**In vivo analysis of torsadogenic potential of an antipsychotic drug paliperidone using the acute atrioventricular block rabbit as a proarrhythmia model**

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**Abstract**

We assessed electrophysiological effects of an atypical antipsychotic drug paliperidone in acute atrioventricular block rabbits. Intravenous administration of paliperidone at a clinically relevant dose (0.06 mg/kg) hardly affected the QT interval or monophasic action potential (MAP) duration, and the higher doses (0.6 and 6 mg/kg) prolonged the QT interval and MAP duration. Meanwhile, premature ventricular contractions with R on T phenomenon were observed in 3 out of 6 animals at the middle dose, and torsades de pointes (TdP) episodes were detected in 2 out of 6 animals at the high dose. Intravenous administration of its chemically related compound risperidone at a clinically relevant dose (0.03 mg/kg) hardly affected the electrophysiological parameters, and the higher doses (0.3 and 3 mg/kg) prolonged the QT interval and MAP duration. Meanwhile, the premature ventricular contractions with R on T were observed in 2 out of 6 animals at the middle dose, and TdP episodes were detected in 4 out of 6 animals at the high dose. These results suggest that paliperidone showed torsadogenic potential at supra-therapeutic doses, whose potency can be estimated to be equal or slightly subordinate in comparison with that of risperidone.

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**1. Introduction**

Paliperidone is a second generation atypical antipsychotic drug used for the treatment of positive and negative symptoms associated with schizophrenia, which is also known as a major active metabolite of risperidone (9-hydroxyrisperidone, Fig. 1) (1,2). For clinical efficacy, safety and tolerability, extended-release tablets containing paliperidone have been prescribed once-daily, which contributes to minimize the 24-h peak-trough variation at steady state plasma concentration (3,4). On the other hand, prolonged release suspension of paliperidone palmitate for injection can be administered once a month after adjustment of the maintenance dose in adult patients stabilized with paliperidone or risperidone (5). In 2014, the Japanese Ministry of Health, Labour and Welfare issued a blue letter (safety advisory) for the prolonged release suspension of paliperidone palmitate (6). Finally, a total of 32 fatal cases had been reported during the Early Post-marketing Phase Vigilance from its launch on November 2013 up to May 2014 (7). Although the risk of all-cause mortality in association with the use of paliperidone was not high in comparison with other investigations, the most common cause of death was sudden death, followed by suicide and neuroleptic malignant syndrome (7).

Paliperidone has been reported to cause a modest increase in the QT interval in clinical practice (3,8), which may be associated with its inhibitory effects on the human *ether-à-go-go*-related gene (hERG) $K^+$ channels, encoding the pore-forming subunit of rapidly activating delayed rectifier $K^+$ current ($I_{kr}$) (9). Since drug-induced QT interval prolongation is often associated with the onset of torsades de pointes (TdP) resulting in a life-threatening ventricular arrhythmia (10,11), further information regarding proarrhythmic potential of paliperidone is needed to better understand causality between the drug and its lethal events. It is noteworthy that the...
pharmacological property of paliperidone on the ventricular repolarization may be essentially in accordance with that of its chemically related compound risperidone (8,12). In this study, we compared proarrhythmic effects of paliperidone with those of risperidone using acute atrioventricular block rabbits, which is adopted as a proarrhythmia model (13).

2. Materials and methods

All experiments were approved by Toho University Animal Care and User Committee and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. A total of 12 New Zealand White rabbits (Sankyo Labo Service, Tokyo, Japan) were used in this study.

2.1. Production of atrioventricular block model

Male New Zealand White rabbits, weighting approximately 3 kg, were initially anesthetized with ketamine hydrochloride (35 mg/kg, i.m.) and xylazine hydrochloride (5 mg/kg, i.m.). After tracheal intubation, 1.5% isoflurane vaporized with 100% oxygen was inhaled with a ventilator (SN-480-5; Shinano, Tokyo, Japan). The tidal volume and respiratory rate were set at 6 mL/kg and 40 strokes/min, respectively. The right femoral artery was cannulated for measurement of the blood pressure. The surface lead II electrocardiogram (ECG) was continuously monitored using a polygraph system (RMP-6008; Nihon Kohden, Tokyo, Japan).

For production of complete atrioventricular block, a catheter ablation technique was carried out according to our previous reports (14,15). A quad-polar electrodes catheter with a tip of 1 mm (6 Fr, D6DR252KT; Biosense Webster, Diamond Bar, CA, USA) was inserted through the left femoral vein and positioned at the tricuspid valve by watching the bipolar electrograms from the distal electrodes pair. The optimal site for the atrioventricular node ablation, namely, the compact atrioventricular node, was determined on the basis of the intracardiac electrogram, of which a very small His deflection was recorded. The power source for atrioventricular node ablation was an electrosurgical generator (SL-1PR; Semco, Tokyo, Japan) delivering continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After proper positioning, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to the return electrode positioned under the animal’s back. The end point of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm. The ventricle was electrically driven with a stimulator (SEC-4103; Nihon Kohden, Tokyo, Japan) at 60 beats/min throughout the experiment. The stimulation pulses were rectangular in shape, of 2 V (approximately twice the threshold voltage) and 3 ms duration.

A monophasic action potential (MAP) recording/pacing combination catheter (5 Fr, interelectrode distance 1 mm; Physio-Tech, Tokyo, Japan) was positioned at the right ventricle through the right jugular vein. The signal was amplified with a differential amplifier (DAM 50, World Precision Instruments, Sarasota, FL, USA). The duration of MAP signals was measured as an interval (ms) at the 90% repolarization level, which was defined as MAP90. Electrophysiological and cardiovascular parameters were continuously monitored with a polygraph system (RMP-6008; Nihon Kohden), and analyzed with a fully automatic analysis system (ecgAUTO; Emka Technologies, Paris, France).

2.2. Experimental protocol

Electrophysiological and cardiovascular parameters were continuously recorded under the ventricular pacing at 60 beats/min. After the basal control assessment (C), a low dose of 0.06 mg/kg of paliperidone was infused over 10 min via the left femoral vein using an infusion pump (PHD 2000 Infusion; Instech Laboratories, Plymouth Meeting, PA, USA), and changes in the blood pressure, ECG and MAP90 were continuously monitored for 60 min. Next, a middle dose of 0.6 mg/kg was additionally infused over 10 min, and each parameter was similarly monitored. Finally, a high dose of 6 mg/kg was additionally infused over 10 min, and changes in the blood pressure, ECG and MAP90 were monitored for 60 min. Also, the effects of risperidone in doses of 0.03, 0.3 and 3 mg/kg were assessed in another series of animals. Each data of the blood pressure, ECG and MAP90 was obtained from the mean of three recordings, which was assessed 5, 10, 15, 20, 25 and 30 min after the low- and middle-dose administration, and 5, 10, 15, 20, 25, 30, 40, 50 and 60 min after the high-dose administration. TdP was defined as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline with 5 or more consecutive beats. Ventricular tachycardia was defined as more than lasting ≥5 consecutive beats. Premature ventricular contraction with R on T phenomenon was defined by prematurity index (16), which was calculated by dividing the coupling interval of the premature ventricular contraction (RR") by the QT interval of the preceding normally conducted beat (=RR"/QT). A premature ventricular contraction with a prematurity index of <1 was considered to represent the R on T phenomenon.

2.3. Beat-to-beat analysis

For assessment of instability of the ventricular repolarization, the MAP90 of 31 consecutive beats was measured before and after the drug administration (14,15). Poincaré plots with MAP90(n) versus MAP90(n + 1) were prepared for each analysis time point.
The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability \((\Sigma |\text{MAP}_{90}(n+1) - \text{MAP}_{90}(n)| /[30 \times \sqrt{2}])\). On the other hand, the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot was determined as long-term variability \((\Sigma |\text{MAP}_{90}(n+1) + \text{MAP}_{90}(n) - 2\text{MAP}_{90}\text{(mean)}| /[30 \times \sqrt{2}])\).

### 2.4. Drug

Paliperidone (molecular weight = 426.48) and risperidone (molecular weight = 410.48) were purchased from Tokyo Chemical Industry (Tokyo, Japan). They were dissolved in 0.5% lactate solution and intravenously administered at an infusion rate of 0.2 mL/kg per min. Heparin sodium, ketamine hydrochloride, xylazine hydrochloride and isoflurane were purchased from AY Pharmaceuticals (Tokyo, Japan), Daiichi Sankyo (Tokyo, Japan), Bayer Yakuhin (Tokyo, Japan) and Mylan Seiyaku (Osaka, Japan), respectively.

### 2.5. Statistical analyses

Data are presented as the mean ± S.E.M. The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Contrast for mean values comparison. A P-value less than 0.05 was considered significant.

### 3. Results

#### 3.1. Torsadogenic action of paliperidone and risperidone

Fig. 2A shows typical tracings of TdP arrhythmias in the acute atrioventricular block rabbit after the high-dose administration of paliperidone (upper panel) or risperidone (lower panel). The arrhythmias followed a premature ventricular contraction that occurred during the preceding T wave, known as R on T phenomenon, as shown in the magnified figure (Fig. 2B).

The time courses of onset of arrhythmias after the administration of paliperidone and risperidone are summarized in Fig. 3A. In the paliperidone group \((n = 6)\), premature ventricular contractions with \(R\) on \(T\) phenomenon were observed in 3 out of 6 animals at the middle dose and all animals at the high dose. At the high dose, episodes of TdP were detected in 2 out of 6 animals, and 1 animal died after the latest TdP that degenerated into ventricular fibrillation. In the risperidone group \((n = 6)\), premature ventricular contractions with \(R\) on \(T\) phenomenon were observed in 2 out of 6 animals at the middle dose and all animals at the high dose. At the high dose, episodes of TdP were detected in 4 out of 6 animals, and 2 animals died after the latest TdP that degenerated into ventricular fibrillation.

#### 3.2. Effects of paliperidone and risperidone on the electrophysiological and hemodynamic parameters

Fig. 3B shows the effects of paliperidone and risperidone on the QT interval, MAP90 (upper panel), mean BP (middle panel) and atrial rate (lower panel). The QT interval and MAP90 were prolonged by paliperidone as well as risperidone in a dose-dependent manner. The extents of increase of the QT interval and MAP90 by paliperidone were less than those in the risperidone group. Paliperidone slightly but significantly decreased the MBP and atrial rate, and similar results were obtained by risperidone except that the MBP transiently increased after the high-dose administration.

#### 3.3. Beat-to-beat analysis for the temporal dispersion of repolarization

Typical results of effects of paliperidone and risperidone on the Poincaré plots of the MAP90 are shown in Fig. 4. Table 1 summarized the short-term and long-term variability of the MAP90 before and after the drug administration. Paliperidone as well as risperidone at middle and high doses significantly increased the short-term variability of the MAP90, which were accompanied with significant prolongation of the MAP90.

### 4. Discussion

We assessed proarrhythmic effects of paliperidone using the acute atrioventricular block rabbit (13), which were compared with those of risperidone. The low dose of paliperidone did not affect hemodynamic or electrophysiological parameters of the rabbit, whereas the middle and high doses exerted proarrhythmic actions, which was similar to those of risperidone as observed in this study.
In the rabbit heart, the expression of KvLQT1 (α-subunit for slowly activating delayed rectifier K⁺ current (Iₖs) channels) and minK (β-subunit for Iₖs channels) has been demonstrated to be lower than that in human, resulting in small density of Iₖs, which is recognized to provide the molecular basis for the sensitivity of the rabbit heart to Iₖs blocker-induced repolarization abnormalities and related arrhythmias (17).

4.1. Experimental doses used in this study

Clinical application of risperidone has provided important evidence that potent 5-HT₂A antagonism combined with milder dopaminergic D₂ antagonism resulted in significantly improved clinical signs and symptoms (18). In animal studies in vivo, subcutaneous administration of paliperidone and risperidone

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**Fig. 3.** Proarrhythmic and electrophysiological effects of paliperidone (n = 6) and risperidone (n = 6) in the acute atrioventricular block rabbit. (A) Summary of proarrhythmic effects of paliperidone (left panel) and risperidone (right panel). Each column indicates the responses of each rabbit to drugs. The columns are marked according to the severity of arrhythmias developed in each minute. Black column means the occurrence of ventricular fibrillation (VF), i.e., death. Red column means the occurrence of ventricular tachycardia (VT) including torsades de pointes (TdP). Orange column means the occurrence of premature ventricular contraction (PVC) with the R on T phenomenon. (B) Time course of the effects of paliperidone and risperidone on the QT interval, MAP duration at 90% repolarization level (MAP₉₀), mean blood pressure (MBP) and atrial rate. In the paliperidone group (left panels), the pre-drug control value (C) of QT interval, MAP₉₀, MBP and atrial rate were 242 ± 13, 180 ± 12 ms, 38 ± 3 mmHg and 273 ± 6 beats/min, respectively, whereas those in the risperidone group (right panels) were 283 ± 12, 220 ± 13 ms, 40 ± 3 mmHg and 355 ± 10 beats/min, respectively. In the experimental group receiving paliperidone, the MAP₉₀ was prolonged in a dose-dependent manner, and obvious but statistically insignificant difference was detected in the MAP₉₀ 10 min after the start of administration of 0.6 mg/kg (P = 0.0522). The maximal changes in the MAP₉₀ at 0.06, 0.6, and 6 mg/kg from pre-drug control value (C) were +5 ± 3, +52 ± 10, and +101 ± 30 ms respectively. The maximal changes in the experimental group receiving risperidone at 0.03, 0.3, and 3 mg/kg were +18 ± 7, +105 ± 18, and +147 ± 28 ms, respectively. Data are presented as mean ± S.E.M. The closed symbols represent the significant differences from each pre-drug control value (C) by P < 0.05.
suppressed tryptamine-induced seizures with ED50 values of 0.11 and 0.056 mg/kg, respectively (19), showing that 5HT2A antagonism by paliperidone is estimated to be roughly half as potent as that by risperidone. On the other hand, intravenously administered 0.03 mg/kg of risperidone over 10 min is estimated to be clinically relevant based on our previous study using halothane-anesthetized animals (12). Thus, the present study for paliperidone can be considered to be carried out under the therapeutic (0.06 mg/kg, i.v.) to supra-therapeutic (0.6–6 mg/kg, i.v.) levels. Although plasma concentrations of testing drugs were not measured in this study, we have already confirmed that the plasma concentration of a clinically relevant dose of risperidone (0.03 mg/kg, i.v.) was 0.125 μM in dogs, whereas those of supra-therapeutic doses (0.3 and 3 mg/kg, i.v.) were 1.176 and 8.223 μM, respectively (12).

4.2. Torsadogenic action of paliperidone

As shown in Figs. 2 and 3, paliperidone increased the number of premature ventricular contractions with R on T phenomenon at the middle dose and induced TdP at the high dose, which were not observed at the low dose. Premature ventricular contractions with R on T phenomenon, associated with early afterdepolarization (EAD)-induced triggered activity, have been known to be able to initiate the development of TdP when transmural dispersion of repolarization is exaggerated (20). The proarrhythmic mechanisms may be associated with electrophysiological effects of paliperidone, showing suppression of hERG K+ channels with IC50 values of 0.528–1.3 μM (9,21–23), slight inhibition of Na+ channels by 15% and L-type Ca2+ channels by 14% at 10 μM, and no effects on the IC50 at 10 μM (22). In our previous study using the same animal model, TdP arrhythmias can be finely detected after the administration of a class III antiarrhythmic drug nifekalant at 3 mg/kg (10 times higher dose for clinical use) (13), whose sensitivity was similar to that of a well-established predictive marker of proarrhythmia, repolarization alternans (25). These results suggest that paliperidone has torsadogenic potential at supra-therapeutic doses, which may partly explain the results of the Early Post-marketing Phase Vigilance for the prolonged release suspension of paliperidone palmitate showing 12 cases of sudden death and 4 cases of suspected sudden death in the 32 fatal cases (7). More importantly, paliperidone was prescribed in combination with other antipsychotics in 25 out of 32 fatal cases. Thus, more careful plan for drug administration might have been required for these patients.

4.3. Comparison with torsadogenic potential of risperidone

Paliperidone has been reported to suppress hERG K+ channels expressed in HEK293 cells with IC50 values of 0.528–1.3 μM (9,21–23). Since the IC50 values of risperidone have been shown to be 0.148–0.85 μM (21,23,26), effects of paliperidone on the hERG K+ channels are estimated to be roughly half as potent as those of risperidone. As shown in Figs. 2 and 3, risperidone increased the number of premature ventricular contractions with R on T phenomenon at the middle dose and induced TdP at the high dose. Similarly, it has been reported that supra-therapeutic dose of
risperidone (3 mg/kg) significantly increased the electrically vulnerable period of the ventricular muscle in the halothane-anesthetized canine model (12). Meanwhile, clinically relevant dose of 0.06 mg/kg of paliperidone or 0.03 mg/kg of risperidone hardly affected the ECG parameters in this study. These in vivo observations are essentially in accordance with a systematic review of case reports, where paliperidone is suggested to be a relatively safe drug in the context of QT interval prolongation and TdP when properly prescribed (27). Since torsadogenic potential of paliperidone can be estimated to be equal or slightly subordinate in comparison with that of risperidone based on the current study, caution has to be paid for patients with TdP risks on the prescription of paliperidone as well as risperidone (27).

4.4. Clinical implications

This study provides information for the torsadogenic potential of paliperidone using the acute atrioventricular block rabbit, which is adopted as a proarrhythmic model (13). Since contribution of Ks channels to repolarization phase in the rabbit ventricular myocytes has been demonstrated to be lower than that in the healthy human (17), the current results may correspond to effects of paliperidone in patients with congenital long QT syndrome type 1 or heart diseases complicating secondarily down-regulated Ks, such as sustained bradycardia (28,29). More importantly, cardiovascular morbidity and mortality are important problems in patients with schizophrenia, because a wide spectrum of reasons, ranging from genes to the environment, are held responsible for causing the cardiovascular risk factors that can lead to shortening the life expectancy of patients with schizophrenia (30). Furthermore, concomitant use of antipsychotics or other drugs with the possibility of prolongation of the QT interval may lead to marked QT prolongation (31,32). Although paliperidone has been reported to cause a modest increase in the QT interval in clinical practice (3,8), we have to recognize that the drug has an inherent proarrhythmic action.

4.5. Conclusions

Paliperidone showed torsadogenic potential at supratherapeutic doses in this study, whose potency can be estimated to be equal or slightly subordinate in comparison with that of risperidone. Caution has to be paid for patients with TdP risks on the prescription of paliperidone as well as risperidone since the drug has an inherent proarrhythmic action.

Conflicts of interest

We declare no conflict of interest.

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References


