Full Paper

Halothane Sensitizes the Guinea-Pig Heart to Pharmacological I_{Kr} Blockade: Comparison With Urethane Anesthesia

Yasue Sakaguchi^{1,2}, Atsushi Sugiyama^{1,*}, Shuji Takao², Yasuki Akie², Akira Takahara¹, and Keitaro Hashimoto¹

¹Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3898, Japan

²Fuji Biomedix Co., Ltd., Kobuchisawa-cho, Kitakoma-gun, Yamanashi 408-0044, Japan

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Abstract. Potential utility of halothane-anesthetized guinea pigs for detecting drug-induced repolarization delay was analyzed in comparison with urethane-anesthesia ($n=4$ for both groups). Basal QT interval was significantly greater under halothane-anesthesia than urethane-anesthesia (192 ± 7 vs 132 ± 5 ms, respectively), whereas the reverse was true for the heart rate (190 ± 7 vs 248 ± 11 beats/min, respectively). The typical I_{Kr} -blocker *dl*-sotalol (0.1 to 3 mg/kg, i.v.) induced dose-related bradycardia and QT interval prolongation under each anesthesia. The extent of maximum prolongation in the QT interval was greater under halothane-anesthesia than urethane-anesthesia ($+101 \pm 15$ vs $+49 \pm 3$ ms, respectively), whereas that of peak change in the heart rate was smaller under the former than the latter (-49 ± 8 vs -63 ± 5 beats/min, respectively). Pretreatment of the animals under urethane-anesthesia with the selective I_{Ks} blocker chromanol 293B ($n=6$) increased the extent of the *dl*-sotalol-induced QT interval prolongation to $+57 \pm 8$ ms, which was only 0.56 times of that under the halothane-anesthesia, whereas the pretreatment increased the peak change in the heart rate to -76 ± 12 ms. These results indicate that the halothane-anesthesia may effectively sensitize the guinea-pig heart to pharmacological I_{Kr} blockade.

Keywords: QT interval prolongation, sotalol, halothane, urethane

Introduction

In order to avoid excessive QT interval prolongation, resulting in the onset of lethal ventricular arrhythmias, almost all new drug candidates are now being carefully evaluated according to the guideline ICH S7B for Safety Pharmacology Studies (1), which strongly recommends a combination of an in vitro and in vivo assay system. The full complement of the mechanism in the in vivo models is more informative with regard to the clinical situation; however, multiple outward potassium currents in the in situ heart, including the transient outward current (I_{to}), inward rectifier current (I_{K1}), and slow (I_{Ks}) and rapid (I_{Kr}) components of delayed rectifier currents, help one another to accomplish the repolarization process in safe, which is recently called repolarization

reserve (2, 3). In the case of small animals, their hearts usually have more inherent repolarization reserve than those in humans because of their faster heart rate (2, 3), which has made such animals less suitable for predicting the drug-induced repolarization delay in clinical patients.

On the other hand, a small in vivo animal model is very important, particularly in the early stage of drug development, since only a limited amount of drug is available due to its cost for synthesis and/or large number of experiments have to be performed in parallel at low cost. Since halothane-anesthesia has been demonstrated to decrease the repolarization reserve in dogs (4, 5), and guinea pigs possess specific ion channels similar to humans except for I_{to} (1), in this study, we assessed the utility of halothane-anesthetized guinea pigs as a simple and rapid screening procedure for evaluating the in vivo QT prolonging property of drug

*Corresponding author. FAX: +81-55-273-6739
E-mail: atsushis@yamanashi.ac.jp

candidates. The typical I_{Kr} channel blocker *dl*-sotalol was selected to test the model (6) since the most common mechanism of QT interval prolongation by pharmaceuticals is inhibition of I_{Kr} (1). Moreover, we compared the extent of *dl*-sotalol-induced repolarization delay with those observed under urethane-anesthesia in the presence and absence of the selective I_{Ks} blocker chromanol 293B to better understand the modulatory effects of anesthetics on various ionic channels.

Materials and Methods

All experiments were performed in accordance with the rules and regulations of the Animal Experiment Ethics Committee of Fuji Biomedix Co., Ltd. and those of the University of Yamanashi.

Induction of anesthesia

Fourteen male Hartley guinea pigs, weighing 300–400 g (Japan SLC, Inc., Hamamatsu), were fasted from the evening on the day before the experiments. The animals were randomly assigned to 3 groups: halothane-anesthetized group, urethane-anesthetized group, and urethane-anesthetized + chromanol 293B-pretreated group. In the halothane-anesthetized group ($n = 4$), guinea pigs were initially anesthetized with 5% halothane in a container under spontaneous respiration. After a tracheal cannula was inserted, 1–2% halothane vaporized with 100% oxygen was inhaled with a rodent ventilator (SN-480-7; Shinano, Tokyo) (7). The tidal volume and respiratory rate were set at 10 ml/kg and 60 strokes/min, respectively. In the urethane-anesthetized group ($n = 4$) and the urethane-anesthetized + chromanol 293B-pretreated group ($n = 6$), the animals were anesthetized with urethane (1.2 g/kg, i.p.) under spontaneous respiration.

Parameters

The right jugular vein was cannulated for drug administration. The surface lead II electrocardiogram (ECG) was continuously monitored using a telemetry system (Data acquisition software, Open ART 2.2; Data Sciences, Inc., Arden Hills, MN, USA) and analyzed using a real time full automatic data analysis system (NOTOCORD-hem3.5; Notocord Systems, Paris, France). In our preliminary experiments, we have confirmed that the heart rates and each of the ECG parameters of the guinea pigs were stable for >3 h under the halothane-anesthesia ($n = 4$) as well as the urethane-anesthesia ($n = 4$).

Experimental protocol

A dose of 0.1 mg/kg of *dl*-sotalol was infused over

10 min using a syringe pump (Harvard Apparatus, Inc., Holliston, MA, USA); and the heart rate, PR interval, QRS width, and QT interval were measured 5, 10, 15, 20, 25, and 30 min after the start of the drug infusion. Next, 0.3 mg/kg of the drug was cumulatively infused over 10 min and each parameter was assessed in the same manner. Then, 1 mg/kg of the drug was cumulatively infused over 10 min and each parameter was assessed in the same manner. Finally, 3 mg/kg of the drug was cumulatively infused over 10 min and each parameter was assessed in the same manner. In the urethane-anesthetized + chromanol 293B-pretreated group, 1 mg/kg of chromanol 293B, which can effectively inhibit I_{Ks} (8), was intravenously administrated 10 min before 0.1 mg/kg of *dl*-sotalol infusion.

Drugs

dl-Sotalol (Sotacor™; Bristol Myers K.K., Tokyo) was suspended in saline to prepare 0.05, 0.15, 0.5, and 1.5 mg/ml solutions, which were filtrated through a Millex-GV filter of 0.22 μ m (Millipore Corp., Billerica, MA, USA). Chromanol 293B (Tocris, Ellisville, MO, USA) was dissolved in dimethylsulfoxide (DMSO) to prepare a 10 mg/ml solution. Halothane (Takeda Pharmaceutical Co., Ltd., Osaka) and urethane (Sigma-Aldrich Co., St. Louis, MO, USA) were purchased.

Data analyses

Each measurement of the ECG parameters was the mean of five consecutive recordings. Corrected QT intervals were calculated using the formulas of Bazett (QTc(B)) (9) and Fridericia (QTc(F)) (10). The data were expressed as the mean $\pm$ S.E.M. Statistical significance within a parameter were evaluated by one-way, repeated-measures ANOVA followed by Contrast for mean values comparison between the pre-drug control values (C) and others, whereas those between the groups were assessed by one-way, factorial ANOVA followed by Bonferroni test. In the urethane-anesthetized + chromanol 293B-pretreated group, a statistical comparison between before (C') and after (C) the administration of chromanol 293B was performed by the paired *t*-test. A *P* value <0.05 was considered to be statistically significant.

Results

Effects on the heart rate

The time courses of changes in the heart rate are summarized in Fig. 1 (upper panels). The pre-drug basal value (C) of the heart rate was 190 ± 7 beats/min in the halothane-anesthetized group, 248 ± 11 beats/min in the urethane-anesthetized group, and 255 ± 7 beats/min in

Fig. 1. Time courses of the heart rate (circles), PR interval (triangles), QRS width (circles), QT intervals (circles), QTc(B) (triangles: corrected by Bazett's formula), and QTc(F) (squares: corrected by Fridericia's formula). The effects of *dl*-sotalol on the halothane-anesthetized guinea pigs ($n=4$), urethane-anesthetized guinea pigs ($n=4$), and urethane-anesthetized + chromanol-293B pretreated guinea pigs ($n=6$) are summarized in the left, center, and right panels, respectively. Data are presented as the mean $\pm$ S.E.M. The closed symbols represent the significant differences from each basal control value (C) by $P < 0.05$.

the urethane-anesthetized + chromanol 293B-pretreated group. The basal heart rate was significantly smaller in the halothane-anesthetized group than in the urethane-anesthetized group or in the urethane-anesthetized + chromanol 293B-pretreated group. Administration of chromanol 293B hardly affected the heart rate.

After the administration of *dl*-sotalol, the heart rate decreased in the halothane- and urethane-anesthetized groups for 5–30 min after the start of 1 mg/kg infusion and for 5–30 min after the start of 3 mg/kg infusion. Meanwhile, in the urethane-anesthetized + chromanol 293B-pretreated group, the heart rate decreased at 10 min after the 0.1 mg/kg, for 5–15 min after the 0.3 mg/kg, for 5–25 min after the 1 mg/kg, and for 5–30 min after the 3 mg/kg infusion. The maximum

reduction in the heart rate from the basal value was -49 ± 8 beats/min (-26%) in the halothane-anesthetized group, whereas it was -63 ± 5 beats/min (-25%) in the urethane-anesthetized group and -76 ± 12 beats/min (-30%) in the urethane-anesthetized + chromanol 293B-pretreated group. The extent (beats/min) of the bradycardic effects tended to be greater in the urethane-anesthetized group and the urethane-anesthetized + chromanol 293B-pretreated group compared with that in the halothane-anesthetized group, which, however, did not achieve statistical significance.

Effects on the ECG parameters

The time courses of changes in the ECG parameters are summarized in Fig. 1 (lower panel), and typical

Halothane-anesthesia

Control

(HR=178 beats/min, QT=212 ms)

10 min after 3 mg/kg of *dl*-sotalol

(HR=139 beats/min, QT=293 ms)

Fig. 2. Typical tracings of surface lead II electrocardiograms before (Control) and 10 min after the start of 3 mg/kg of *dl*-sotalol infusion of a halothane-anesthetized guinea pig.

tracings of the effects of *dl*-sotalol on the ECG of the halothane-anesthetized group are depicted in Fig. 2. The pre-drug basal values (C) of the PR interval, QRS width, QT interval, QTc(B), and QTc(F) were 73 ± 6 , 30 ± 1 , 192 ± 7 , 342 ± 9 , and 282 ± 8 ms in the halothane-anesthetized group; 61 ± 4 , 25 ± 3 , 132 ± 5 , 268 ± 5 , and 212 ± 5 ms in the urethane-anesthetized group; and 63 ± 2 , 33 ± 2 , 132 ± 4 , 271 ± 6 , and 213 ± 6 ms in the urethane-anesthetized + chromanol 293B-pretreated group, respectively. The QT interval, QTc(B), and QTc(F) were significantly greater in the halothane-anesthetized group than those in the urethane-anesthetized group or in the urethane-anesthetized + chromanol 293B-pretreated group, whereas no significant difference was detected in the PR interval or QRS width among the groups. Administration of chromanol 293B slightly but significantly prolonged the PR interval, whereas it hardly affected the QRS width, QT interval, QTc(B), or QTc(F).

After the administration of *dl*-sotalol, the QT interval, QTc(B) and QTc(F) were significantly prolonged in each group. In the halothane-anesthetized group, significant prolongation was detected in the QT interval and QTc(F) at 10 min after the 0.3 mg/kg, for 5–30 min after the 1 mg/kg, and for 5–30 min after the 3 mg/kg infusion and in the QTc(B) for 10–15 min after the 0.3 mg/kg, for 5–30 min after the 1 mg/kg, and for 5–30 min after the 3 mg/kg infusion. In the urethane-

anesthetized group, significant prolongation was detected in the QT interval, QTc(B), and QTc(F) for 5–20 min after the 1 mg/kg and for 5–30 min after the 3 mg/kg infusion. On the other hand, in the urethane-anesthetized + chromanol 293B-pretreated group, a significant prolongation was detected in the QT interval at 10 min after the 0.1 mg/kg, at 10 min after the 0.3 mg/kg, for 5–25 min after 1 mg/kg, and for 5–30 min after the 3 mg/kg infusion and in the QTc(B) and QTc(F) for 5–10 min after the 0.1 mg/kg, at 10 min after the 0.3 mg/kg, for 5–25 min after the 1 mg/kg, and for 5–30 min after the 3 mg/kg infusion. The PR interval and QRS width were slightly but significantly shortened by the administration of *dl*-sotalol in the urethane-anesthetized + chromanol 293B-pretreated group, whereas such effects were not detected in the other groups (Fig. 1).

The maximum increases in the QT interval after the 0.1, 0.3, 1, and 3 mg/kg infusion from the respective basal values were $+9 \pm 6$ (+5%), $+25 \pm 11$ (+13%), $+62 \pm 7$ (+32%), and $+101 \pm 15$ ms (+53%) in the halothane-anesthetized group; $+3 \pm 10$ (+2%), $+4 \pm 2$ (+3%), $+27 \pm 5$ (+17%), and $+49 \pm 3$ ms (+37%) in the urethane-anesthetized group; and $+12 \pm 5$ (+9%), $+16 \pm 4$ (+12%), $+31 \pm 6$ (+24%), and $+57 \pm 8$ ms (+44%) in the urethane-anesthetized + chromanol 293B-pretreated group, respectively. The extent (ms) of the QT interval prolongation was >2 times greater in the halothane-anesthetized group compared with those in the urethane-anesthetized group. In the urethane-anesthetized + chromanol 293B-pretreated group, pretreatment with chromanol 293B slightly increased the *dl*-sotalol-induced QT interval prolongation compared with that in the urethane-anesthetized group. However, the extent was only 0.56 times of that observed under the halothane-anesthesia.

Discussion

In this study, we examined potential utility of halothane-anesthetized guinea pigs as a model for detecting the drug-induced QT interval prolongation in comparison with the well-established urethane-anesthetized guinea pigs (8). The basal QT interval was significantly greater under the halothane-anesthesia than under the urethane-anesthesia, whereas the reverse was true for the heart rate. Administration of *dl*-sotalol induced dose-related bradycardia and QT interval prolongation under both anesthetics. The extent of the changes in QT interval prolongation was >2 times greater under the halothane-anesthesia than the urethane-anesthesia, whereas that of the heart rate was slightly smaller in each animal under the former than the

latter. Pretreatment of animals under the urethane-anesthesia with chromanol 293B enhanced the *dl*-sotalol-induced QT interval prolongation. However, the enhancement of the QT interval prolongation was small compared with that under the urethane-anesthesia alone, which could not mimic the effects of the halothane-anesthesia. Also, these results well reflect the clinical experience with *dl*-sotalol, in which QT interval at baseline was significantly longer in patients with excessive QT-interval prolongation, resulting in a pre-disposition to torsades de pointes (6).

Several kinds of anesthetics have been used on various animals to analyze the effect of *dl*-sotalol on the QT interval. For example, in a study using pentobarbital-anesthetized guinea pigs with a similar experimental protocol as used in this study (11), intravenous administration of 3 mg/kg of *dl*-sotalol prolonged the QTc(B) and QTc(F) by 64 and 71 ms, respectively, whereas those values in the present study were 109 and 109 ms under the halothane-anesthesia and 49 and 52 ms in the urethane-anesthesia, respectively. Also, in our recent study using the halothane-anesthetized dogs (12), 0.02, 0.2, and 2 mg/kg, i.v. of *dl*-sotalol prolonged the QT interval by 5 (+2%), 36 (+11%), and 118 ms (+36%), respectively. Thus, the present results together with these previous reports indicate that the sensitivity of the assay models for the drug-induced QT-interval prolongation may be in the order of halothane-anesthetized dogs and guinea pigs > pentobarbital-anesthetized guinea pigs > urethane-anesthetized guinea pigs. Thus, the halothane-anesthesia can be considered to effectively sensitize the guinea pig heart to pharmacological I_{Kr} blockade like in the canine heart (4, 5).

Mechanisms underlying the marked prolongation of the QT interval under the halothane-anesthesia would need comments. First, the halothane-anesthesia has been shown to suppress the cardiomechanical function including the heart rate, ventricular contraction, blood pressure, and cardiac output (4), whereas such a profile was not reported for the urethane-anesthesia (13). On the other hand, *dl*-sotalol has been shown to be primarily eliminated into the urine without hepatic metabolism (14). So, it will be possible that the pharmacokinetic profile of *dl*-sotalol is different between the two anesthetic conditions; namely, elimination rate of the drug from the systemic circulation may be slower under the halothane-anesthesia, which might amplify the drug action on the heart. Second, it is well known that the contribution of I_{Kr} or I_{Ks} to ventricular repolarization is rate-dependent; that is, I_{Kr} plays a major role in ventricular repolarization with relatively slower heart rate. Upon increasing the heart rate, I_{Ks} becomes more predominant and replaces I_{Kr} as a repolarizing current. In

the present study, the heart rate is significantly slower under the halothane-anesthesia than that under the urethane-anesthesia, which may partly explain the increased sensitivity under the halothane-anesthesia. Third, the high sensitivity under the halothane-anesthesia can be explained in relation to its modulatory effects on various ionic channels. Previous electrophysiological studies have indicated that the clinical anesthetic concentration of halothane inhibits several outward potassium currents including I_{Kr} , I_{Ks} , I_{K1} , and I_{to} (15–18), whereas no information is available for the urethane-anesthesia regarding such modulatory effects on various ionic currents. Under the block of these outward currents by halothane, the ventricular repolarization would be largely dependent on the unblocked remaining potassium currents. Since the enhancement of the *dl*-sotalol-induced QT-interval prolongation by the pretreatment with chromanol 293B was small, the increased sensitivity under the halothane-anesthesia is also likely due to the inhibition of multiple repolarization currents in the ventricle.

In conclusion, the halothane-anesthesia may sensitize the guinea-pig heart to pharmacological I_{Kr} blockade, which may be viewed as a useful influence, since it can unmask the latent QT-interval prolonging action of a drug. Also, the halothane-anesthetized guinea-pig model is a relatively simple, rapid, and low-cost method for predicting the in vivo I_{Kr} blocking property of drug candidates.

References

- 1 The ICH Steering Committee. The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). The guideline was recommended for adoption at Step 4 of the ICH process on 12 May 2005 (<http://www.ich.org/>).
- 2 Biliczki P, Virag L, Iost N, Papp JG, Varro A. Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of repolarization reserve. *Br J Pharmacol.* 2002;137:361–368.
- 3 Lengyel C, Dezső L, Biliczki P, Horvath C, Virag L, Iost N, et al. Effect of a neuroprotective drug, eliprodil on cardiac repolarisation: importance of the decreased repolarisation reserve in the development of proarrhythmic risk. *Br J Pharmacol.* 2004;143: 152–158.
- 4 Takahara A, Sugiyama A, Satoh Y, Wang K, Honsho S, Hashimoto K. Halothane sensitizes the canine heart to pharmacological I_{Kr} blockade. *Eur J Pharmacol.* 2005;507:169–177.
- 5 Satoh Y, Sugiyama A, Takahara A, Ando K, Wang K, Honsho S, et al. The antipsychotic and antiemetic drug prochlorperazine delays the ventricular repolarization of the in situ canine heart. *J Pharmacol Sci.* 2005;97:101–106.

- 6 Hohnloser SH, Woosley RL. Sotalol. *N Eng J Med*. 1994; 331:31–38.
- 7 Noda Y, Hashimoto K. Development of a halothane-adrenaline arrhythmia model using in vivo guinea pigs. *J Pharmacol Sci*. 2004;95:234–239.
- 8 Yang Z, Shi G, Li C, Wang H, Liu K, Liu Y. Electrophysiologic effects of nicorandil on the guinea pig long QT1 syndrome model. *J Cardiovasc Electrophysiol*. 2004;15:815–820.
- 9 Bazett HC. An analysis of the time-relations of electrocardiograms. *Heart*. 1920;7:353–370.
- 10 Fridericia LS. Die systolendauer in elektrokardiogramm bei normalen menschen und bei herzfranken. *Acta Med Scand*. 1920;53:469–486.
- 11 Testai L, Calderone V, Salvadori A, Breschi MC, Nieri P, Martinotti E. QT prolongation in anaesthetized guinea-pigs: an experimental approach for preliminary screening of torsadogenicity of drugs and drug candidates. *J Appl Toxicol*. 2004;24: 217–222.
- 12 Satoh Y, Sugiyama A, Wada K, Nomura H, Hashimoto K. In vivo electropharmacological effects of a class III antiarrhythmic drug *dl*-sotalol assessed using halothane anesthetized canine model. *J Pharmacol Sci*. 2004;94 Suppl 1:265P.
- 13 Warren DJ, Ledingham JG. Renal circulatory responses to general anaesthesia in the rabbit: studies using radioactive microspheres. *Clin Sci Mol Med*. 1975;48:61–66.
- 14 Hanyok JJ. Clinical pharmacokinetics of sotalol. *Am J Cardiol*. 1993;72:19A–26A.
- 15 Davies LA, Hopkins PM, Boyett MR, Harrison SM. Effects of halothane on the transient outward K^+ current in rat ventricular myocytes. *Br J Pharmacol*. 2000;131:223–230.
- 16 Stadnicka A, Bosnjak ZJ, Kampine JP, Kwok WM. Modulation of cardiac inward rectifier K^+ current by halothane and isoflurane. *Anesth Analg*. 2000;90:824–833.
- 17 Shibata S, Ono K, Iijima T. Sevoflurane inhibition of the slowly activating delayed rectifier K^+ current in guinea pig ventricular cells. *J Pharmacol Sci*. 2004;95:363–373.
- 18 Li J, Correa AM. Kinetic modulation of HERG potassium channels by the volatile anesthetic halothane. *Anesthesiology*. 2002;97:921–930.