

Sensitivity to detect QT interval prolongation in a Telemetrized Beagle Dog: Cardiovascular studies with Moxifloxacin

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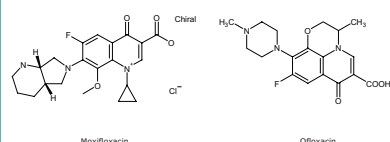
Abstract

Moxifloxacin (MOX) was used to evaluate the sensitivity and validity of the dog telemetry model as a preclinical predictor of QT interval prolongation in humans. Cardiovascular (hemodynamic and electrocardiographic) monitoring, via telemetry implants, was conducted for 2hr pre-dose and 24hr post-dosing with MOX with a toxicokinetic evaluation in a separate group of dogs. In both studies, MOX was administered orally by gavage in 0.5% methylcellulose at 0, 10, 30, and 100mg/kg. Inherent variability of the model was assessed with administration of 4 doses of vehicle (0.5% methylcellulose) alone in all 4 dogs to mimic a dose-escalation paradigm. QT was corrected for heart rate (QT_c) using an individual animal correction (Miyazaki and Tagawa, 2002). MOX produced significant dose-related increases in QT_c at doses of 10 (C_{max} = 7.0±0.5µM), 30 (C_{max} = 20.4±1.8µM), and 100 (C_{max} = 39.5±5.91µM) mg/kg with peak increases of 14 (6%), 27 (12%), and 45 (20%) msec, respectively (p≤0.05 vs. vehicle). QT_c increases (3 to 6%) at 10mg/kg of MOX were comparable to QT/QT_c increases (4.0±5.1%) in humans at a therapeutic dose (400mg, C_{max} = 7.5±2.7µM) (Demolis et al., 2000). The small change in QT_c (3%) at 10mg/kg of MOX was not due to the variability in QT_c observed in dogs as no significant QT_c changes occurred on days 2, 3, and 4 in a study with vehicle alone. In conclusion, the dog telemetry model exhibits high sensitivity to detect small but significant QT/QT_c increases with MOX in the same range of therapeutic plasma concentrations attained in humans.

INTRODUCTION

- Novel Cardiac & non-cardiac drugs may be associated with
 - QT interval prolongation
 - potentially fatal polymorphic tachycardia – Torsades de Pointes (TdP)
- Potential of novel drugs to cause TdP in humans is difficult to predict in preclinical studies
 - Human condition complicated by genetics, pre-existing diseases, & medications
- Delayed ventricular repolarization (QT interval prolongation) is used as a surrogate marker of TdP
 - Efficient, sensitive and reliable preclinical methods of evaluation are required to detect/predict QT interval prolongation in humans
- Moxifloxacin: a fluoroquinolone antibiotic
 - Potential to produce small but significant QT prolongation in humans
 - Mediated most likely by its inhibitory activity of hERG
- Moxifloxacin employed as positive control for Thorough Clinical QT studies
 - Ability to produce moderate QT changes with little or no risk of TdP
- We have developed a telemetry model in beagle dogs
 - Use of Moxifloxacin to assess the sensitivity and efficiency of this preclinical model to detect and/or predict QT interval prolongation in humans

Figure 1. Structures of Moxifloxacin and of the internal standard Ofloxacin



OBJECTIVES

- To evaluate the electrocardiographic effects of escalating oral doses of Moxifloxacin in conscious dogs
- To further evaluate the normal variability of the model/study design with repeated administration of vehicle (0.5% Methylcellulose in deionized water)

METHODS

Cardiovascular Studies

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). Moxifloxacin was administered orally in 0.5% methylcellulose at 0, 10, 30, 100 (5 mL/kg). Each dog received all 4 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 dogs using the same paradigm as in the moxifloxacin study.

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.
- Three female and one male beagle dogs instrumented with a Konigsberg cardiovascular implant were used in the moxifloxacin study and 1 male and 3 females were used in the vehicle study (Age: ~1 to 3 years; Weight: ~8 to 14 kg).
- Dogs received 300 g/day of PMI Certified Canine Diet. Water was available *ad libitum*. They were fed approximately 3 to 4 hours post dose.

- Dogs were individually housed in stainless-steel dog runs in an environmentally controlled, HEPA-filtered room with a 12-hour light cycle. On the days of data collection, dogs were transferred to a separate dog room equipped for telemetry recording.

- 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 (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 dog and fit with a linear regression (Miyazaki and Tagawa, 2002).

- Shown in equation 1 is the association of QT with heart rate which was analyzed by linear regression on a logarithmic scale. Shown in equation 2 is the slope estimate used in an analysis of covariance (ANCOVA) model to correct QT (equation 2), HR_{ref} = mean heart rate for each dog.

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

$$\text{Equation 2: } \log(QT_c) = \log(QT) - \beta \cdot [\log(HR) - \log(HR_{ref})]$$

- Data was collected for ≥20 hours prior to the vehicle dose, ≥2 hours pre-dose of moxifloxacin and ≥24 hours post-dose with moxifloxacin or vehicle. Data were reported as mean 15 minute values ± standard error of the mean.

Statistical Analysis:

- In order to account for inherent differences in baseline cardiovascular values between dogs, 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 hours prior to dosing.
- The post dose period was divided into 21 time intervals: twelve 15-minute intervals for the first three hours, three 60-minute intervals for hours 3 – 6, three 120-minute intervals for hours 6 – 12 and three 240-minute intervals for the hours 12 – 24 after dosing.
- Each dog at each time interval post dose contributed one observation to the analysis.
- The NOSTASOT (NO STATISTICAL Significance Of Trend) method (Tukey et al., 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≤0.05 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≤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.

Pharmacokinetic Studies

- In a separate study, moxifloxacin was administered orally in 0.5% methylcellulose at 10, 30 and 100 mg/kg to 2 female and 2 male dogs.

- Each dog received all 4 doses using a dose escalation paradigm identical to the cardiovascular study design.

- Samples of blood were collected from the dogs dosed with moxifloxacin at the following time points: Predose, and 0.5, 1, 2, 4, 6, 8, and 24 hours postdose

- Approximately 0.5 mL of blood was collected into chilled EDTA blood tubes at each time point and plasma samples were stored at ≤ -60°C.

- Moxifloxacin was isolated from dog samples by acetonitrile precipitation procedure, and was identified by electrospray LC-MS/MS analysis with a SCIEX API 4000 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 Figure 1, was achieved on a Aqua C18 column (Phenomenex, 2 x 50mm, 5µm 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 402.4→358.3 and for Ofloxacin was m/z 362.2→318.1 at a 500°C source temperature, 4500 V ionspray voltage, collision energy and declustering potential ranged from 25–30 eV and 75–90 V respectively.

- A dwell time of 150 ms was used for each transition. Daily calibration curves ranging from 0.0249 µM to 2.49 µM 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/x2 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® (Pharsight, Cary, NC).

Figure 2: Average Change in Heart Rate and QT_c following 4 doses of 0.5% Methylcellulose in Deionized Water

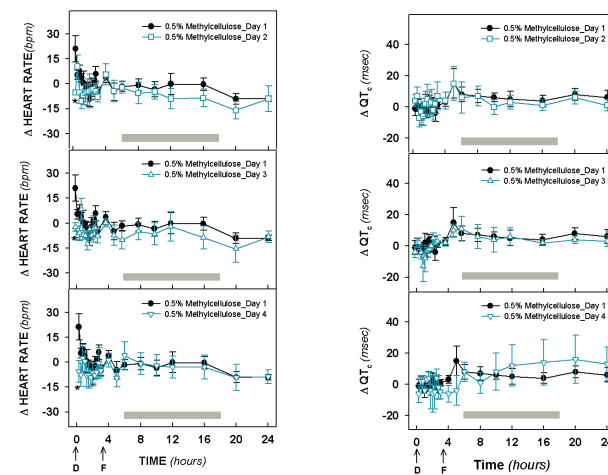
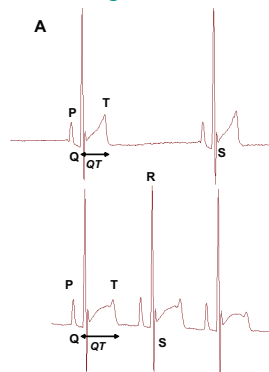


Figure 2: Left Panel: Average change (normalized to pre-dose baseline) in heart rate following 4 doses of methylcellulose in deionized water

Right Panel: Average change (normalized to pre-dose baseline) in QT_c following 4 doses of methylcellulose in deionized water

Arrows represent time of dosing (D) and feeding (F). — Represents an approximate period of darkness. Data is represented at mean ± SEM. Statistical significance represented as *p≤ 0.05.

Figure 3A:



Upper Panel: Representative electrocardiogram from dog #41042108 following 0.5% methylcellulose alone.

Lower Panel: Representative electrocardiogram from dog #41042108 approximately 16 - 20 hours post administration of 100 mg/kg Moxifloxacin

Table 1: Statistically significant increases (minimal and maximal detected changes) at 10, 30, and 100 mg/kg of Moxifloxacin in QT_c interval

Moxifloxacin (mg/kg)	* Minimum ↑QT _c			* Maximum ↑QT _c		
	Time (hr)	msec	%	Time (hr)	msec	%
10	2.75 - 3	6	3	1-1.25	14	6
30	1.25-1.5	15	7	1-1.25	27	12
100	0.25-0.5	9	4	16-20	45	20

* Statistical significance p≤ 0.05 relative to vehicle

Results

Figure 4: Pharmacokinetic profiles (over 24 hours) of Moxifloxacin at 10 mg/kg (A), 30 mg/kg (B), and 100 mg/kg (C)

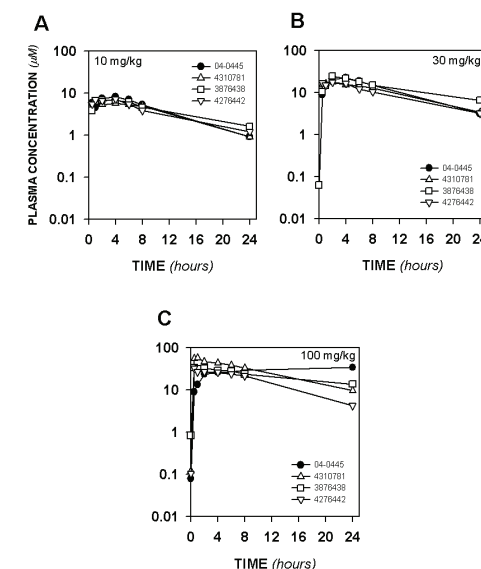


Figure 3B: Increased Heart Rate at 100 mg/kg of Moxifloxacin and increased QT_c interval at 10, 30 and 100 mg/kg of Moxifloxacin

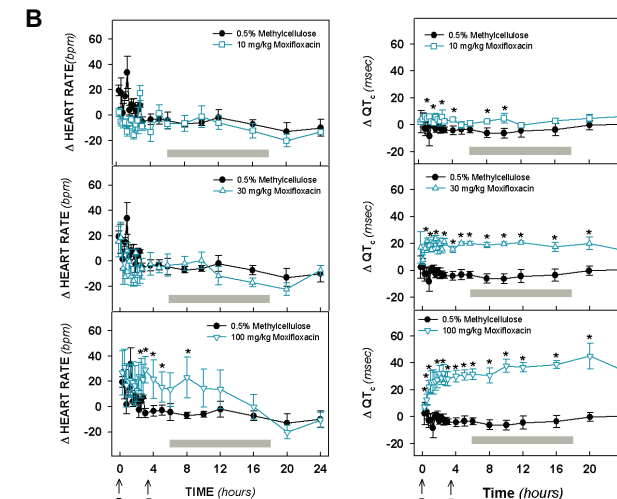


Figure 3B:

Left Panel: Average change (normalized to pre-dose baseline) in heart rate, in conscious dogs, following 10, 30 and 100 mg/kg of Moxifloxacin in 0.5% methylcellulose in deionized water.

Right Panel: Average change (normalized to pre-dose baseline) in QT_c in conscious dogs, following 10, 30 and 100 mg/kg of Moxifloxacin in 0.5% methylcellulose in deionized water.

Arrows represent time of dosing (D) and feeding (F). — Represents an approximate period of darkness. Data is represented at mean ± SEM. Statistical significance represented as *p≤ 0.05.

Table 2: Pharmacokinetics, in beagle dogs, following a single oral dose of 10, 30 and 100 mg/kg Moxifloxacin

	MOXIFLOXACIN (mg/kg)		
	10	30	100
AUC _{0-24hr} (µM.hr)	94.6 ± 3.67	268 ± 21.5	578 ± 68.6
C _{max} (µM)	6.99 ± 0.493	20.4 ± 1.75	39.5 ± 5.91
T _{max} (hr)	3.5 ± 0.50	2.5 ± 0.5	6.5 ± 5.8

CONCLUSIONS

- Moxifloxacin produced significant dose-related ↑QT_c at
 - 10 mg/kg (C_{max} = 7.0 ± 0.5µM)
 - Peak Increases: +14 msec (6%)
 - Comparable ↑QT/QT_c interval (4.0 ± 5.1%) observed in humans at therapeutic doses of 400mg (Demolis et al., 2000)
 - similar to the C_{max} attained at 400 mg in humans (C_{max} = 7.5 ± 2.7 µM) (Demolis et al., 2000)
 - 30 mg/kg (C_{max} = 20.4 ± 1.8µM)
 - Peak Increases: +27 msec (12%)
 - similar to the C_{max} at 400 mg in humans (C_{max} = 7.5 ± 2.7 µM) (Demolis et al., 2000)
 - 100 mg/kg (C_{max} = 39.5 ± 5.91µM), Peak Increases: +45 msec (20%)
- No statistically significant changes in QT_c interval on days 2, 3, and 4 following vehicle alone
 - small ↑QT_c (3%) at 10 mg/kg of Moxifloxacin was not due to the variability in QT_c observed in dogs in an escalating study design
- The dog telemetry model exhibits high sensitivity to detect small but significant increases in QT/QT_c interval (~3%) with Moxifloxacin
 - in the same range of therapeutic plasma concentrations attained in humans

- Superior electrocardiogram quality & appropriate use of QT correction factors
 - contributors to the reproducibility, reliability & sensitivity

The dog telemetry model should be considered an important preclinical predictor of QT prolongation of novel human pharmaceuticals!