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Original article

Application of a probabilistic method for the determination of drug-induced QT prolongation in telemetered cynomolgus monkeys: Effects of moxifloxacin

Henry H. Holzgrefe [a,*], Icilio Cavero [b], Lewis V. Buchanan [a], Michael W. Gill [a], Stephen K. Durham [a]

[a] *Bristol-Myers Squibb Pharmaceutical Research Institute, United States*
[b] *Via dei Bacchettoni 19B, 55100 Lucca, Italy*

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Abstract

Introduction: Moxifloxacin is the most widely used positive reference agent in clinical cardiac repolarization safety studies, but it has not been characterized in the cynomolgus monkey. This important experimental animal species exhibits pronounced heart rate variability, complicating the temporal evaluation of QT interval data. **Methods:** Digitized epicardial ECGs and aortic blood pressures were collected for 20 h in telemetered cynomolgus monkeys ($n=6$) following the administration of either vehicle or moxifloxacin (10 or 50 mg/kg, p.o.). Moxifloxacin plasma concentrations were determined 4 h postdose. ECG intervals were analyzed by computerized algorithms. Individual probabilistic QT rate-corrections (QTc) were derived from the slopes of predose log-transformed QT–RR data where each QT value was the mean of >250 beats/RR increment. The resulting QTc was used to determine the repolarization effects of moxifloxacin, expressed as the placebo-adjusted change in QTc (ΔQTc), and as the integrated response from 0 to 12 h ($AUC_{0\rightarrow12}$) postdose. **Results:** No ΔQTc effect was produced by 10 mg/kg moxifloxacin. However, moxifloxacin (50 mg/kg; 5.86±0.5 μg/mL C_{max}) significantly prolonged the RR interval by 50 to 112 ms from 3.5 to 7.5 h postdose and ΔQTc by ≥7.2 ms from 1.83 to 9.17 h, with a maximal ΔQTc effect of +26.4 ms. No notable effects on either systemic blood pressure or body temperature occurred with either dose. **Discussion:** Probabilistic QT rate-corrections appear to have eliminated the confounding effects of heart rate, provided for a stable QTc baseline, and enabled the demonstration of an exposure-dependent QTc prolongation by moxifloxacin. The duration and magnitude of the QTc effect paralleled moxifloxacin pharmacokinetics, and C_{max} values were similar to those achieved clinically in thorough QT/QTc studies. Thus, novel probabilistic QT rate-corrections may offer highly robust assessments of repolarization risk in both nonclinical and clinical investigations.

Keywords: Cynomolgus monkey; ECG; Epicardial; Methods; Moxifloxacin; Probabilistic QT correction method; QT prolongation; QT rate-correction; Telemetry

1. Introduction

QT–RR relationships have been extensively modeled and analyzed employing a battery of mathematical constructs varying from the simple to the very complex (Malik, 2001; Malik, Hnatkova, & Batchvarov, 2004a). While some of these models have improved upon the accuracy of classic heart rate correction formulae based upon observations initially made by Bazett (1920) and Fridericia (1920), none have achieved the *ultimate precision* (Malik et al., 2004a) that is necessary to satisfy current regulatory requirements. Indeed, a recent ICH guidance document for clinical studies on cardiac repolarization (E14) requires the reliable detection of threshold QTc changes in the range of 5–10 ms (ICH, 2005; Cavero & Crumb, 2006; Darpo, Nebout, & Sager, 2006).

In a recent FDA analysis of 19 drugs submitted for approval, the nonclinical data derived from the *in vitro* (hERG channel) and *in vivo* (QTc interval) assays recommended by ICH S7B were poorly predictive (≤50%) of the clinical outcome, assessed as a placebo-adjusted QTc increase of ≥5 ms. As such, the FDA currently holds that nonclinical assays are insufficiently sensitive

* Corresponding author. Bristol-Myers Squibb, P.O. Box 4755, Mail Stop J4, Syracuse, NY 13221-4755, United States. Tel.: +1 315 432 2564.
E-mail address: HenryHolzgrefe@Hotmail.com (H.H. Holzgrefe).

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to accurately predict repolarization liabilities in man. For this reason, a thorough QT/QTc study is now required for virtually all newly developed pharmaceutical agents (Cavero & Crumb, 2006; Darpo et al., 2006).

The low predictability for nonclinical repolarization assays in the FDA experience was probably due, at least in part, to the use of experimental protocols which are known to provide potentially flawed results. This has been especially true in the case of heart rate corrections of the raw QT interval (Cavero & Crumb, 2005, 2006). Importantly, Malik has observed that "…all proposed models of QT/RR regression represent a convention of data analysis rather than the nature of the physiological process" (Malik, 1996). In an effort to generate a QT/RR model that more correctly represented the true nature of electrophysiological repolarization, we recently proposed that individual raw QT intervals may not be accurately quantified as discrete measurements, but may be precisely described only as probabilistic values derived from a relatively large range of values (Holzgrefe et al., 2007). In order to more fully characterize the robustness of our probabilistic QT rate-correction, we examined the *in vivo* effects of the clinical QT prolonging reference agent, moxifloxacin, at typical therapeutic concentrations (Camm, 2005; Extramiana et al., 2005). Studies on myocardial repolarization, assessed as changes in the rate-corrected QT (QTc), were performed in the telemetered cynomolgus monkey, a species commonly used in nonclinical cardiac safety studies.

The purposes of this study were threefold: In the cynomolgus monkey: (1) to demonstrate and characterize the implementation of probabilistic QTc analysis in a repolarization cardiovascular safety study; (2) to fully characterize the temporal repolarization effects of moxifloxacin assessed by probabilistic QTc determinations; and (3) to characterize the temporal hemodynamic effects of moxifloxacin.

2. Methods

2.1. Regulatory guidelines

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Animal Care and Use Committee at Bristol-Myers Squibb Company, Syracuse, New York.

2.2. Animal preparation

Six cynomolgus monkeys (3 males, subjects a–c [5.87± 0.12 kg] and 3 females, subjects d–f [4.63±0.0.06 kg]) were instrumented with radiotelemetry devices (Model TL11M3-D70-PCTP, Data Sciences International [DSI], St. Paul, Minnesota) employing epicardial ECG lead attachment as previously described (Holzgrefe et al., 2007). ECG signal fidelity was verified intraoperatively to assure proper lead function. One telemetry pressure transducer was inserted into the femoral artery for aortic blood pressure, while a second pressure transducer was placed in the left ventricle (*left ventricular radiotelemetry pressure data are not a part of the current study and are not reported herein*). Core body temperature was monitored by a sensor located within the telemetry implant and secured to the internal abdominal wall. All surgical procedures were performed in accordance with the manufacturer's recommendations.

2.3. Drug preparation and analysis

Moxifloxacin hydrochloride (Avelox®) tablets were obtained as a commercial pharmaceutical preparation (Bell Medical Services, Inc., Marlboro, NJ). Moxifloxacin tablets were ground and prepared as a suspension (20 mg/mL) in 0.5% methylcellulose and administered in ascending gavage doses of 10 and 50 mg/kg. Two days prior to each drug treatment, animals received a corresponding volume-equivalent of vehicle. Moxifloxacin doses were separated by 14 days. Venous blood samples were obtained 4 h after dosing for the determination of free moxifloxacin plasma concentrations (Tandem Labs, West Trenton, NJ). Collection of telemetry data commenced 1.5–2 h prior to dosing and continued for 20 h postdose.

2.4. Telemetered ECG acquisition

Animals were housed individually in stainless-steel cages of appropriate size, offered feed daily and purified tap water *ad libitum* via an automated watering system. Room environmental conditions were controlled with respect to temperature and humidity with alternating 12 h light/dark cycles. ECGs and aortic blood pressures were digitized at the maximum sampling rate (500 Hz) of the DSI telemetry transmitter for 21 h/day. Digitized data were archived for subsequent offline analysis with a Ponemah P3Plus data acquisition and analysis system (version 4.1, Data Sciences International, Valley View, Ohio).

The absolute temporal resolution for any discrete signal digitized at 500 Hz is ±2 ms. In the current study, probabilistic determinations were employed to estimate the means of QT interval populations where the number of cardiac cycles (n) was ≥ 250 (Holzgrefe et al., 2007). As the precision of the mean for any population improves by $1/\sqrt{n}$, probabilistic raw QT estimates are characterized by sub-millisecond precision, even though the raw signals are acquired with a characteristic precision of ±2 ms for the individual complexes. All data were filtered through a 1–100 Hz bandpass filter which is embedded into the DSI telemetry transmitter, and is not adjustable by the end user. The DSI transmitter bandpass filter is implemented as a 1-pole high-pass filter with a 3 dB corner at 0.5 Hz, and a 1-pole low-pass filter with a 3 dB corner at 270 Hz. The transmitter bandpass filter is designed to include, at a minimum, the bandwidth from 1 to 100 Hz. Additional *post hoc* frequency filters were not employed during automated ECG analysis.

In this study, all interval measurements within the ECG complexes were fully automated. Aberrant waveforms (<1% of all ECG complexes), due to either physiologic or environmental noise, were objectively identified and rejected employing a *post hoc* combination of Ponemah P3Plus ECG algorithm performance descriptors (raw ECG values, *good* and *bad waves*, and T-wave count). These methods yielded discrete, noise-free filtered datasets in which reproducibility approached 100%.