Journal of Pharmacological and Toxicological Methods 61 (2010) 271–276

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Sensitivity of common marmosets to detect drug-induced QT interval prolongation: Moxifloxacin case study

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ARTICLE INFO

Article history:
Received 11 June 2009
Accepted 18 January 2010

Keywords:
Common marmoset
Telemetry
Moxifloxacin
QT interval prolongation
Probabilistic QT analysis
Safety pharmacology

ABSTRACT

Introduction: Moxifloxacin is the most widely used positive reference agent in clinical cardiac repolarization studies, but it has not been characterized in common marmosets which are uniquely suited to studies in early-stage development due to their small size and minimal test article requirements. The purpose of this study was to evaluate the sensitivity of the common marmoset to detect moxifloxacin-associated QT interval prolongation. **Methods:** Eight telemetered common marmosets were monitored for 24 h following oral administration of moxifloxacin by gavage at 0, 10, 30, and 100 mg/kg using a Latin square design. Concurrently, a pharmacokinetic evaluation in 8 non-telemetered animals was conducted. A rate-corrected QT (QTc) interval was derived using an individual probabilistic QT rate-correction. ΔΔQTc (placebo-adjusted QTc change from the individual baseline) was calculated and the relationship between pharmacokinetics (PK) and pharmacodynamics (PD) was analyzed. **Results:** A slight, but not significant, increase in ΔΔQTc was detected with 10 mg/kg of moxifloxacin. Moxifloxacin at 30 and 100 mg/kg elicited dose-dependent increases in ΔΔQTc of 14.0 ± 3.6 and 35.0 ± 6.2 ms, respectively, with associated total moxifloxacin C_{max} values of 6.5 ± 0.5 and 16.5 ± 1.6 μg/mL, respectively. From the PK/PD relationship, the plasma concentration which would attain ΔΔQTc of 5 to 10 ms was estimated to be 1.67–3.73 μg/mL. The results were consistent with typical clinical trial results (ΔΔQTc of 6.6–14.8 ms at 2.5–3.5 μg/mL). **Conclusions:** The present study demonstrates that the common marmoset is highly sensitive to moxifloxacin-associated changes in cardiac repolarization, assessed as ΔΔQTc. As such, this species is suitable for precise and reliable detection of small, but significant, drug-associated increases in QTc interval. Thus, the common marmoset should be regarded as a validated animal model for the detection of QT risk in early-stage drug development and represents an important addition to the current *in vivo* armamentarium.

1. Introduction

Several classes of non-cardiac drugs have been found to induce QT interval prolongation which is associated with a rare, but life-threatening, arrhythmia known as Torsades de Pointes (TdP) (De Ponti, Poluzzi, & Montanaro, 2001). Current regulatory preclinical safety guidance from the International Conference on Harmonization (ICH) (ICH S7B, 2005) requires *in vivo* assessment of the possible effects on the QT interval for new chemical entities in early-stage development. Common marmosets represent a valuable experimental animal for early-stage repolarization safety studies because of their small size, minimal test article requirements, and demonstrated sensitivity for the detection of drug-induced QT interval prolongation (Tabo et al., 2008).

In the ICH guidance for the clinical evaluation of drug-induced QT interval prolongation (ICH E14, 2005), the threshold of regulatory concern for changes in the rate-corrected QT (QTc) interval was set at 5 ms because of the established association of QT prolongation with TdP. The reliable detection of such a small change in nonclinical conscious cardiovascular studies requires robust methods to precisely quantify the QT interval and effectively dissociate it from the confounding effects of heart rate. In addition to the well-established rate-dependence of the QT interval, an intrinsic rate-independent QT variability has recently been described in the dog, cynomolgus monkey, and common marmoset (Holzgrefe et al., 2007; Tabo et al., 2008). Holzgrefe et al. (2007) proposed a novel probabilistic analytical method to adjust for rate-independent QT variability. However, the ability to detect drug-induced QT interval prolongation using probabilistic QT analysis has not yet been evaluated in common marmosets.

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doi:10.1016/j.vascn.2010.01.006

The ICH E14 guidance requires reliable detection of QT interval changes using a positive control to validate assay sensitivity. Moxifloxacin, a fluoroquinolone antibiotic known to prolong the QT interval (Balfour & Wiseman, 1999), has been used as a standard positive reference compound to validate test systems for the ability to detect small, but significant, increases in QTc intervals (Shah, 2005). Similarly, it is necessary to characterize the sensitivity of new animal models to moxifloxacin-induced QT interval prolongation to demonstrate the appropriateness of a given preclinical assay to predict QT risk in humans (Chaves et al., 2006, 2007; Chen et al., 2005; Holzgrefe, Cavero, Buchanan, Gill, & Durham, 2007).

Accordingly, the current study was designed to assess the sensitivity of common marmosets to detect moxifloxacin-induced QT interval prolongation employing probabilistic QT analysis and compare the results with corresponding clinical outcomes.

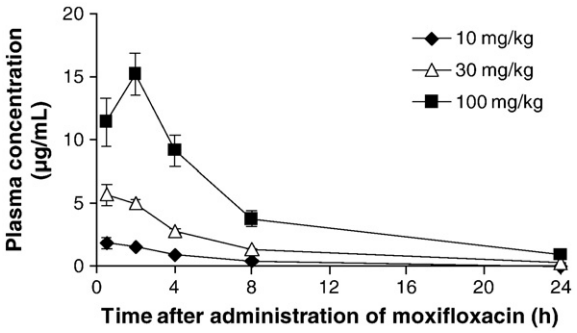

Fig. 1. Pharmacokinetic profiles of moxifloxacin at 10 (♦), 30 (△) and 100 mg/kg (■) in non-telemetered common marmosets. Data points represent the mean ± SEM of 8 animals.

2. Methods

2.1. Test drug

Moxifloxacin hydrochloride (moxifloxacin) was obtained from Chemos GmbH (Regenstauf, Germany).

2.2. Animals

Common marmosets obtained from Clea Japan, Inc. (Tokyo, Japan) were used in this study. All animals were housed individually in stainless steel cages placed in an animal room on a 12:12 h light–dark cycle (lighting: 07:00–19:00) with a temperature range of 24–31 °C and a relative humidity range of 35–75%. The animals were given solid food once daily with water available *ad libitum*. On the dosing day, the food was given between 5 h and 7 h post-dose. The care of the animals and the present protocols complied with the "General Consideration for Animal Experiments" promulgated in Chugai Pharmaceutical Co., Ltd. and was approved by the Institutional Animal Care and Use Committee.

2.3. Telemetry study

2.3.1. Surgical procedures

Eight telemetered common marmosets (4 males and 4 females) weighing 291.2–387.9 g, implanted with a telemetry transmitter (TL11M2-C50-PXT, Data Sciences International, St Paul, MN, USA), were used in this study. Briefly, the transmitter was implanted in the peritoneal cavity of each animal under isoflurane anesthesia. A telemetric transducer for measuring blood pressure was inserted into the abdominal aorta. The electrocardiogram (ECG) electrodes were implanted subcutaneously over the sternum and between the left 7th and 8th ribs (approximate lead II configuration). The animals were treated with analgesics (buprenorphine), antiphlogistics (flunixin meglumine) and antibiotics (cefazolin sodium) after surgery and allowed to recover for at least one month before use in this study.

2.3.2. Experimental protocol

Animals were administered vehicle and moxifloxicin at 10, 30, and 100 mg/kg in a Latin square design. Moxifloxacin was prepared as a suspension in 0.5% methylcellulose solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 5 mL/kg of the suspension was orally administered to the animals by gastric gavage at approximately 10:00 AM. The animals were fasted overnight before administration. Each animal was treated with the different dose levels with at least a 1-week interval between doses. Blood pressure and ECG signals were recorded with Dataquest ART data acquisition system (Data Sciences International) at 0.5 and 1 kHz, respectively, continuously from 2 h before until 24.5 h after dosing. The recordings were performed under quiet conditions except during scheduled periods of feeding, dosing and cleaning of the animal rooms and cages.

2.3.3. Cardiovascular parameters analyzed

The data obtained were automatically analyzed using HEM data analysis software (Notocord Systems SAS, Croissy sur Seine, France). All beat-to-beat QT and RR data were logged as the average of 10-s intervals and each mean value was used for the calculation of each individual QT rate-correction. The individual rate-corrected QT interval (QTc) was

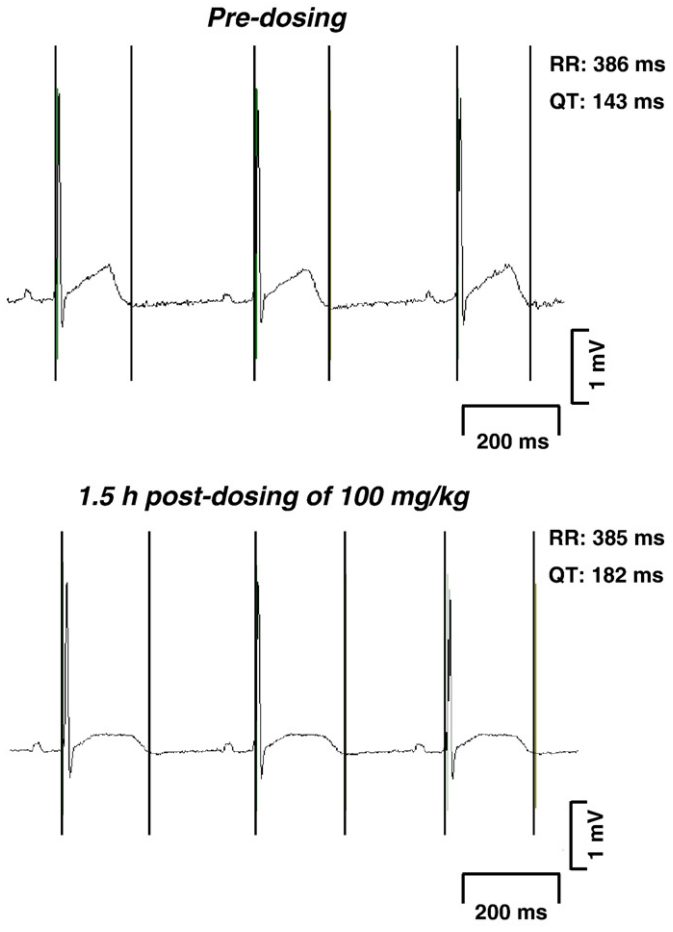

Fig. 2. Representative ECG traces in a single female common marmoset before (Pre) and 1.5 h after oral administration of moxifloxacin at 100 mg/kg, including fiduciary marks of the beginning of QRS complex and the end of the T-wave. The values of the RR and QT intervals are shown on each waveform.