Torsadogenic Potential of HCN Channel Blocker Ivabradine Assessed in the Rabbit Proarrhythmia Model

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INTRODUCTION

Ivabradine is an inhibitor of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, approved as a heart rate-reducing drug for the treatment of coronary artery disease and chronic heart failure.1) This drug possesses antiarrhythmic properties, as shown in the isolated cardiac preparations from humans or dogs; this property may be advantageous in the treatment of patients with heart disease liable to cardiac rhythm disturbance.2) The antiarrhythmic effects of ivabradine have also been experimentally shown in acute myocardial ischemia of rats.3,4) However, clinical case reports of torsade de pointes arrhythmias have also been shown in acute experimental heart failure in a rabbit model.5) To clarify torsadogenic potential of ivabradine itself, we investigated electrophysiological effects of ivabradine in this study by using a proarrhythmia model of acute atrioventricular (AV) block rabbit, which detects drug-induced torsade de pointes arrhythmias with high sensitivity and specificity.7–9)

MATERIALS AND METHODS

All animal experimental procedures were approved by the Toho University Animal Care and User Committee (Approval No. 20-55-331). Experiments were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. New Zealand White rabbits were purchased from Japan SLC (Hamamatsu, Japan).

Production of Acute AV Block Model Male rabbits (approximately 3 kg) were anesthetized with intramuscular administration of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg), and then inhaled 1.5% of isoflurane vaporized with pure oxygen via a tracheal tube using a ventilator (SN-480-5; Shinano, Tokyo, Japan). For measuring blood pressure (BP), heparinized catheter was inserted into the right femoral artery under the monitoring of the surface lead II electrocardiogram (ECG). Complete AV block was produced by a catheter ablation technique, as described previously.7–9) The endpoint of the catheter ablation was the development of complete AV block with the onset of stable idioventricular escape rhythm.

Electrophysiological Measurement A monophasic action potential (MAP) recording/pacing combination catheter (Physio-Tech, Tokyo, Japan) was inserted through the right jugular vein to record the MAP of the right ventricle. The duration of MAP signals was measured as an interval (ms) at the 90% repolarization level, defined as MAP90.

TDP was judged as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting ≥5 consecutive beats. Premature ventricular contraction with R on T phenomenon was defined with a prematurity index of <1, which was calculated by dividing the coupling interval of premature ventricular beat (RR') by the QT interval of the preceding normally conducted beat (=RR'/QT).10) For assessing the instability of the ventricular repolarization, beat-to-beat analysis using the MAP90 of 31 consecutive beats was performed before drug administration and at which a maximal change in MAP90 was detected (or just before induction of the first TdP). Short-term variability of repolarization, a surrogate parameter that reliably identifies proarrhythmic risk in preclinical animal models, was obtained using Poincaré plots of MAP90(n) versus MAP90(n + 1), as described in our previous studies.7–9)

Experimental Protocol All experiments were done under idioventricular rhythm. After the basal control assessment (C), ivabradine at 0.01 mg/kg was intravenously infused over 10 min, and cardiovascular variables were continuously monitored until 30 min after the start of the infusion. Subsequently, ivabradine at 0.1 mg/kg was additionally infused, and cardiovascular variables were monitored similarly. Next, ivabradine

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Ivabradine hydrochloride was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and was dissolved in saline. Heparin sodium, isoflurane, ketamine hydrochloride and xylazine hydrochloride were purchased from AY pharmaceuticals (Tokyo, Japan), Mylan Seiyaku (Osaka, Japan), Daiichi Sankyo (Tokyo, Japan) and Bayer Yakuhin (Tokyo, Japan), respectively.

Statistical Analysis  Data are shown as mean ± standard error of the mean (S.E.M.). The statistical significance within a parameter was evaluated by one-way repeated-measures ANOVA, followed by Dunnett’s test for comparison of each treatment with a pre-drug control, which was analyzed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, U.S.A.). A p-value <0.05 was considered significant.

RESULTS

Torsadogenic Action of Ivabradine  Figure 1A shows the typical tracing of TdP arrhythmia during the administration of 0.1 mg/kg of ivabradine to an acute AV block rabbit. The arrhythmia followed a premature ventricular contraction that occurred during the preceding T wave, known as R on T phenomenon (PVC with R on T phenomenon). Figure 1B summarizes the time course showing the onset of arrhythmias after the administration of ivabradine. After the administration of 0.01 mg/kg, there were no proarrhythmic signs including PVCs with R on T phenomenon. After the administration of 0.1 mg/kg, episodes of short type and 2 episodes of the longer type were discontinuously observed at 7–10 and 15–19 min after the administration of 0.1 mg/kg, whereas 1 episode of the short type and 1 episode of longer type were continuously observed at 2–5 min after the high-dose administration. In rabbits #2 and #5 receiving the high dose, ventricular standstill was continuously observed after the termination of latest TdP, leading to cardiac arrest. ECG: electrocardiogram, MAP: monophasic action potential, BP: blood pressure. (Color figure can be accessed in the online version.)

Table 1. Effects of Ivabradine on the Beat-to-Beat Variability of Ventricular Repolarization in the Rabbit with Acute Atrioventricular Block

| Ivabradine (mg/kg) | Control | 0.01 | 0.1 | 1 |
|-------------------|---------|------|-----|---|
| STV (ms)          | 2.3 ± 0.9 | 3.0 ± 0.8 | 2.9 ± 0.6 | 5.4 ± 1.5 |
| LTV (ms)          | 1.9 ± 0.7 | 2.7 ± 0.8 | 2.3 ± 0.5 | 4.3 ± 1.1 |
| MAP90 (ms)        | 177 ± 33 | 190 ± 35 | 202 ± 38 | 228 ± 44* |

Poincaré plots of MAP90(n) versus MAP90(n+1) were prepared for each analysis time point, and short-term variability (Σ|MAP90(n+1)−MAP90(n)|/[30×√2]) and long-term variability (Σ|MAP90(n+1)+MAP90(n)−2MAP90(mean)|/[30×√2]) were calculated. Data are presented as mean ± S.E.M. of 5 experiments except for the dose of 1 mg/kg (n = 4) because the MAP90 of 31 consecutive beats was not obtained due to arrhythmias after the drug administration in the #5 animal. STV: short-term variability, LTV: long-term variability, MAP90: monophasic action potential duration at 90% repolarization level. *p < 0.05, significantly different from corresponding pre-drug control.

Fig. 1. Proarrhythmic Effects of Ivabradine

(A) Typical tracings of ivabradine-induced torsade de pointes (TdP) in the rabbit with acute atrioventricular block (#5). The arrhythmia appeared at 7 min 20 s after the administration of 0.1 mg/kg, which terminated spontaneously. (B) Summary of proarrhythmic effect of ivabradine. Each column indicates the responses of each rabbit to drug, and the columns are marked in accordance with the severity of arrhythmias developed in each minute. Orange, red and black columns represent the occurrence of premature ventricular contraction with R on T phenomenon (R on T PVC), TdP and cardiac arrest (CA), respectively. In rabbit #2, 2 episodes of short type of TdP (<10 s) and 3 episodes of longer type of the arrhythmias (≥10 s) were discontinuously observed at 15–16, 17–19 and 20–22 min after the high-dose administration. In rabbit #5, 2 episodes of the short type and 2 episodes of the longer type were discontinuously observed at 7–10 and 15–19 min after the administration of 0.1 mg/kg, whereas 1 episode of the short type and 1 episode of longer type were continuously observed at 2–5 min after the high-dose administration. In rabbits #2 and #5 receiving the high dose, ventricular standstill was continuously observed after the termination of latest TdP, leading to cardiac arrest. ECG: electrocardiogram, MAP: monophasic action potential, BP: blood pressure. (Color figure can be accessed in the online version.)

at 1 mg/kg was additionally infused, and cardiovascular variables were monitored until 60 min.

Materials  Ivabradine hydrochloride was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and was dissolved in saline. Heparin sodium, isoflurane, ketamine hydrochloride and xylazine hydrochloride were purchased from AY pharmaceuticals (Tokyo, Japan), Mylan Seiyaku (Osaka, Japan), Daiichi Sankyo (Tokyo, Japan) and Bayer Yakuhin (Tokyo, Japan), respectively.
Parameters  
Hemodynamic and electrophysiological effects of ivabradine (n = 5) are summarized in Fig. 2. No significant change was detected in the any of the parameters after the administration of 0.01 or 0.1 mg/kg. The high dose of 1 mg/kg significantly increased the QT interval, corrected QT interval (QTc), QRS width and MAP90 but decreased the atrial and ventricular rates. Meanwhile, there was no significant change in the mean BP.

DISCUSSION  
Proarrhythmic effects of ivabradine were investigated using the acute AV block rabbit model.10) Ivabradine did not affect cardiovascular variables at the low dose, whereas its middle and high doses exerted proarrhythmic actions. Although ivabradine has been demonstrated to act as an aggravating factor for proarrhythmic risk in the isolated rabbit heart,11) this is the first report demonstrating that ivabradine itself has torsadogenic actions in the in vivo experimental condition.

Ivabradine is clinically supplied as oral tablets containing 2.5, 5 and 7.5 mg in Japan, whereas intravenous administration of 0.1 to 0.25 mg/kg of ivabradine has been used for previous clinical investigations.12,13) Thus, the present study used 0.01 to 1 mg/kg, i.v. of ivabradine that can be considered to be carried out under subtherapeutic to supratherapeutic levels. Based on pharmacokinetic parameters obtained from human and animals, maximum plasma concentrations of ivabradine at 1 mg/kg can be estimated to be 88–410 nM in this study,14–16) which is relatively higher than Cmax values of 13.5–45 nM in healthy human after the administration of oral tablets containing 2.5–7.5 mg.14) In this study, ivabradine induced TdP in 1 out of 5 rabbits at 0.1 mg/kg and in 2 out of 5 rabbits at 1 mg/kg (Fig. 1), which is roughly corresponded to a therapeutic to supratherapeutic dose range. In addition, the short-term variability of MAP90, a predicting marker for the onset of TdP,17) tended to increase in rabbits, as shown in Table 1, which is essentially similar to the property of other torsadogenic drugs as demonstrated in the AV block rabbit model.7,9)
As shown in Fig. 2, the high dose of ivabradine significantly prolonged the QT interval and MAP_{90} with decrement of atrial and ventricular spontaneous rate of AV block rabbit heart, whereas no significant change in these parameters was detected at low and middle doses. In previous in vitro electrophysiological studies, IC_{50} of ivabradine for HCN4 and human ether-a-go-go related gene (hERG) K^+ currents have been demonstrated to be 2.2 and 3.5 μM, respectively, which may reflect our current results that ivabradine affected automaticity and ventricular repolarization from the same dose of 1 mg/kg. Importantly, as shown in Table 2, TdP arrhythmias could be only observed in the 2 rabbits that showed more potent suppressive effects on ventricular automaticity (#2, #5), which might depend on a physiological condition of HCN channels that are functionally regulated by cAMP. This may provide significant information on distinctive torsadogenicity of ivabradine because there is a possibility that reverse use-dependent QT-interval prolongation by hERG K^+ channel blockade could be enhanced by pharmacological inhibition of HCN channels.

Based on Table 2, TdP arrhythmias appeared when advanced bradycardia was present as an individual-specific reaction, thus the TdP risk may be low in the heart rate-reducing condition. Moreover, ivabradine is positioned as a Conditional Risk, as suggested by an online proarrhythmia model. Torsade de pointes risk may be low in the heart rate-reducing condition. This may provide significant information on distinctive torsadogenic potential of ivabradine because there is a possibility that reverse use-dependent QT-interval prolongation by hERG K^+ channel blockade could be enhanced by pharmacological inhibition of HCN channels.

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In conclusion, the present study suggests that the torsadogenic potential of ivabradine may become evident when its expected bradycardic action appears more excessively.

Conflict of Interest The authors declare no conflict of interest.

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