tubes at each time point and plasma samples were stored at $\leq -50^\circ\text{C}$.

Moxifloxacin was isolated from monkey samples by acetonitrile precipitation procedure, and was identified by electrospray LC-MS/MS analysis with a SCIEX API 4000

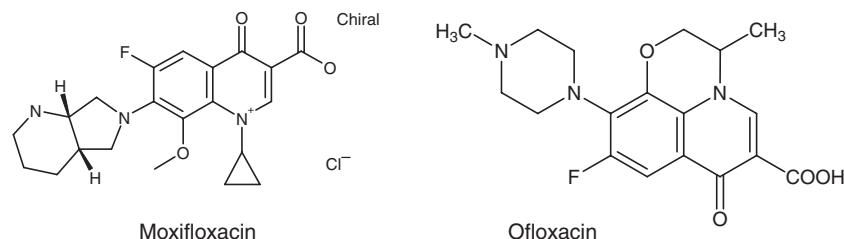


Fig. 1. Chemical structures of Moxifloxacin and Ofloxacin (the internal standard).

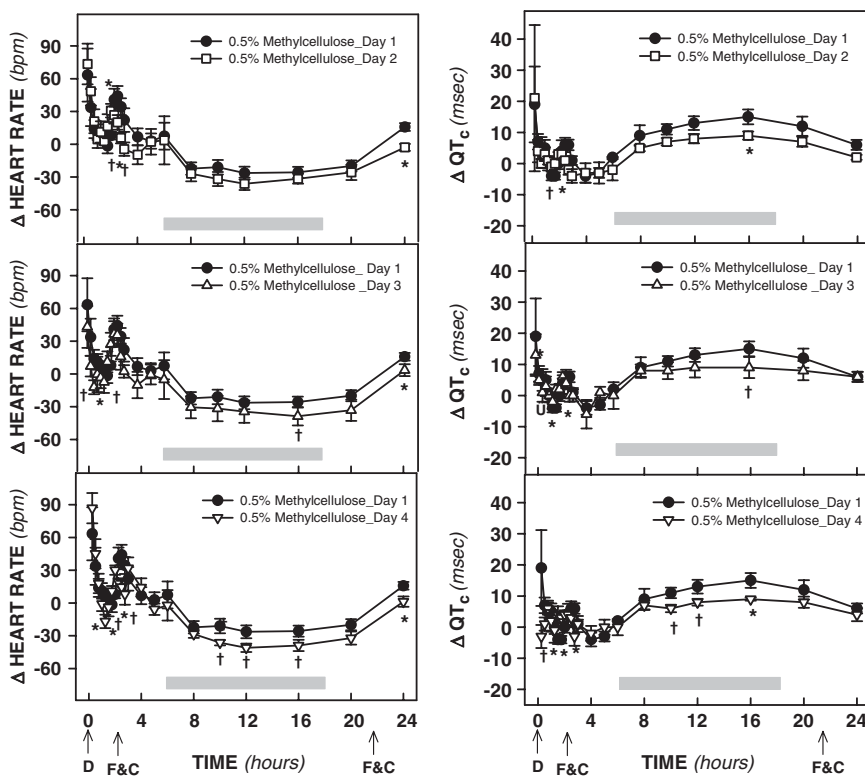


Fig. 2. *Left Panel*: Average changes in heart rate following repeated doses of 0.5% methylcellulose in deionized water. *Right Panel*: Average changes in QT_c interval following repeated doses of 0.5% methylcellulose in deionized water. Arrows represent time of dosing (D), feeding and cleaning (F&C). represents an approximate period of darkness. Data is represented as mean \pm SEM. Statistical significance represented as * $p \leq 0.05$, $\dagger p \leq 0.1$.

triple quadrupole mass spectrometer equipped with an Agilent 1100 HPLC pump and Leap CTC PAL autosampler. Chromatographic separation for Moxifloxacin and the Ofloxacin (Internal Standard), for structures see Fig. 1, was achieved on an Aqua C18 column (Phenomenex, 2×50 mm, $5 \mu\text{m}$ particle size) using a gradient consisting of water with 0.1% formic acid and acetonitrile with 0.1% formic acid. Spectra were acquired in the positive ionization mode by multiple reaction monitoring (MRM). The MRM transition for Moxifloxacin was m/z 402.5 \rightarrow 358.1 and for Ofloxacin was m/z 362.3 \rightarrow 318.1 at a 550°C source temperature, 5500 V ionspray voltage, collision energy and declustering potential ranged from 25–30 eV and 80–90 V, respectively. A dwell time of 150 ms was used for each transition. Daily calibration curves ranging from 0.0125 to $2.49 \mu\text{M}$ were constructed using the ratios of the observed peak areas of Moxifloxacin and the internal standard. Plasma concentrations of Moxifloxacin in unknown samples were determined by Watson LIMS software (Thermo/Informatics, Philadelphia, PA) using a $1/x^2$ weighted linear regression equation of the peak area ratio against concentration for the calibration curve.

Pharmacokinetic calculations were generated with noncompartmental analysis of moxifloxacin plasma concentrations versus time in WinNonlin $^{\text{C}}$ (Pharsight, Cary, NC).

3. Results

In general, repeated oral administration of moxifloxacin (maximum dose = 175 mg/kg) and 0.5% methylcellulose in deionized water were well tolerated by the monkeys with no abnormal physical signs observed at all dose levels.

Fig. 2 shows the average changes in heart rate and corrected QT interval (QT_c) (Miyazaki's correction) over 4 days of dosing with vehicle alone (0.5% methylcellulose). Increases in heart rate were observed during times of stress and excitation (dosing, cleaning and feeding). Decreases in heart rate were observed at night (grey bar represents approximate period of darkness; Fig. 2, Left Panel). Increases in QT_c interval were also observed at night ($\sim +10$ to $+15$ ms) as compared to the pre-dose baseline (morning; Fig. 2, Right Panel). When compared to day 1 of vehicle treatment, sporadic but statistically significant changes (range: 11% to 25%) in heart rate were observed on days 2, 3, and 4 (Fig. 2, Left Panel). Similarly, when compared to day 1 of

Fig. 3. (A) *Upper Panel*: Representative electrocardiogram from Monkey # 04-R470 at pre-dose baseline. *Lower Panel*: Representative electrocardiogram from Monkey #04-R470 approximately 16–20 h post administration of 175 mg/Kg of Moxifloxacin. (B) *Left Panel*: Average changes in heart rate, in rhesus monkeys, following 10, 30, 100 and 175 mg/kg of Moxifloxacin in 0.5% methylcellulose in deionized water. *Right Panel*: Average changes in QT_c interval, in rhesus monkeys, following 10, 30, 100 and 175 mg/kg of Moxifloxacin in 0.5% methylcellulose in deionized water. Arrows represent time of dosing (D), feeding and cleaning (F&C). represents an approximate period of darkness. Data is represented as mean \pm SEM. Statistical significance represented as * $p \leq 0.05$, $\dagger p \leq 0.1$.

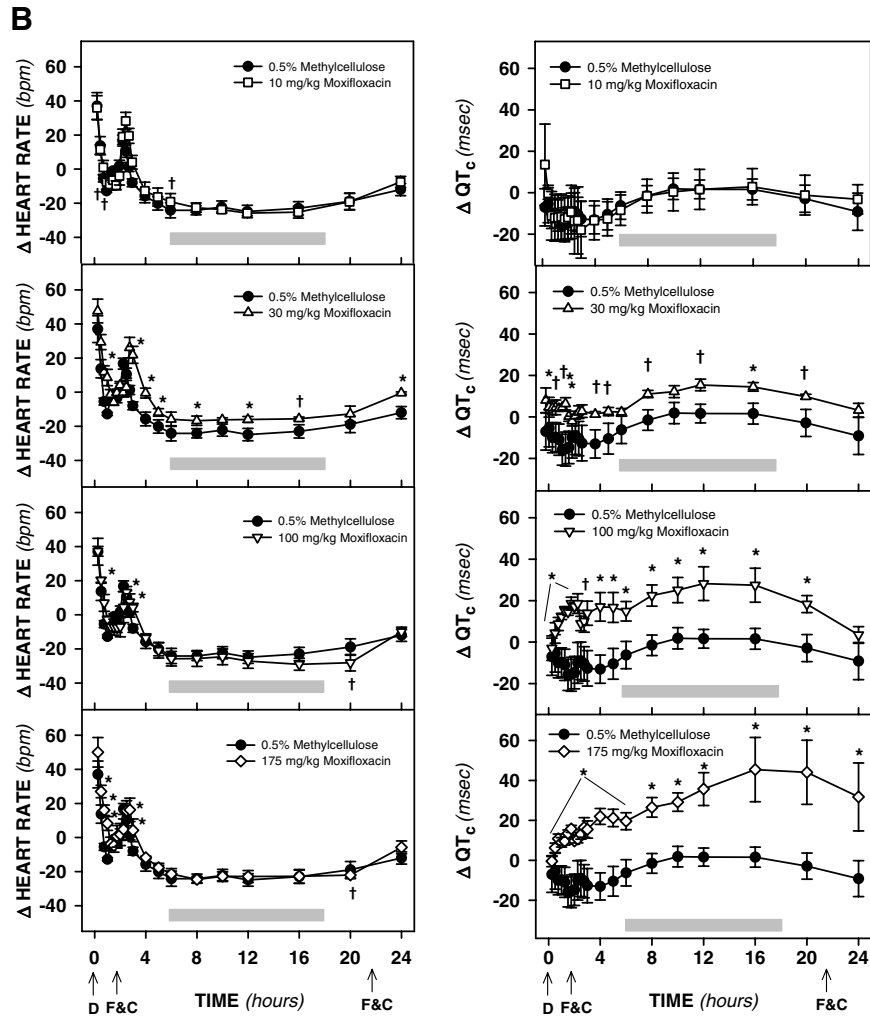
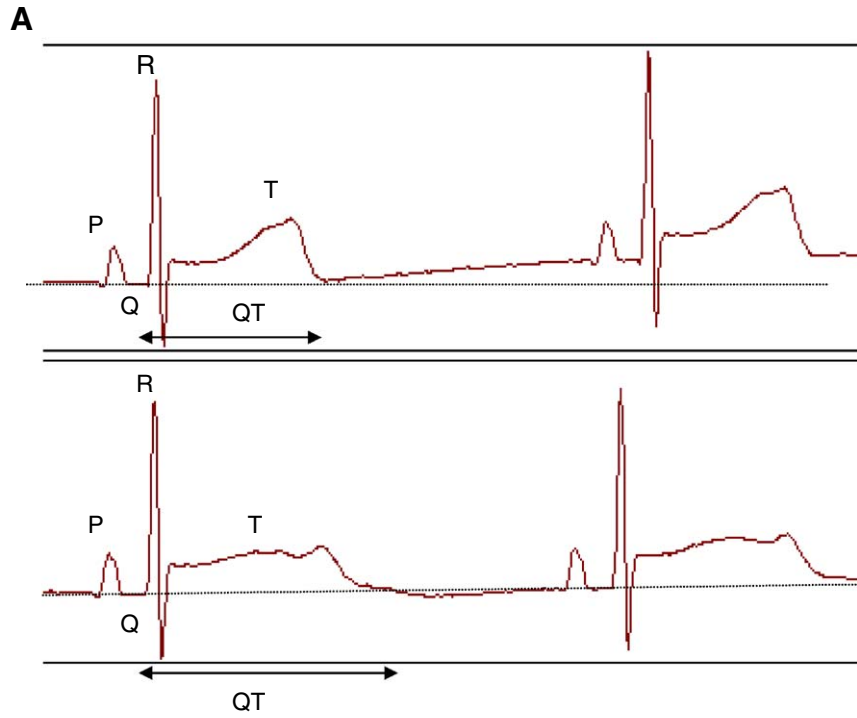


Table 1
Statistically significant increases (minimal and maximal detected change), at 30, 100, and 175 mg/kg, in QT_c interval

Moxifloxacin (mg/kg)	*Minimum ↑QT _c			*Maximum ↑QT _c		
	Time (h)	ms	%	Time (h)	ms	%
10	–	–	–	–	–	–
30	0.25–0.5	10	4	1.5–1.75	22	8
100	0.25–0.5	10	4	10–12	27	10
175	0.25–0.5	12	5	16–20	47	18

– No treatment-related effect; *statistical significance $p \leq 0.05$ relative to vehicle.

vehicle treatment, sporadic but statistically significant changes (range: 1% to 3%) in QT_c interval were also observed on days 2, 3, and 4 (Fig. 2, Right Panel).

Fig. 3A shows representative electrocardiographic (ECG) tracing from rhesus monkeys at pre-dose baseline and approximately 16 to 20h post 175 mg/kg moxifloxacin. Telemetry based ECG tracings from Rhesus monkeys were found to be of superior quality allowing for validation of automated measurements of QT interval measures according to Good Laboratory Practices (Fig. 3A). Fig. 3B plots average changes in heart rate and QT_c interval following 10, 30, 100 and 175 mg/kg of moxifloxacin. Sporadic, but statistically significant changes in heart rate were observed at all dose levels (Fig. 3B, Left Panel). These changes were not dose-dependent and were within the normal limits of variability observed with vehicle alone (Fig. 2), and therefore were not considered to be pharmacologically mediated. Significant, dose-dependent increases in QT_c interval were observed at 30, 100 and 175 mg/kg of Moxifloxacin (Fig. 3B, Right Panel). The maximum and minimum detectable increases in QT_c interval with moxifloxacin are summarized in Table 1. Moxifloxacin-induced increases in QT_c interval ranged from 10 to 47 ms (4% to 18%) and, exceeded the range of normal variability in QT_c observed with vehicle treatment alone (Table 1). The averaged mean arterial pressure, PR and QRS intervals following vehicle and moxifloxacin at approximately 4h post dose (approximate T_{\max}) are summarized in Table 2. Mean arterial pressure, PR, and QRS intervals remained unchanged (compared to vehicle) at all doses of moxifloxacin (Table 2).

Fig. 4 shows the pharmacokinetic profiles for each monkey over 24h after administration of moxifloxacin at doses of 10, 30, 100 and 175 mg/kg. The averaged ($n=4$ monkeys) pharmacokinetic parameters at each dose level are summarized in Table 3. Monkey #02-0001 pharmacokinetic profile, at 175 mg/kg of moxifloxacin, was markedly different from the oral pharmacokinetic profile of the other three monkeys in the group and was therefore not included in the pharmacokinetic calculations of peak plasma concentration and exposure at 175 mg/kg. Moxifloxacin levels in plasma rose rapidly at all dose levels. Plasma concentrations were sustained up to 8h post-dose at the 175 mg/kg dose group (Fig. 4). Mean maximum plasma concentrations (C_{\max}) were achieved at 3.5 to 5.3h post-dose (Table 3). Moxifloxacin elimination was rapid with mean trough (24-h) concentrations that were approximately 6% to 13% of the respective C_{\max} values between doses of 10 to 100 mg/kg. At a

dose of 175 mg/kg, moxifloxacin elimination appeared slow with a mean trough (24-h) concentration that was approximately 50% of the respective C_{\max} value; however, this apparent slow elimination may have been caused by prolonged absorption of moxifloxacin. Mean systemic exposure (AUC_{0-24h}) values of moxifloxacin were approximately dose proportional in all dose groups; however, mean C_{\max} values were less than dose proportional in all dose groups (Table 3).

4. Discussion

Several cardiac and non-cardiac drugs can delay the action potential duration and lead to QT interval prolongation (Fermini & Fossa, 2003; Redfern et al., 2003). QT interval prolongation is not dangerous per se, but can lead to a potentially fatal polymorphic tachycardia called Torsades de Pointes (Fermini & Fossa, 2003). Due to the potentially fatal nature of TdP, QT interval prolongation is used as a surrogate indicator of drug induced TdP in animals and humans (ICH, 2001, 2005). Therefore, there is a critical need for well-characterized preclinical models with the ability to detect and predict QT interval prolongation in humans. In this study, we have developed a primate model in rhesus monkeys with cardiovascular telemetry measurements and have tested the sensitivity of the model to detect QT changes using moxifloxacin, an agent well-known to prolong QT in humans (Camm, 2005; Demolis et al., 2000; Noel et al., 2003). The variability within the model and the study design was also evaluated with repeated doses of vehicle.

The central focus of this study was to validate and characterize our preclinical primate telemetry model as a predictor of electrocardiographic (ECG) anomalies, particularly QT interval prolongation for human pharmaceuticals. Primate telemetry is an expensive investment preclinically, but the initial investment in surgical time and expense is well-warranted given the usefulness of the model for preclinical cardiovascular safety evaluations and its ability to predict human outcomes. Moxifloxacin, a fluoroquinolone antibiotic, has been shown to produce reproducible increases in QT/QT_c interval in normal healthy human volunteers with little to no risk of TdP (Demolis et al., 2000; Noel et al., 2003). Therefore, it has been employed as a positive control agent to evaluate the sensitivity for detection of QT changes in human Thorough Clinical QT studies (TCQT) (Camm, 2005; Demolis et al., 2000; Noel et al., 2003). In this study, moxifloxacin administered orally to rhesus monkeys resulted in dose-dependent increases in QT/QT_c interval at 30, 100, and 175 mg/kg, which were analogous to

Table 2
Mean Arterial Pressure (MAP), PR and QRS interval at 4h post dose ($\sim T_{\max}$) of Moxifloxacin

	Vehicle	Moxifloxacin (mg/kg)			
		10	30	100	175
MAP (mmHg)	103±3	106±2	105±5	105±5	101±4
PR (ms)	83±2	92±9	83±1	84±1	82±2
QRS (ms)	49±1	49±1	49±2	48±2	49±1

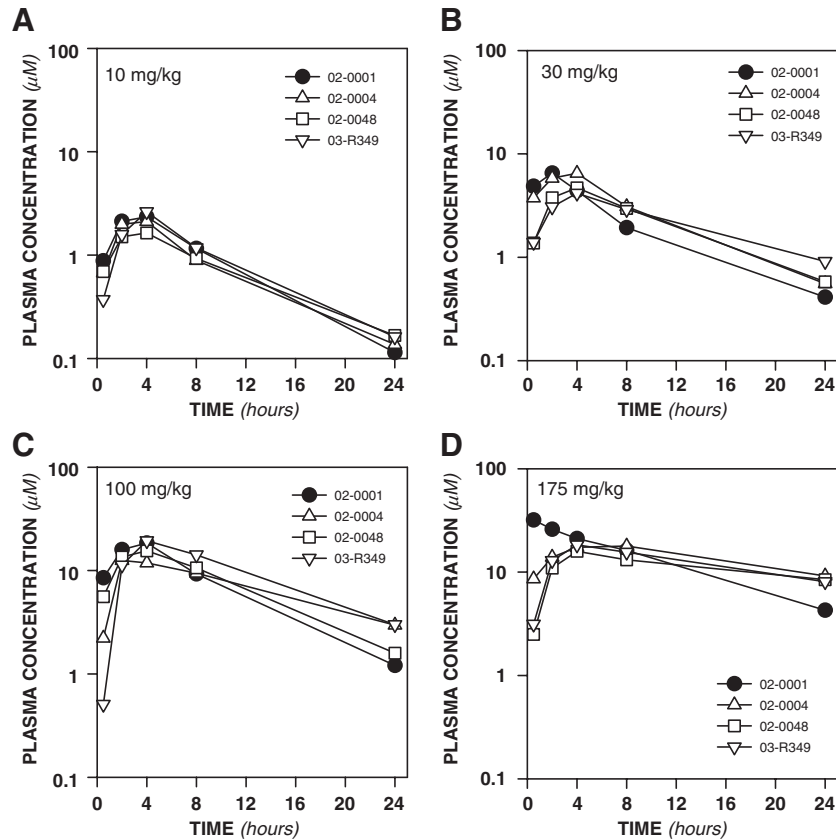


Fig. 4. Pharmacokinetic profiles (over 24h) of Moxifloxacin at 10mg/kg (A), 30mg/kg (B), 100mg/kg (C), and 175mg/kg (D).

the effects observed in humans (Demolis et al., 2000; Noel et al., 2003). At 30mg/kg of moxifloxacin in the monkeys, increases in QT_c interval ranging between 10 to 22ms (4% to 8%) were comparable to the increases in QT/QT_c interval ($4.0 \pm 5.1\%$) observed in humans at a therapeutic dose of 400mg (Demolis et al., 2000). Additionally, the maximal plasma concentration attained at 30mg/kg ($C_{max} = 5.5 \pm 0.6 \mu M$) was also similar to the maximal plasma concentration attained at 400mg in humans ($C_{max} = 7.5 \pm 2.7 \mu M$) (Demolis et al., 2000). At 100mg/kg of moxifloxacin in monkeys, increases in QT_c interval ranged from 10 to 27ms (4% to 10%) and were also comparable to the increases in QT/QT_c interval ($4.5 \pm 3.8\%$) observed in humans at supra-therapeutic dose of 800mg (Demolis et al., 2000). Similarly, the maximal plasma concentration attained at 100mg/kg ($C_{max} = 16.5 \pm 1.6 \mu M$) was

also comparable to the maximal plasma concentration attained at 800mg of moxifloxacin in humans ($14 \pm 3 \mu M$) (Demolis et al., 2000). The incidence of TdP with moxifloxacin is very low (4 per 7.7 million cases) in humans and most reported cases are primarily accompanied by other concomitant risk factors for TdP (Camm, 2005). Similarly, in this monkey study (free of other concomitant TdP risk factors) moxifloxacin treatment even at the highest exposure ($AUC = 302.2 \pm 20.0 \mu M h$) did not produce a single episode of TdP.

An important characteristic of the monkey telemetry model is its ability to detect increases in the QT/QT_c interval analogous to those observed in humans, as supported by the results of moxifloxacin treatment in this study. Additionally, the monkey telemetry model offers several other advantages as a preclinical cardiovascular safety model for studying novel human pharmaceuticals. These include the ease of data collection, similar circadian patterns in heart rate and the QT/QT_c interval as compared to humans, limited stress on the animals, superior ECG tracings and low intra-animal variability. Unlike other conscious electrocardiographic evaluations in which monkeys are acclimated to experimental conditions for long periods of time (~6 months) in order to obtain high quality data (Hassimoto & Harada, 2004), no acclimation or training are required for this model because the monkeys are remotely monitored in telemetry cages similar to their standard housing environment. This limits direct investigator involvement to short periods of dosing, feeding or cleaning and reduces the time animals may experience excitability to a minimum,

Table 3
Pharmacokinetics, in rhesus monkeys, following a single oral dose of 10, 30, 100 and 175 mg/kg of Moxifloxacin

	Moxifloxacin (mg/kg)			
	Sexes combined			
	10	30	100	175 ^a
AUC_{0-24h} ($\mu M h$)	21.9 ± 1.3	58.2 ± 3.6	202.6 ± 14.4	302.2 ± 20.0
C_{max} (μM)	2.2 ± 0.2	5.5 ± 0.6	16.5 ± 1.6	17.3 ± 0.7
T_{max} (h)	4.0 ± 0.0	3.5 ± 0.5	3.5 ± 0.5	5.3 ± 1.3

Data represented as mean \pm SEM.

^a 02-0001 was not included in the pharmacokinetic calculations.

negating the need for sedatives or anesthesia commonly used in other conscious electrocardiographic studies (Atkins & Dickie, 1986; Gonder, Gard, & Lott, 1980). In this study, physiologically relevant heart rates that averaged ~ 130 bpm at pre-dose baseline were maintained on each day of the study. Additionally, circadian heart rate decreases of approximately 30 bpm (-23%) were observed during periods of sleep as compared to pre-dose baseline (periods of consciousness), and these were similar to those reported in humans (Smetana, Batchvarov, Hnatkova, Camm, & Malik, 2003). In this study, monkeys were monitored for approximately 24h post dose, but cardiovascular parameters can be easily monitored for much longer durations allowing for safety evaluations of drugs or their corresponding metabolites which exhibit very long half lives.

Unlike other primate ECG studies, a single lead ECG was obtained in this telemetry model (Atkins & Dickie, 1986; Gonder et al., 1980; Hassimoto & Harada, 2004). While multiple leads are useful for detecting differential electrical abnormalities in the heart, the single high quality lead in these studies provided excellent recordings for the main intent of assessing QT interval changes. Additionally, multiple leads may lead to extensive data analysis and resource utilization, but a single lead allows for a reduction in unnecessary use of resources for data analysis while maintaining the quality of ECG recordings and QT interval measures. In these studies, the ECG tracings were of superior quality highlighting a well defined T wave which allowed for standardized automated measurements of QT interval. Unlike restrained ECG studies, the lower heart rates maintained on this telemetry study allowed for a clear delineation of the end of T wave due to the lack of fused T and P waves often observed at heart rates >200 bpm (Hassimoto & Harada, 2004). Therefore, the standardized automated measures of QT interval were found to be reproducible within each monkey from day to day as was evident by the low intra-animal variability in heart rate (coefficient of variation (CV%) $<10\%$) and QT_c interval (CV% $<2\%$) observed during a large portion of the monitoring period. However, as expected during periods of excitability (dosing, cleaning, and feeding), the number of ECG waveforms recognizable by the computer algorithm were significantly reduced (data not shown), leading to large variability during these stressful time periods. Nevertheless, these disruptive periods of excitability were significantly reduced in a telemetry model as compared to a restrained ECG study.

Since the intra-animal variability in our monkey telemetry model was low (CV% $<2\%$ to 10%), it allowed us to maximize our statistical power with a small group of animals ($n=4$). In the vehicle study, the high statistical power lead to sporadic, but significant changes in heart rate and QT_c interval with repeated single doses of vehicle alone. The low background variability of QT_c ($\leq 3\%$) in this model allows for the reliable detection of small but significant treatment-related changes of $>3\%$.

Circadian patterns of corrected QT interval (QT_c) are evident in humans ($\sim +12$ ms increases at night) (Noel et al., 2003), and herein the monkeys exhibited increases in QT_c interval of approximately 10 to 15ms at night consistent with other published reports (Gauvin et al., 2006). These circadian changes

in QT_c interval may be attributable to heart rate correction techniques. Therefore, in attempt to account for normal circadian patterns, statistical comparisons between moxifloxacin treatment and vehicle were made at the same time of day. QT/RR relationships from conscious telemetry measures in dogs, monkeys, and humans have been shown to be variable and fixed relationships to normalize QT do not sufficiently account for circadian or individual variations (Gauvin et al., 2006; Miyazaki & Tagawa, 2002; Smetana et al., 2003). In an attempt to address this concern, subject-specific correction techniques that account for circadian and individual variations in QT/RR relationships have been successfully applied to telemetry data from conscious dogs (Miyazaki & Tagawa, 2002). More recently, this correction technique has also been applied to conscious telemetry data from monkeys and has proven to be a very effective heart rate correction technique for QT interval measures in cynomolgus monkeys, when compared to other standard correction techniques like Bazett's, Fridericia's, and Vande Water's correction (Gauvin et al., 2006). Additionally, since QT/RR or QT/HR relationships differ greatly among humans and animals, and because it is recommended by the ICH guidelines that subject specific QT corrections are employed, a subject specific analysis of covariance (ANCOVA) model to correct QT was employed in this study (Gauvin et al., 2006; ICH, 2005; Malik, 2002; Miyazaki & Tagawa, 2002; Smetana et al., 2003).

In conclusion, we have demonstrated the utility of conscious telemetrized rhesus monkey as a reproducible, sensitive and reliable model for electrocardiographic assessment of QT interval prolongation. This model exhibited low intra-animal variability and high degree of sensitivity to detect small but significant QT/QT_c interval increases ($\sim 4\%$) with MOX in the same therapeutic plasma concentration range attained in humans. Therefore, the primate telemetry model should be considered an important preclinical predictor of QT prolongation of novel human pharmaceuticals.

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