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Torsadogenic action of cisapride, *dl*-sotalol, bepridil and verapamil analyzed by the
chronic atrioventricular block cynomolgus monkeys:
Comparison with that reported in the CiPA in silico mechanistic model

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Abstract

In order to bridge the gap of information between the in silico model and human subjects, we evaluated torsadogenic risk of cisapride, *dl*-sotalol, bepridil and verapamil selected from 12 training compounds in the comprehensive in vitro proarrhythmia assay (CiPA) using the chronic atrioventricular block monkeys. Cisapride (0, 1 and 5 mg/kg, n=5 for each dose), *dl*-sotalol (0, 1, 3 and 10 mg/kg, n=5 for each dose), bepridil (0, 10 and 100 mg/kg, n=4 for each dose), verapamil (0, 1.5, 15 and 75 mg/kg, n=4 for each dose) were orally administered to the monkeys in conscious state. Five mg/kg of cisapride, 1, 3 and 10 mg/kg of *dl*-sotalol and 100 mg/kg of bepridil prolonged $\Delta\Delta Q_{TcF}$, which was not observed by verapamil. Torsade de pointes was induced by 5 mg/kg of cisapride in 2 out of 5 animals, by 10 mg/kg of *dl*-sotalol in 5 out of 5 and by 100 mg/kg of bepridil in 2 out of 4, which was not induced by verapamil. These torsadogenic doses were normalized by their maximum clinical daily ones to estimate torsadogenic risk. The order of risk was *dl*-sotalol > bepridil \geq cisapride > verapamil in our study. Since the order was bepridil \geq *dl*-sotalol > cispride > verapamil in CiPA in silico mechanistic model validation, sympathetic regulation on the heart may play a pivotal role in the onset of torsade de pointes in vivo.

Keywords: atrioventricular block, cynomolgus monkey, CiPA, in silico model, torsade de pointes

Introduction

When ventricular repolarization is delayed and the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including torsade de pointes. Thus, much emphasis has been placed on the potential proarrhythmic effects of pharmaceuticals that are associated with QT interval prolongation on the development of pharmaceuticals (ICH harmonised tripartite guideline, 2005). The international council on harmonization (ICH) S7B regulatory guidelines describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization, including the in vitro I_{Kr} /hERG assay, in vivo QT assay and optional follow-up studies, which constitute the integrated risk assessments (ICH harmonised tripartite guideline, 2005). Furthermore, in the comprehensive in vitro proarrhythmia assay (CiPA) being classified into one of the follow-up studies, multi-ion channel pharmacology data are obtained in vitro, which are integrated into a human cardiomyocyte model in silico. The CiPA will predict proarrhythmia risk within the framework of ICH S7B regulatory guidelines (ICH E14/S7B Implementation Working Group, 2020; ICH harmonised tripartite guideline, 2005; Li et al., 2019). However, it is not fully validated to what extent this CiPA in silico mechanistic model can estimate the proarrhythmic effects of pharmaceuticals in patients with labile repolarization.

The chronic atrioventricular block dog model is known to be useful for qualitatively and quantitatively predicting the risk of drug-induced torsade de pointes in human (Sugiyama 2008; Takahara et al., 2006; Thomsen et al., 2006; Volders et al., 1999). Moreover, the chronic atrioventricular block dog model has been shown to be able to simulate the pathophysiology in a patient who is the most sensitive to I_{Kr} blocker-induced torsade de pointes, by which proarrhythmia risk of drugs can be classified into high, low and no risk (Sugiyama 2008). However, expression level of major metabolic enzymes such as CYP3A4 in dogs is known to vary from that in human (Martignoni et al., 2006), while primate models may generally prove more predictive when metabolites and/or metabolic pathways of a drug are unique to primates (Sugiyama 2008). Furthermore, the chronic atrioventricular block monkey model has been shown to detect the drug-induced torsade de pointes attacks (Sugiyama 2008; Goto et al., 2020a; Goto et al., 2020b). Unlike the canine model, attack of torsade de pointes induced by drug interventions can be spontaneously terminated in the monkey model. Thus, the monkey model is more predictive of human responses than the canine one, especially when a repeated-measures design was employed, also significantly reducing the total number of animals (Sugiyama 2008; Goto et al., 2020a;

Goto et al., 2020b).

In order to bridge the gap of information between the in silico model and human subjects, we analyzed torsadogenic risk of cisapride, *dl*-sotalol, bepridil and verapamil selected from 12 CiPA training compounds (Li et al.,2019), which was compared with those reported by the in silico model and the chronic atrioventricular block dogs (Li et al.,2019; Oros et al., 2010; Sugiyama 2008).

Materials and methods

All experiments were approved by the Committee for Research at Ina Research Inc. (Nagano, Japan) (No. IN05012 and IN09023), and performed in accordance with the Guidelines for the Care and Use of laboratory Animals of Ina Research, Inc. Experiments were performed using cynomolgus monkeys. Animals were obtained from Kears Co., Ltd. (Osaka, Japan) and Ina Research Philippines, Inc. (Muntinlupa, the Philippines). The animals were kept in individual cages with a 12 h light (7:00-19:00) to dark (19:00-7:00) cycle. The ventilation provided a total air exchange rate of 16-22 times per hour. The room temperature was maintained at 22-28°C, and relative humidity was 40-80%. The animals had access to tap water *ad libitum* and received 100 g of food pellets (PS, Oriental Yeast Co., Ltd., Tokyo, Japan) once a day. A timeline showing the experimental paradigm from production of atrioventricular block to recording of drug effects and sampling of the blood is schematically depicted in Fig. 1.

Production of chronic atrioventricular block

Cynomolgus monkeys were anesthetized with pentobarbital sodium (25 mg/kg, *i.v.*). After intubation with a cuffed endotracheal tube, the respiration was controlled using a volume-limited ventilator (SN-480-5; Shinano Manufacturing Co., Ltd., Tokyo, Japan) with room air. Tidal volume and respiratory rate were set at 10 mL/kg and 15 breaths/min, respectively. The surface lead II electrocardiogram was continuously monitored. The atrioventricular block was induced as previously described (Sugiyama 2008; Goto et al., 2020a; Goto et al., 2020b). A clinically available 5-French quad-polar electrodes catheter (Cordis-Webster Inc., CA, USA) was inserted through the right femoral vein under sterile condition, of which tip was positioned across the tricuspid valve under the guide of bipolar electrogram from the distal electrode pair. The optimal site for the atrioventricular node ablation; namely, the compact atrioventricular node, was determined on the basis of the intracardiac electrogram, in which a small notch indicating the His bundle electrogram could be recorded between the atrial and ventricular electrograms and atrium/ventricular voltage ratio could become >1.5 during a respiratory cycle (Fig. 2). The power source for the atrioventricular nodal ablation was obtained from an electrosurgical generator (MS-1500; Senko Medical Instrument Manufacturing Co., Ltd., Tokyo, Japan), which delivers continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After determining the location, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to an

indifferent patch electrode positioned on the animal's back, which was continued then for 30 s if junctional ectopic complexes were induced. The endpoint of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm.

We kept the animals for >12 months during the experimental period with the highest standard of the health management, dietary control and recreation in addition to performing the refinement of the animal experimentation, implementing the 3Rs principles.

Experimental protocol

Electrocardiogram equivalent to lead II was recorded using Holter electrocardiograph with analysis system (QR2100 and HS1000; Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan) over 24 h without anesthesia. The data acquisition was started 1 h before each drug or vehicle was administered. Five to ten recordings of consecutive complexes were used to calculate the mean for the electrocardiographic indices. The QT interval was corrected with Fridericia's formula: $QTcF = QT / (RR/1000)^{1/3}$, in which RR was given in ms (Fridericia 1920). Torsade de pointes was defined as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting ≥ 6 consecutive beats (Satoh and Zipes, 1996).

Experiments were performed in conscious state using 10 cynomolgus monkeys of either sex of 3-9 years old, weighing 3-6 kg, of which atrioventricular node had been ablated >6 months before (Table 1). Cisapride, *dl*-sotalol, bepridil and verapamil in each dose were suspended with 0.5% methylcellulose solution into a final volume of 5 mL/kg for oral administration. Either cisapride in doses of 1 and 5 mg/kg ($n=5$), *dl*-sotalol in doses of 1, 3 and 10 mg/kg ($n=5$), bepridil in doses of 10 and 100 mg/kg ($n=4$) or verapamil in doses 1.5, 15 and 75 mg/kg ($n=4$) were orally administered. The effects of the drugs on the idioventricular rate, QT and QTcF were assessed at 0, 1, 2, 3, 4, 6, 8, 12 and 21 h after the start of administration for cisapride and *dl*-sotalol, whereas those were done at 0, 1, 2, 3, 4, 6, 8, 12 and 24 h after the start of administration for bepridil and verapamil. The dosing interval between the drugs as well as that between the doses was set ≥ 1 week. The vehicle was orally administered to each animal, and each variable was assessed in the same manner.

The blood was sampled from hind limb vein in a volume of 1.2 mL at 0, 1, 2, 4, 8 and 24 h after the start of administration for bepridil and verapamil, which were centrifuged at $1,600 \times g$ for 10 min at 4°C. The plasma was stored at -95°C to -65°C until the drug concentration was measured, which was performed at Ina Research Inc.

using a validated, high-performance liquid chromatography system (Prominence UFLC; Shimadzu Corporation, Kyoto, Japan). The lower limit of quantification for bepridil and verapamil was 0.25 and 0.1 ng/mL, respectively.

Drugs

The following drugs were purchased: pentobarbital sodium (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), *dl*-sotalol (Sotacor[®], Bristol-Myers Squibb Company, Tokyo, Japan), bepridil (Bepricor[®], Schering-Plough Corporation, Osaka, Japan) and verapamil (Vasolan[®], Eisai Co., Ltd., Tokyo, Japan), whereas cisapride was obtained from Yamanashi Research Center of Clinical Pharmacology at University of Yamanashi (Yamanashi, Japan).

Statistical analyses

The change in QTcF from its basal control value (0) was expressed as Δ QTcF at each time point. The time-matched difference in Δ QTcF at each time point between zero-administered and each dose of drug-administered groups was defined as $\Delta\Delta$ QTcF. Data are expressed as mean \pm SEM. The statistical significances within a parameter were examined by one-way, repeated-measures analysis of variance (ANOVA). When a *p* value <0.05 was confirmed by ANOVA, a post-hoc test for multiple comparisons (Contrasts) was used to determine the statistical significance between the basal control value (0) and the other ones after the drug administration. We assessed the effects of different doses of a drug on $\Delta\Delta$ QTcF using the same animal populations by using a cross-over design. We did not perform statistical analysis for the dose-dependent effects of a drug on $\Delta\Delta$ QTcF, since main purpose of this study was not to show the drug-induced QT-interval prolongation but to find the doses that could induce torsade de pointes. A *p* value <0.05 was considered to be statistically significant.

Results

Clinical picture of the animals

One animal (animal #7) died 2 h after the administration of 75 mg/kg of verapamil due to acute congestive heart failure, in which torsade de pointes was not observed. Accordingly, statistical analysis of the effects of 75 mg/kg of verapamil on $\Delta\Delta Q_{TcF}$ was performed by using data from 3 surviving animals. Vomiting was induced in #9 and #10 animals 1 h after the administration of 100 mg/kg of bepridil, whereas it was observed in #8, #9 and #10 animals 2 h, 4 h and 1 h after the administration of 75 mg/kg of verapamil, respectively.

Electrocardiographic variables

Time courses of changes in $\Delta\Delta Q_{TcF}$ are summarized in Fig. 3. Cisapride in a dose of 5 mg/kg prolonged $\Delta\Delta Q_{TcF}$ for 1-4 h with peak change of $+59\pm 10$ at 3 h, which was not observed after the administration of 1 mg/kg. *dl*-Sotalol in doses of 1, 3 and 10 mg/kg prolonged $\Delta\Delta Q_{TcF}$ for 1-12 h, for 1-21 h and for 1-21 h with peak change of $+75\pm 21$ at 2 h, $+123\pm 29$ at 2h and $+166\pm 21$ at 2 h, respectively. Bepridil in a dose of 100 mg/kg prolonged $\Delta\Delta Q_{TcF}$ for 4-12 h with peak change of $+94\pm 39$ at 12 h, which was not observed after the administration of 10 mg/kg. Verapamil in a dose of 1.5, 15 or 75 mg/kg did not alter $\Delta\Delta Q_{TcF}$.

Proarrhythmic effects

The number of episodes of torsade de pointes in each treatment group is summarized in Fig. 4, whereas typical tracing of electrocardiogram showing the onset and termination of torsade de pointes observed at 10.5 h after the administration of 100 mg/kg of bepridil is depicted in Fig. 5. Torsade de pointes was induced by 5 mg/kg of cisapride in 2 out of 5 animals with the total number of episodes of 46 in #3 and 1 in #4; by 10 mg/kg of *dl*-sotalol in 5 out of 5 ones with that of 51 in #1, 6 in #2, 19 in #3, 78 in #5 and 5 in #6; and by 100 mg/kg of bepridil in 2 out of 4 ones with that of 1 in #8 and 10 in #9. Each attack of torsade de pointes was spontaneously terminated; thus, no animal died by arrhythmia during the experimental period, enabling to perform cross-over study protocol.

Pharmacokinetic variables

The time courses of changes in the plasma concentration of bepridil and verapamil are summarized in Table 2. The peak concentrations for 10 and 100 mg/kg

of bepridil were 102.2 ng/mL (265.7 nmol/L) at 1 h and 144.9 ng/mL (376.8 nmol/L) at 2 h after drug administration, respectively (n=3 for each dose), whereas those for 1.5, 15 and 75 mg/kg of verapamil were 0.2 ng/mL (0.5 nmol/L) at 1 h, 20.8 ng/mL (42.3 nmol/L) at 1 h and 299.3 ng/mL (609.6 nmol/L) at 2 h, respectively (n=3 for each dose). The plasma concentration of bepridil or verapamil of #7 animal could not be obtained due to technical error during the assay. It should be noted that the concentration of bepridil rose again at 8 h after the oral administration of 100 mg/kg, which was greater than that at 1 h.

Discussion

We orally administered cisapride, *dl*-sotalol, bepridil and verapamil to the chronic atrioventricular block monkeys, and confirmed qualitatively similar torsadogenic profile to those reported with the in silico model validation and with the chronic atrioventricular block dogs, although their torsadogenic risk was quantitatively different among them as discussed below (Li et al., 2019; Oros et al., 2010; Sugiyama 2008). In addition, each attack of torsade de pointes was spontaneously terminated as previously described (Goto et al., 2020a; Goto et al., 2020b; Sugiyama 2008), enabling to assess multiple drug doses within a small number of animals as described in Table 1.

Rationale for the drug doses

Clinically recommended daily oral doses of cisapride, *dl*-sotalol, bepridil and verapamil according to the drug information from the manufacturer along with their maximum daily doses per kg are summarized in Table 3. Since we could not measure the plasma concentration of cisapride or *dl*-sotalol, we looked into previous reports and our preliminary study data. In a previous study using cynomolgus monkeys (Ando et al., 2005), an oral dose of 1, 3 and 10 mg/kg of cisapride attained the peak plasma concentrations of 33, 121 and 420 ng/mL, respectively. When estimated by the linear regression, 5 mg/kg of cisapride would provide 205.6 ng/mL (441.3 nmol/L). In our preliminary study using a limited number of experiments with the chronic atrioventricular block monkeys (n=2), orally administered 10 mg/kg of *dl*-sotalol showed peak plasma concentrations of 3.9 (14.3) and 4.6 µg/mL (17.1 µmol/L), respectively. Since protein binding ratio of cisapride, *dl*-sotalol, bepridil and verapamil were described to be 98%, 0%, 98.74% and 93.7%, respectively in the drug information from the manufacturer, peak concentrations of their unbinding form after the administration of 5 mg/kg of cisapride, 10 mg/kg of *dl*-sotalol, 100 mg/kg of bepridil and 75 mg/kg of verapamil could be calculated as 8.8 nmol/L, 15.7 µmol/L, 4.7 nmol/L and 38.4 nmol/L, respectively. Since IC₅₀ value for I_{Kr} has been reported to be 10.1 nmol/L for cisapride, 110.6 µmol/L for *dl*-sotalol, 50 nmol/L for bepridil and 288 nmol/L for verapamil (Dutta et al., 2017), I_{Kr} can be suppressed by each drug in the order of cisapride (0.871) >> *dl*-sotalol (0.142) ≥ verapamil (0.134) > bepridil (0.094) when free plasma peak concentration/IC₅₀ values were used, which were depicted within the parenthesis.

Electrocardiographic variables

Cisapride, *dl*-sotalol and bepridil prolonged $\Delta\Delta\text{QTcF}$ in a dose-related manner, but verapamil did not prolong it in any dose as shown in Fig. 3. Bepridil and verapamil are generally classified into Ca^{2+} channel blockers. The inhibitory action of bepridil on I_{Kr} was 56 times greater compared with that on I_{CaL} , whereas that of verapamil was only 0.7 times in vitro (Dutta et al., 2017), explaining significant prolongation of $\Delta\Delta\text{QTcF}$ by bepridil and no effect on it by verapamil. These results indicate that the balanced inhibition between inward and outward currents for verapamil in vitro could be extrapolated into the in vivo condition.

Proarrhythmic effects

Using previously described stratification of torsadogenic risk of drugs with the chronic atrioventricular block dogs (Sugiyama 2008), we newly developed the classification criteria consisting of ‘high’, ‘intermediate’ and ‘low/no’, and determined the risk of cisapride, *dl*-sotalol, bepridil and verapamil in the monkey model as summarized in Table 3. *dl*-Sotalol induced torsade de pointes by ≤ 3 times of the maximum clinical daily dose, indicating it may have high risk. Cisapride or bepridil did not induce torsade de pointes by ≤ 3 times of the maximum clinical daily dose, but they induced torsade de pointes by > 3 times of it, indicating they will have intermediate risk. Verapamil did not induce torsade de pointes by any dose, indicating it has low/no risk.

In our previous study using the chronic atrioventricular block dogs (Sugiyama 2008), cisapride and *dl*-sotalol were classified into high risk; and bepridil was judged as intermediate risk with the currently defined criteria (Table 4). The results of *dl*-sotalol and bepridil in dogs were essentially in accordance with the present study; however, cisapride was classified into intermediate risk in this study. This discrepancy in magnitude of the torsadogenic risk could be understood as follows. Cisapride undergoes extensive “first-pass” metabolism by CYP3A4 system to cardio-inactive metabolite in humans, whereas cynomolgus monkeys do not have CYP3A4 but express CYP3A8 (Martignoni et al., 2006) which functions like human CYP3A4. Since dogs do not express CYP3A4 or its alternative enzymes (Martignoni et al., 2006), the plasma concentration of cisapride may be higher in dogs than in monkeys, thus making the risk of torsade de pointes greater in dogs. Indeed, approximately 10 times higher plasma concentrations were observed in dogs than in monkeys after the oral administration of cisapride (Ando et al., 2005; Toyoshima et al., 2005). Verapamil did not prolong the QT interval as previously reported (Sugiyama 2008), explaining its lack of torsadogenic action in the chronic atrioventricular block monkey model. The similar result was

reported by others using the chronic atrioventricular block dogs (Oros et al., 2010).

In the CiPA in silico mechanistic model validation (Li et al., 2019; Redfern et al., 2003), the order of torsadogenic risk was bepridil \geq *dl*-sotalol > cisapride > verapamil (Table 4). Meanwhile, that was *dl*-sotalol > bepridil \geq cisapride > verapamil in this study, which was in accordance with that in the in silico model except that torsadogenic risk of bepridil was less great than that of *dl*-sotalol in our study. Concomitant β -adrenoceptor blockade has been shown to increase the torsadogenic action of I_{Kr} blockers in vivo, since it may reduce physiological I_{Ks} function and can decrease the heart rate, enhancing I_{Kr} inhibition (Goto et al., 2019). In this study, *dl*-sotalol would block β -adrenoceptor, whereas bepridil might have increased the sympathetic tone via its hypotensive action (Ishizaka et al., 2008), which may explain why torsadogenic risk of *dl*-sotalol was greater than bepridil in vivo. In addition, late onset of torsade de pointes by bepridil (Fig. 4) may partly depend on its unique pharmacokinetics (Table 2) possibly due to enterohepatic circulation and/or attenuation of the hypotension-induced, reflex-mediated increase of sympathetic tone.

Study limitation

First, the relatively small number of animals of 4-5 for each drug may be a limitation of this study. However, our previous studies using the chronic atrioventricular block monkeys have demonstrated that the number of 3-6 animals would be enough to find hidden drug-induced torsade de pointes (Sugiyama 2008; Goto et al., 2020a; Goto et al., 2020b). Second, it had been empirically known that sensitivity of the model animals for the drug-induced torsade de pointes may be hardly altered by their age by itself, but can be rather determined by the duration after the production of atrioventricular block. Indeed in this experiment, we observed the onset of drug-induced torsade de points in 8 animals of 3, 3, 3, 4, 6, 6, 7 and 8 years old, which was not induced in 2 animals of 5 and 9 years old. However, further studies need to be performed to confirm this hypothesis.

Conclusion

Torsadogenic risks of the drugs in a monkey model were qualitatively similar to those assessed by the chronic atrioventricular block dogs and by the CiPA in silico mechanistic model. However, the magnitude of torsadogenic risk may vary among them, which could be in part explained by the lack of expression of metabolic enzyme in dogs, and by the absence of sympathetic regulation on the heart in the in silico model. Thus, the use of monkey model could make up disadvantage of the chronic

atrioventricular block dogs; moreover, it would bridge the gap of information between the in silico model and human subjects.

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Figure legends

Fig. 1

Schematic representation of experimental protocol. (A) A timeline showing the experimental paradigm from the production of atrioventricular (AV) block to the assessment of drug effects. (B) Time points for the analysis of electrocardiogram (ECG) and the blood sampling after each drug administration.

Fig. 2

Electrocardiogram (ECG) before and after radiofrequency ablation of the atrioventricular (AV) node. (A) Actual tracings of surface lead II ECG and His bundle (His) electrogram from the ablation site before the radiofrequency ablation. (B) Typical tracing of ECG after the radiofrequency ablation. Note that the P wave and QRS complex were observed with their respective automaticity rate after the onset of AV conduction block. P: P wave; and QRS: QRS complex. A: atrial; H: His bundle; and V: ventricular electrograms.

Fig. 3

Time courses of the $\Delta\Delta\text{QTcF}$ after the oral administration of 1 and 5 mg/kg of cisapride (n=5), 1, 3 and 10 mg/kg of *dl*-sotalol (n=5), 10 and 100 mg/kg of bepridil (n=4), and 1.5, 15 and 75 mg/kg of verapamil (n=4 for 1.5 and 15 mg/kg, n=3 for 75 mg/kg). Statistical analysis of the effects of 75 mg/kg of verapamil on $\Delta\Delta\text{QTcF}$ was performed by using data from 3 surviving animals, since one animal (#7) died from acute congestive heart failure at 2 h after the drug administration. Data are presented as mean \pm SEM. The closed symbols represent significant differences from their respective basal control values (0) just before the drug administration by $p < 0.05$.

Fig. 4

The number of episodes of torsade de pointes (TdP) after the administration of 5 mg/kg of cisapride (n=5), 10 mg/kg of *dl*-sotalol (n=5), 100 mg/kg of bepridil (n=4), and 75 mg/kg of verapamil (n=4). Red rectangles: #1 animal; closed ones: #2 animal; grey ones: #3 animal; green ones: #4 animal; blue ones: #5 animal; open ones: #6 animal; yellow ones: #8 animal; and brown ones: #9 animal.

Fig. 5

Typical tracing of electrocardiogram equivalent to lead II just before bepridil

administration (Control, top) and 10.5 h after the administration of 100 mg/kg of bepridil (10.5 h after 100 mg/kg of bepridil, bottom) in #9 animal.

Table 1. Summary of background in the animals used for each treatment.

Treatment	#1 Female (6 years)	#2 Male (3 years)	#3 Male (3 years)	#4 Male (4 years)	#5 Female (3 years)	#6 Male (6 years)	#7 Male (9 years)	#8 Male (7 years)	#9 Female (8 years)	#10 Female (5 years)
Cisapride (1, 5 mg/kg, p.o.)	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5					
<i>dl</i> -Sotalol (1, 3, 10 mg/kg, p.o.)	Exp 1	Exp 2	Exp 3		Exp 4	Exp 5				
Bepidil (10, 100 mg/kg, p.o.)							Exp 1	Exp 2	Exp 3	Exp 4
Verapamil (1.5, 15, 75 mg/kg, p.o.)							Exp 1	Exp 2	Exp 3	Exp 4

shows the animal number. The number in parenthesis indicates age of each animal when the drug assessment was performed. Exp means Experiment number for each drug treatment.

Table 2. The time courses of plasma drug concentrations for bepridil and verapamil.

Parameters	Drugs	Dosage of drugs (mg/kg)	Time after the administration (h)				
			1	2	4	8	24
Plasma concentration (ng/mL)	Bepridil (n=3)	10	102.2±28.2	94.5±22.3	56.5±17.0	11.5±4.6	1.6±1.5
		100	128.6±50.6	144.9±68.0	127.8±50.2	134.6±73.1	68.0±34.7
	Verapamil (n=3)	1.5	0.2±0.1	0.2±0.0	ND	ND	ND
		15	20.8±6.8	14.6±6.1	4.8±2.0	1.8±1.2	0.1±0.1
		75	196.4±92.6	299.3±138.0	139.9±54.4	33.0±15.1	3.0±2.3

ND: not detected due to below the detection limit.

Table 3. Risk stratification of drugs assessed by the chronic atrioventricular block monkeys.

Drugs	Clinical daily dose	Maximum clinical daily dose	$\leq 3x$ of maximum clinical daily dose			$> 3x$ of maximum clinical daily dose		Possible risk
			Drug dose (Incidence of TdP)			Drug dose (Incidence of TdP)		
Cisapride	7.5-20 mg	0.33 mg/kg	1 mg/kg (0/5)			5 mg/kg (2/5)		Intermediate
<i>dl</i> -Sotalol	80-320 mg	5.33 mg/kg	1 mg/kg (0/5)	3 mg/kg (0/5)	10 mg/kg (5/5)			High
Bepidil	100-200 mg	3.33 mg/kg	10 mg/kg (0/4)			100 mg/kg (2/4)		Intermediate
Verapamil	120-240 mg	4 mg/kg	1.5 mg/kg (0/4)			15 mg/kg (0/4)	75 mg/kg (0/4)	Low/No

Possible risk of cisapride, *dl*-sotalol, bepidil and verapamil in the monkey model was determined using new stratification of torsadogenic risk of drugs with the atrioventricular block dogs based on our previous report (Sugiyama, 2008). Maximum clinical daily dose (mg/kg) was obtained by the following equation: upper limit of clinical daily dose (mg)/60 (kg). Incidence of TdP: the number of animals that showed torsade de pointes (TdP)/the number of experiments.

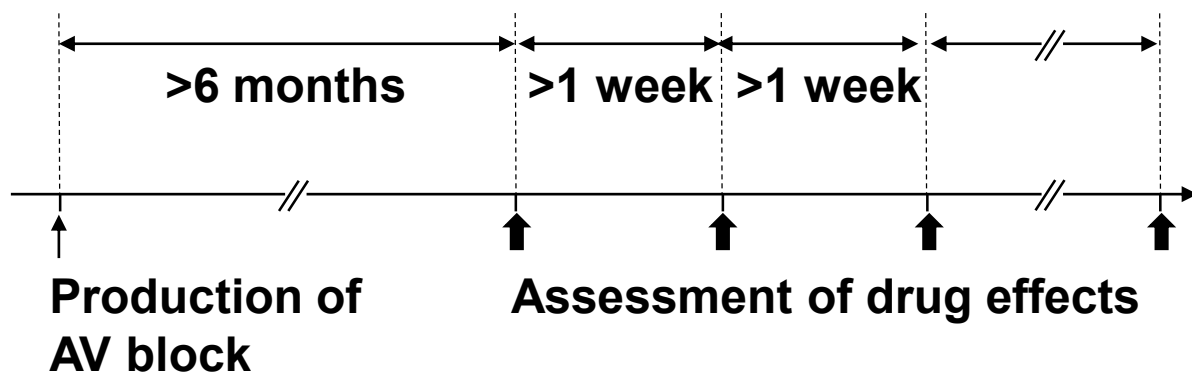
Table 4. Correlations of possible risks of drugs for inducing TdP among the chronic AV block monkeys, dogs and the CiPA in silico mechanistic model.

Possible risk	AV block monkeys	AV block dogs (Sugiyama, 2008)			CiPA in silico mechanistic model (Li et al., 2019)		
	Drugs	Drugs	$\leq 3\times$ of maximum clinical daily dose		$>3\times$ of maximum clinical daily dose		
			Dose (Incidence of TdP)		Dose (Incidence of TdP)		
High	<i>dl</i> -Sotalol	<i>dl</i> -Sotalol Cisapride	3 mg/kg (3/4) 1 mg/kg (1/6)	10 mg/kg (3/4)	Bepridil <i>dl</i> -Sotalol	<0.0581 $\mu\text{C}/\mu\text{F}$	
Intermediate	Bepridil Cisapride	Bepridil	3 mg/kg (0/4)		30 mg/kg (3/4)	Cisapride	0.0581 <Intermediate <0.0671
Low/No	Verapamil					Verapamil	0.0671 <

Drugs were given p.o. in the AV dogs. Maximum clinical daily dose (mg/kg) was obtained by the following equation: upper limit of clinical daily dose (mg)/60 (kg). AV: atrioventricular; CiPA: the comprehensive in vitro proarrhythmia assay; and Incidence of TdP: the number of animals that showed torsade de pointes (TdP)/the number of experiments.

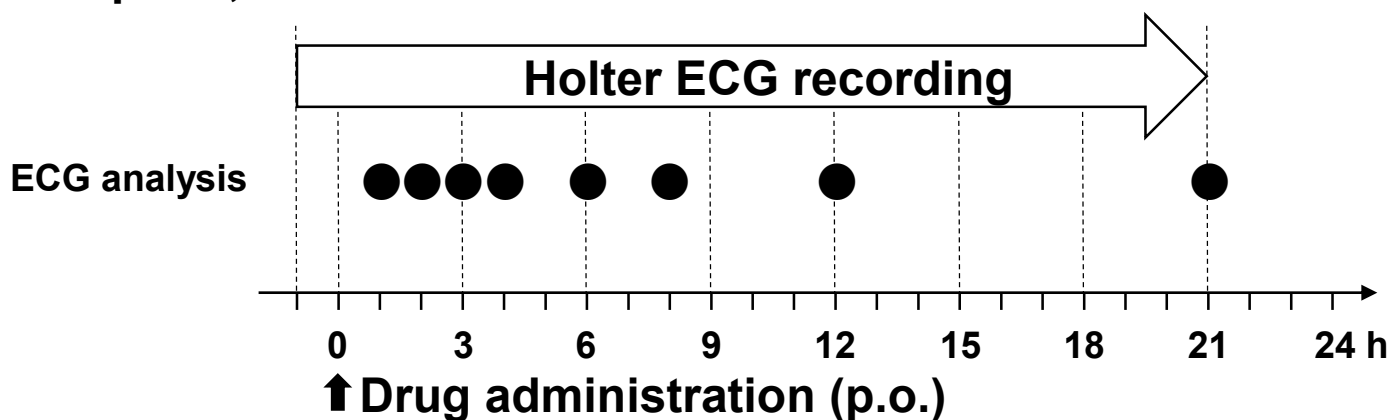
Figure 1

A



B

Cisapride, *d*/-Sotalol



Bepridil, Verapamil

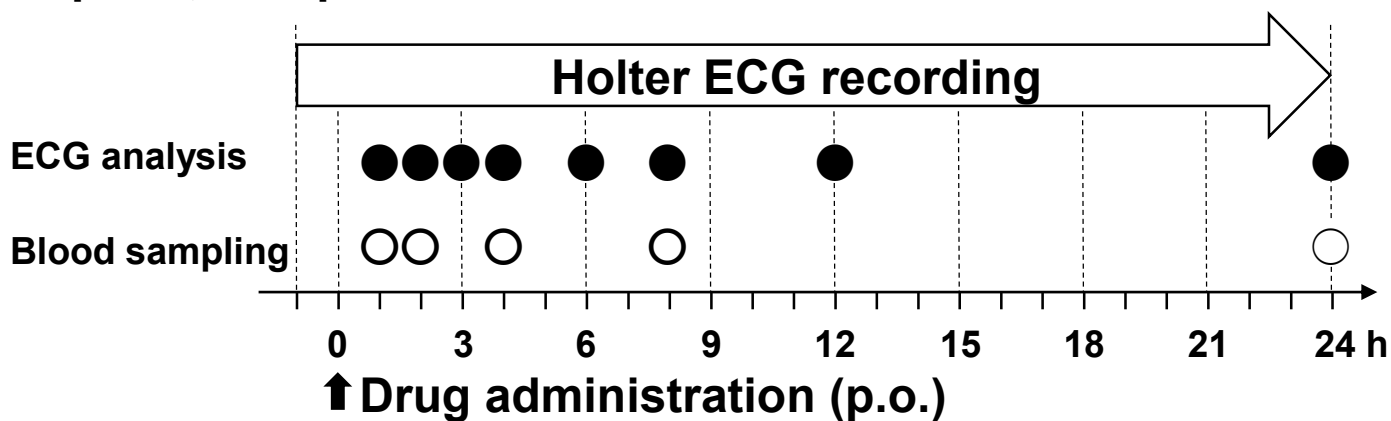
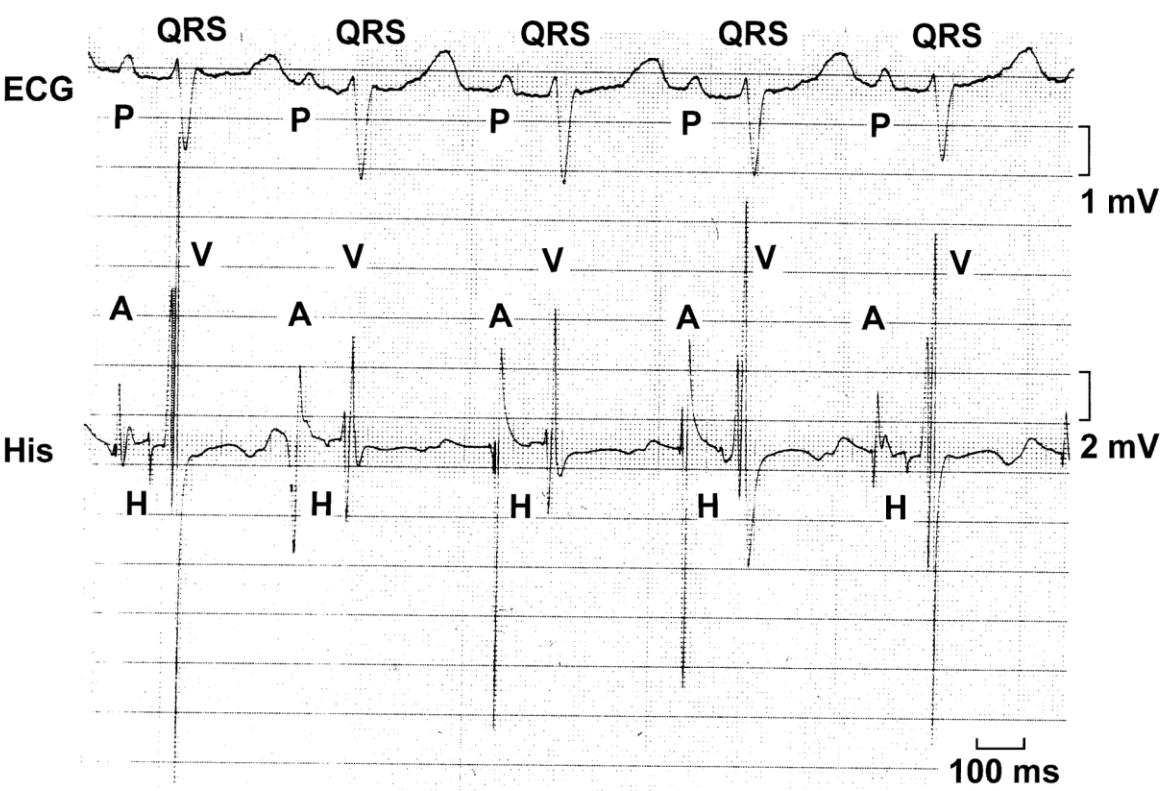


Figure 2

A Before AV node ablation



B After AV node ablation

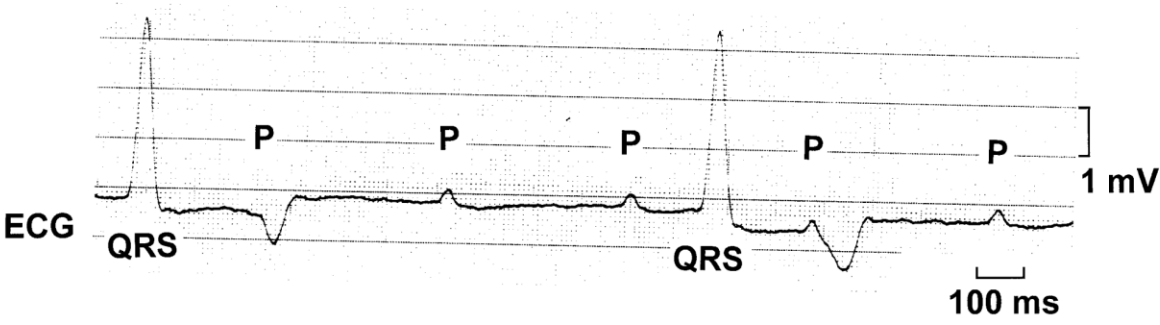


Figure 3

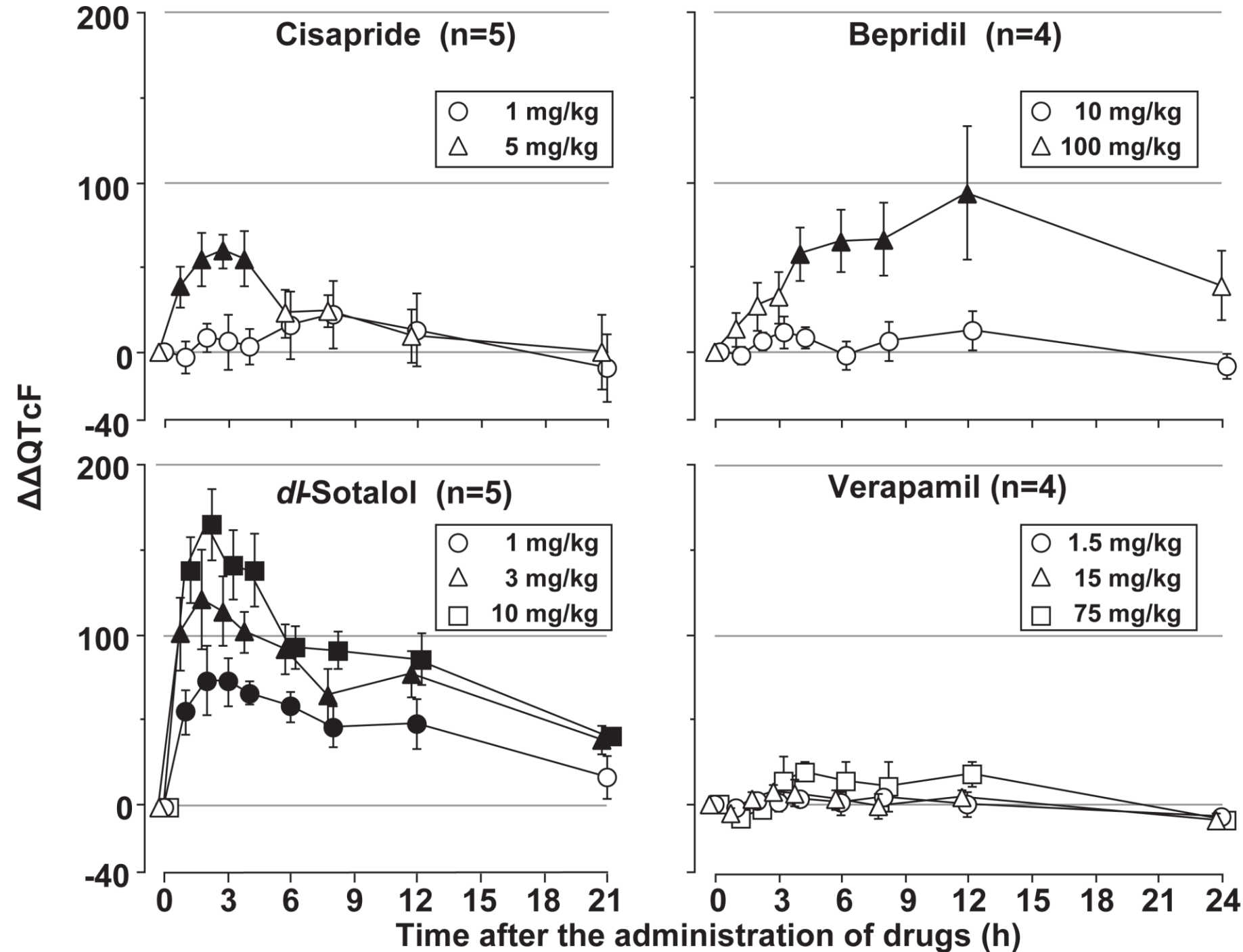


Figure 4

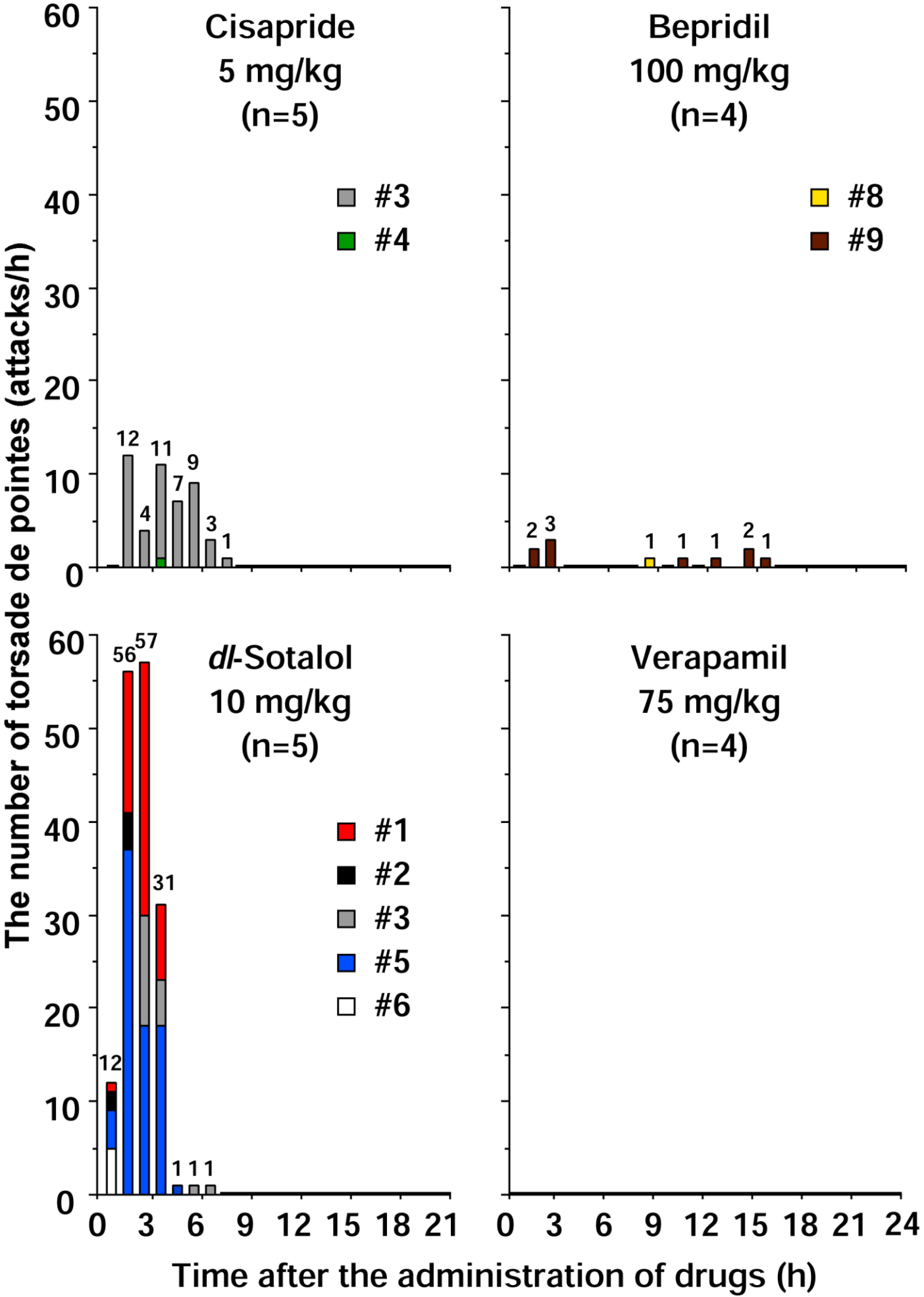
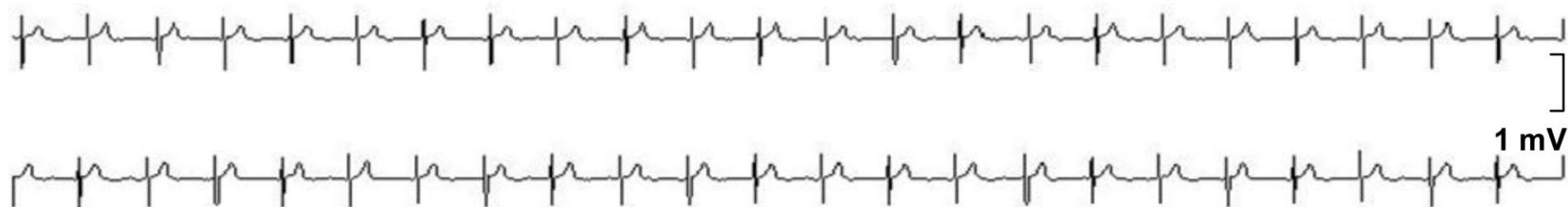


Figure 5

Control



10.5 h after 100 mg/kg of bepridil

