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Translational science approach for assessment of cardiovascular effects and proarrhythmogenic potential of the beta-3 adrenergic agonist mirabegron

Cees Korstanje[a,*], Masanori Suzuki[b], Koichiro Yuno[c], Shuichi Sato[d], Masashi Ukai[e], Marlowe J. Schneidkraut[f], Gan X. Yan[g]

[a] Drug Discovery Sciences and Management – EU, Astellas Pharma B.V., Leiden, Netherlands
[b] Research Quality Assurance, Astellas Pharma Inc., Ibaraki, Japan
[c] Drug Safety Research Laboratories, Astellas Pharma Inc., Ibaraki, Japan
[d] Drug Repurposing and Application Management, Astellas Pharma Inc., Ibaraki, Japan
[e] Translational Science Research Labs, Astellas Pharma Inc., Ibaraki, Japan
[f] Drug Discovery Sciences and Management – US, Astellas Research Institute of America Northbrook, IL 60062, USA
[g] Lankenau Medical Center and Lankenau Institute for Medical Research, Wynnewood, PA, USA

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ABSTRACT

Introduction: Translational assessment of cardiac safety parameters is a challenge in clinical development of beta-3 adrenoceptor agonists. The preclinical tools are presented that were used for assessing human safety for mirabegron.

Methods: Studies were performed on electrical conductance at ion channels responsible for cardiac repolarization (I_{Kr}, I_{Ks}, I_{to}, I_{Na}, and $I_{Ca,L}$), on QT-interval, subendocardial APD_{90}, $T_{peak\text{-}end}$ interval, and arrhythmia's in ventricular dog wedge tissue in vitro and on cardiovascular function (BP, HR, and QT_c) in conscious dogs.

Results: In conscious dogs, mirabegron (0.01–10 mg/kg, p.o.) dose-dependently increased HR, reduced SBP but DBP was unchanged. Propranolol blocked the decrease in SBP and attenuated HR increase at 100 mg/kg mirabegron. Mirabegron, at 30, 60, or 100 mg/kg, p.o., had no significant effect on the QT_c interval. In paced dog ventricular wedge, neither mirabegron nor metabolites M5, M11, M12, M14, and M16 prolonged QT, altered transmural dispersion of repolarization, induced premature ventricular contractions, or induced ventricular tachycardia. Mirabegron nor its metabolites inhibited I_{Kr}, I_{Ks}, I_{to} I_{Na}, or $I_{Ca,L}$ at clinically relevant concentrations.

Discussion: Up to exposure levels well exceeding human clinical exposure no discernible effects on ion channel conductance or on arrhythmogenic parameters in ventricular wedge resulted for mirabegron, or its main metabolites, confirming human cardiac safety findings. In vivo, dose-related increases in HR with effects markedly higher than seen clinically, was mediated in part by cross-activation of beta-1 adrenoceptors. This non-clinical cardiac safety test program therefore proved predictive for human cardiac safety for mirabegron.

1. Introduction

Mirabegron is a selective beta-3 adrenoceptors (AR) agonist developed for the treatment of overactive bladder (OAB) (Hatanaka et al., 2013; Takasu et al., 2007). Beta-3-adrenoreceptors have been identified in various tissues in both humans and other species. At present, there are limited, and sometimes contradictory reports on the physiological role of beta-3 adrenoceptors which made clinical development of mirabegron a challenge (Michel & Korstanje, 2016). For example, Bosch et al. (2002) reported that stimulation of beta-3 adrenoreceptors in guinea-pig ventricular myocytes inhibited the slow delayed rectifier potassium current (I_{Ks}) (Bosch et al., 2002), while other authors have indicated an effect on human heart function (Conrath & Opthof, 2002; Gauthier, Tavernier, Charpentier, Langin, & Le Marec, 1996; Moens, Yang, Watts, & Barouch, 2010; Morimoto, Hasegawa, Cheng, Little, & Cheng, 2004; Pott et al., 2006). In addition, since the pharmacological agents to assess beta-3 adrenoreceptor function also have beta-1 and beta-2 AR activity, a clear cardiovascular safety profile of

Abbreviations: APD_{90}, action potential duration measured at 90% repolarization; $I_{Ca,L}$, calcium activated current via L-channel; I_{Na}, sodium activated current; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{Ks}, slowly activating delayer rectifier potassium current; I_{to}, inward-rectifier potassium current; Mx, metabolite number of parent drug; MRHD, maximal registered human dose; PVC, premature ventricular contractions; TdP, torsade de pointe; $T_{p\text{-}e}$, T_{peak} to T_{end} interval; QT_{cM}, QT corrected according to Matsunaga

* Corresponding author at: P.O. Box 344, 2300 AH Leiden, The Netherlands.
E-mail addresses: cees.korstanje@astellas.com (C. Korstanje), masanori-suzuki@astellas.com (M. Suzuki), koichiro.yuno@astellas.com (K. Yuno), shuichi.sato@astellas.com (S. Sato), masashi.ukai@astellas.com (M. Ukai), marlowe.schneidkraut@astellas.com (M.J. Schneidkraut), yanganxin@comcast.net (G.X. Yan).

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beta-3 adrenoreceptor activation is difficult to discern since cross-reactivity via beta-1 and beta-2 ARs may complicate the cardiac effects (Magnano, Holleran, Ramakrishnan, Reiffel, & Bloomfield, 2002; Nam, Burashnikov, & Antzelevitch, 2005). These cardiovascular effects would be of concern for the clinical development of drugs that treat non-life-threatening indications such as OAB (Michel, Harding, & Bond, 2011). In order to address these potential concerns, an extended cardiovascular safety analysis of mirabegron was performed.

Required testing for cardiovascular safety of small molecule drugs are outlined in the International Council for Harmonisation (ICH) guidance documents (ICH S7A and ICH S7B: ICH website). Considerations for species selection include: a metabolic profile comparable to that of humans, pharmacokinetics of the parent and metabolites that result in systemic exposures equal to or greater than that seen clinically at the therapeutic dose, similar or higher anticipated sensitivity of assessment parameters compared to man. For this study, dogs were chosen as the test species since the dog was shown to be sensitive to the cardiovascular effects of beta AR agonists (Morimoto et al., 2004; Shen, Cervoni, Claus, & Vatner, 1996; Zhou et al., 2008) and showed cardiovascular responses to mirabegron. In addition to the ICH S7A-based screening battery, which includes in vivo hemodynamic and ECG assessments in vivo, the current investigation also evaluated the effects of mirabegron and its metabolites on an expanded ion channel panel as well as the proarrhythmic potential in the dog ventricular wedge preparation (Liu et al., 2006; Vos, 2008).

2. Methods

2.1. In vivo cardiovascular assessment

Experiments were conducted in two separate studies at Environmental Biological Life Sciences Research Center, Inc. (Shiga, Japan). Procedures and treatment of the animals were in accordance with institutional guidelines of Environmental Biological Life Sciences Research Center, Inc. Effects on blood pressure (systolic blood pressure [SBP], diastolic blood pressure [DBP]) and heart rate (HR) were assessed in the first study in conscious beagle dogs (3 males and 2 females), weighing 9.0–10.8 kg upon starting the study. In a second study, effects on blood pressure (SBP, DBP), HR and electrocardiogram (ECG) were assessed in conscious male and female beagle dogs (20 animals total) weighing 8.4–12.2 kg. For both studies catheterization in the femoral artery for blood pressure and HR assessment was done under pentobarbital (30 mg/kg, i.v.) anesthesia. Ampicillin was administered intramuscularly at a dose of 50 mg/body for 3 days. At least 3 days after surgery animals were trained to experimental conditions such that conscious and unconstrained measurements could be reliably obtained. The dogs were orally administered mirabegron after fasting of approximately 18 h and were fed 8 h after dosing. In the first study control (lactose 60 mg/kg), 0.01, 0.03, 0.3, 3, and 10 mg/kg mirabegron were administered to the group of 5 dogs with intervals of at least 7 days. In the second group the dogs received only one dose of mirabegron (30, 60, or 100 mg/kg), or 100 mg/kg mirabegron together with propranolol 1 mg/kg i.v., immediately after oral dosing of mirabegron. Blood pressure was assessed via a pressure transducer (DX-360, Nihon Kohden, Japan) connected to the femoral catheter. HR was recorded with an instantaneous HR unit (AT-601G, Nihon Kohden, Japan), and ECG was measured as lead II. QT and QTc interval were determined from the wave shape on the ECG recordings. QTc interval was calculated by the following equations for Bazett: $QTc = QT / RR^{1/2}$ (ms); for Matsunaga: $QTc = \log 600 * QT / \log RR$ (ms).

2.2. Isolated perfused ventricular wedge experiments

The studies were conducted at Main Line Health Heart Center, Wynnewood, USA and were approved by the Institutional Animal Care and Use Committee of Main Line Health Heart Center. Beagle dogs (16 animals total) were heparinized (300 U/kg, i.v. sodium heparin) then euthanized with sodium pentobarbital (80–95 mg/kg, i.v.). A left thoracotomy was performed, the heart was excised, and then placed in cold (4 °C) normal Tyrode's solution. Next, a small branch of the coronary artery was cannulated and the tissue was cleared of blood by perfusing with Tyrode's solution. The unperfused areas of the ventricle were easily identified from the reddish appearance due to the remaining blood in the tissue and were removed. The preparation was placed in a small tissue bath and arterially perfused at a mean perfusion pressure of 35–45 mmHg with Tyrode's solution containing 4 mM K^+ buffered with 95% O_2 and 5% CO_2 and warmed to 35.7 ± 0.1 °C. Bipolar silver electrodes insulated except for the tips and applied to the endocardial surface and were used to stimulate the isolated tissue. The ventricular wedge was initially perfused for 60 min, to allow sufficient time for the tissue to become electrophysiologically stable (Liu et al., 2006; Yan et al., 2001) before the start of the study.

The preparations were stimulated at basic cycle lengths (BCL) of 1000 and 2000 ms (60 and 30 BPM). ECG and transmembrane action potentials were sampled via a D/A converter (CED 1401, Cambridge, UK), stored in electronic media (CD disks and external/central hard drivers), and analysed using Spike 2 software (CED, UK). The T wave end, the T wave peak and QRS duration were manually measured in a blinded manner (Yan and Antzelevitch, 1998). The T wave end was defined as the point at which the final downslope of the T wave crosses the isoelectric line, the T wave peak as the point at which the maximal potential occurs, and the QRS duration as the time interval from the initial deflection to the termal deflection of the QRS. Transmembrane action potential duration at 90% (APD_{90}) was analysed using Spike 2 (CED, UK).

The ventricular tissue was perfused with Tyrode's perfusate during the control reading period followed by increasing concentrations of mirabegron, isoprenaline, or vehicle control. For mirabegron 7.6, 76, 252, and 756 nM were tested, and for isoprenaline 1, 10, and 100 nM. At each concentration, the tissue was stimulated at a BCL of 2000 ms for approximately 15 min before a 5 min period of ECG and action potential readings were taken. This was followed by stimulation at a BCL of 1000 ms for 5 min followed by ECG determinations. Vehicle-treated tissues were perfused with Tyrode's perfusate for the full 180 min period with readings taken at comparable time points to the mirabegron treated tissues. In the vehicle-treated tissues, the final challenge was sotalol (100 μM) which served as a positive ECG control. Isoprenaline served as a positive control for rate changes.

In a second study, with identical protocol 5 mirabegron metabolites designated M5, M11, M12, M14, or M16 were tested. Each metabolite was evaluated at 4 concentrations in a range of approximately 4.5–340 nM (with differences caused by metabolite MW ranging from 295 to 661) in separate ventricular wedge preparations. A vehicle group was studied in parallel, with a final challenge with sotalol (100 μM).

2.3. Cardiac ion channel assessments

Mirabegron effects on human ether-a-go-go (hERG) potassium ion channel (I_{Kr}) current were investigated in human embryonal kidney (HEK) 293 cells stably expressing hERG channels (K_v11.1 protein). Cells were verified for their hERG current before the experiment and were cultured on coverslips for about 28–57 h prior to the patch-clamp experiment. hERG currents were recorded under voltage-gated conditions using the whole-cell patch-clamp technique (Hamill, Marty, Neher, Sakmann, & Sigworth, 1981). The resting membrane potential of the cell was held at −70 mV and depolarizing pulses were applied every 15 s in order to observe hERG currents. After the peak amplitude of several tail currents reached a steady state, test article solutions were applied by superfusion onto the cell for 10 min at a flow rate of 5 mL/min. Effects of test article concentrations were determined by changes obtained in peak amplitude of the tail current induced by a partially repolarizing pulse from 0 to −50 mV for 750 ms following the