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Original article

Evaluation of drug-induced QT prolongation in a halothane-anesthetized monkey model: Effects of sotalol

Tomomichi Ishizaka *, Yu Yoshimatsu, Minori Ozawa, Tomofumi Kimotsuki, Wataru Takasaki, Sunao Manabe, Mitsuya Yasuda

Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., 717, Horikoshi, Fukuroi, Shizuoka 437-0065, Japan

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ABSTRACT

Introduction: Cynomolgus monkeys are used in *in vivo* models of safety pharmacological studies to evaluate the effects of drug candidates on the cardiovascular system. Models using halothane-anesthetized animals have been used for the detection of drug-induced QT interval prolongation, but few studies with anesthetized monkeys have been reported. **Methods:** The electrophysiological changes induced by *dl*-sotalol, a representative class III antiarrhythmic drug, were assessed in halothane-anesthetized monkeys ($n=4$) or conscious and unrestrained monkeys ($n=4$). **Results:** In terms of basal characteristics, the QT interval was longer and the heart rate (HR) was lower under anesthesia than those under conscious conditions. Intravenous administration of 0.1 to 3 mg/kg *dl*-sotalol to anesthetized monkeys decreased the HR and prolonged the QT interval, monophasic action potential (MAP) duration and ventricular effective refractory period in a dose-dependent manner. In addition, reverse use-dependent prolongation of MAP duration was detected by electrical pacing, whereas the terminal repolarization period was hardly affected at any dose. Oral administration of 3 to 30 mg/kg *dl*-sotalol to conscious monkeys also decreased the HR and prolonged the QT interval in a dose-dependent manner. When compared at similar plasma concentrations of sotalol, the extent of QT interval prolongation under halothane anesthesia was equal to or greater than that under conscious conditions. **Discussion:** The sensitivity for detection of drug-induced QT prolongation under halothane anesthesia may be satisfactory compared with that under conscious conditions. The present examinations indicated the usefulness of a model using halothane-anesthetized monkeys for evaluation of drug-induced QT interval prolongation.

1. Introduction

Acquired QT interval prolongation induced by some medicines that have the potential to inhibit rapidly activating delayed rectifier K^+ currents (I_{kr}) suggests a higher risk of life-threatening ventricular arrhythmias, such as torsades de pointes (Belardinelli, Antzelevitch, & Vos, 2003; Roden, 2004). Therefore, drug candidates should be monitored carefully in non-clinical safety pharmacological studies before human use, according to ICH guidelines S7A and S7B (ICH, 2001, 2005). The use of conscious and unrestrained animals is recommended in guideline S7A for the *in vivo* QT assays. On the other hand, guideline S7B suggests that an anesthetized animal model is optimal model for follow-up studies to evaluate in detail the drug-induced prolongation of ventricular repolarization.

Under conscious and unrestrained conditions, frequent changes in heart rate, associated with locomotor activity, add to the difficulties in evaluating the drug-induced QT interval prolongation in monkeys. On the other hand, under anesthesia, control of the ventricular rate by electrical pacing may avoid these difficulties. Also, the intracardiac electrocardiogram provides valuable information, such as monophasic action potential (MAP) duration, ventricular effective refractory period (VERP) and terminal repolarization period (TRP), which can be used to assess the ventricular repolarization and refractoriness (Sugiyama & Hashimoto, 2002). In addition, halothane reportedly decreases the repolarization reserve, making halothane-anesthetized animal models sensitive to drug-induced QT interval prolongation (Chiba et al., 2004; Takahara, Sugiyama, & Hashimoto, 2005; Takahara et al., 2005). Thus, studies in anesthetized cynomolgus monkeys may be useful for follow-up assessments of drug-induced QT prolongation, in case this species is judged to be one of the best species for toxicological assessments of promising test substances. However, information is still limited regarding the evaluation of drug-induced changes in ventricular repolarization using anesthetized cynomolgus monkeys.

In the present study, in order to assess the utility of an anesthetized monkey model in the safety pharmacology field, the changes in ventricular repolarization induced by an I_{kr} channel blocker were

* Corresponding author. Tel.: +81 538 42 4356; fax: +81 538 42 4350.
E-mail address: ishizaka.tomomichi.du@daiichisankyo.co.jp (T. Ishizaka).

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investigated in halothane-anesthetized monkeys in comparison with conscious and unrestrained monkeys. We chose the representative class III antiarrhythmic drug *dl*-sotalol because its potential to delay ventricular repolarization by blockade of I_{kr} in humans is well-known (Anderson & Prystowsky, 1999). In addition, it has high bioavailability after oral administration, no significant first-pass metabolism and minimal protein binding (Anderson & Prystowsky, 1999). Regarding other species, effects of *dl*-sotalol in dogs and guinea pigs under halothane-anesthetized conditions have been investigated extensively and thus comparison among these three non-rodent species is possible (Sakaguchi et al., 2005; Ishizaka et al., 2008).

2. Methods

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd.

2.1. Halothane-anesthetized monkey model

2.1.1. Experimental animals

A total of 8 cynomolgus monkeys (4 males and 4 females) of Chinese origin were supplied from Hamri Co., Ltd. (Ibaraki, Japan). Four cynomolgus monkeys (2 males and 2 females) each were used in the *dl*-sotalol and vehicle groups. The mean age and body weight were approximately 4.5 ± 0.3 years old and 3.3 ± 0.1 kg in the *dl*-sotalol group and 5.5 ± 1.5 years old and 4.1 ± 0.8 kg in the vehicle group, respectively. Neither parameter was significantly different between the two groups. The animals were housed individually in stainless steel cages, except during the examination, in the following environmental conditions: room temperature, 24 °C; relative humidity, 60%; illumination, 150 to 300 luces; lighting, 12-h lighting (7:00 to 19:00); and ventilation, 10 to 15 air changes/h.

2.1.2. Anesthesia

The animals were anesthetized with intramuscular administration of 10 mg/kg ketamine hydrochloride (Ketalar® Intramuscular 500 mg: Sankyo Co., Ltd., Tokyo, Japan) and intubated with a cuffed endotracheal tube. Thereafter, the animals inhaled 1% halothane (Fluothane®, Takeda Chemical Industries, Osaka, Japan) vaporized with 100% oxygen with a ventilator (Anesthesia ventilator PRO-55S combined with PRO-55V: Acoma Medical Industry Co., Ltd., Tokyo, Japan). The tidal volume and respiratory rate were set at 15 mL/kg and 30–40 stroke/min, respectively.

2.1.3. Measurement parameters

The heart rate, PR interval, QRS width and QT interval were measured from the surface lead II ECG obtained from the limb electrodes. Corrected QT intervals (QTc) were calculated with Bazett's formula [$QTcB = QT / (RR / 1000)^{1/2}$] (Bazett, 1920) and Fridericia's formula [$QTcF = QT / (RR / 1000)^{1/3}$] (Fridericia, 1920). Bazett's formula was employed because it was reported to be reasonably adequate for the corrected QT interval in this species (Koga, Kuwano, Kito, & Kanefuji, 2007). Also, it was used by the Japan Pharmaceutical Manufacturers Association for database construction of *in vivo* QT assays in cynomolgus monkeys (Omata, Kasai, Hashimoto, Hombo, & Yamamoto, 2005). Fridericia's formula was used in a halothane-anesthetized guinea pig model, which was evaluated with *dl*-sotalol. In addition, both Fridericia's formula and Bazett's formula were used in that study, making interspecies comparison obtainable.

A bi-directional steerable electrodes catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum in the right ventricle through the left femoral vein for obtaining MAP signals and ventricular pacing. The signals were amplified with a DC preamplifier (300, EP Technologies Inc). The right ventricle was electrically driven through the electrode catheter using a cardiac stimulator (SEC-4103; Nihon Kohden Corporation, Tokyo, Japan) at each assessment time point. The stimulation pulses were rectangular, 1–2 V (i.e., about twice the threshold voltage) and 1 ms in duration. The duration of the MAP signal was measured as an interval, along a line horizontal to the diastolic baseline, from the monophasic action potential upstroke to the desired repolarization level. The interval (ms) at 90% repolarization was defined as MAP_{90}. The MAP_{90} was measured during the sinus rhythm ($MAP_{90(sinus)}$) and at pacing cycle lengths of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The effective refractory period of the ventricle (VERP) was assessed by programmed electrical stimulation to the right ventricle. The pacing protocol consisted of five beats of basal stimuli at a cycle length of 400 ms followed by an extra stimulus at various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5-ms decrements until refractoriness occurred. The above parameters were continuously monitored using a polygraph system (RM-6000; Nihon Kohden Corporation) and recorded on a thermal array recorder (RTA-1200; Nihon Kohden Corporation) at a paper speed of 100 mm/s at each time point. The above parameters were measured manually on the paper trace. Each measurement of the electrocardiogram and MAP was the mean of 3 consecutive recordings. The duration of the TRP was calculated by the difference between the $MAP_{90(CL400)}$ and the VERP at the same site using calculation software (Microsoft Office Excel 2003: Microsoft Corporation, Tokyo, Japan).

2.1.4. Dose formulation

dl-Sotalol (molecular weight = 272.08) from commercially sourced Sotacor® tablets (Bristol-Myers Squibb, Tokyo, Japan) was dissolved in 0.9% saline and the solution was filtered using a sterilizing filter (0.22 μm, Millex®-GV, Nihon Millipore K.K., Tokyo, Japan). The filtered solutions were prepared at concentrations of 0.05, 0.15, 0.5 and 1.5 mg/mL *dl*-sotalol.

2.1.5. Experimental protocol

In the sotalol group, doses of 0.1, 0.3, 1 and 3 mg/kg of *dl*-sotalol (cumulative dose 4.4 mg/kg) were administered successively by intravenous infusion at about 35 min intervals between infusions. Each dose of *dl*-sotalol was infused over 10 min using a syringe pump (Harvard Apparatus, Inc., Holliston, MA, USA). The cardiovascular parameters were assessed before the start of the infusion, and at 5, 10, 20 and 30 min after the start of the each infusion. Each dose was administered intravenously at a volume of 2 mL/kg. A sample of 0.5 mL arterial blood was drawn at 10, 20 and 30 min after the start of the infusion at each dose for the plasma drug concentration assay.

In the vehicle group, the same volume of 0.9% saline was administered at the same duration (2 mL/kg over 10 min at each administration point) at similar intervals as in the sotalol group, and the cardiovascular parameters were assessed in a similar manner in order to validate the halothane-anesthetized cynomolgus monkey model.

2.2. Conscious monkey model

2.2.1. Experimental animals

Four cynomolgus monkeys (2 males and 2 females) supplied from the same supplier as that of the above halothane-anesthetized monkeys were used and the mean age and body weights were approximately 4.3 ± 0.3 years old and 2.8 ± 0.2 kg, respectively. The animals were housed individually as mentioned above.

2.2.2. Measurement parameters

The heart rate, PR interval, QRS width and QT interval were measured from the surface lead II ECG obtained using a Holter's electrocardiograph (Digital Quick Corder QR2100, Fukuda M·E Kogyo Co., Ltd., Tokyo, Japan) attached to each animal. Ten consecutive