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Measurement of J–T_{peak}c along with QT-Interval Prolongation May Increase the Assay Sensitivity and Specificity for Predicting the Onset of Drug-Induced Torsade de Pointes: Experimental Evidences Based on Proarrhythmia Model Animals

Ai Goto[1] · Mihoko Hagiwara-Nagasawa[2] · Ryuichi Kambayashi[2] · Koki Chiba[1] · Hiroko Izumi-Nakaseko[1,2] · Atsuhiko T. Naito[1,2] · Yasunari Kanda[3] · Atsushi Sugiyama[1,2]

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Abstract

dl-Sotalol which can block both K^+ channel and ß-adrenoceptor has been shown to prolong the J–T_{peak}c of electrocardiogram in beagle dogs but tended to shorten it in *microminipigs*, although the drug prolonged the QT interval in both animals under physiologically maintained experimental condition. In order to estimate how the changes in the J–T_{peak}c in the normal hearts would be reflected in the pathologic hearts, we compared proarrhythmic effects of *dl*-sotalol by using proarrhythmia models of beagle dogs and *microminipigs*, of which atrioventricular node had been ablated > 2 months and 8–9 weeks before, respectively ($n = 4$ for each species). *dl*-Sotalol in an oral dose of 10 mg/kg induced torsade de pointes in three out of four beagle dogs, which degenerated into ventricular fibrillation. In *microminipigs*, the same dose did not trigger torsade de pointes at all, whereas intermittent ventricular pauses were observed in each animal after the drug treatment. These results indicate that assessment of the J–T_{peak}c along with the QT-interval prolongation in healthy subjects may provide reliable information of risk prediction for patients susceptible to the drug-induced torsade de pointes.

Keywords *dl*-Sotalol · J–T_{peak}c · Torsade de pointes · Beagle dogs · *Microminipigs*

Introduction

Clinical as well as non-clinical studies have suggested that the drug-induced torsade de pointes may develop particularly when there is larger inward current compared with outward one during the early repolarization phase of the ventricle, since it may trigger early afterdepolarization [1–7]. Accordingly, the J–T_{peak}c prolongation in the electrocardiogram has been expected as a new surrogate marker for estimating such an imbalance [1–7]. However, it is still unknown that assessment of the J–T_{peak}c along with the QT-interval prolongation in healthy subjects may provide reliable information of risk prediction for patients susceptible to the drug-induced torsade de pointes.

dl-Sotalol has both ß-adrenoceptor and I_{Kr} blocking actions [8], which has been shown to prolong the J–T_{peak}c of electrocardiogram in beagle dogs but tended to shorten it in *microminipigs*, although the drug prolonged the QT interval in both animals under physiologically maintained condition [9, 10]. *dl*-Sotalol has been used as a positive control drug

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Ai Goto and Mihoko Hagiwara-Nagasawa have contributed equally to this work.

✉ Atsushi Sugiyama
atsushi.sugiyama@med.toho-u.ac.jp

[1] Department of Pharmacology, Toho University Graduate School of Medicine, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540, Japan

[2] Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540, Japan

[3] Division of Pharmacology, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

to confirm QT-interval prolonging effect in various animal models [9–12], whereas *d*-sotalol rather than *dl*-sotalol has been adopted to induce torsade de pointes in proarrhythmia models [13–15], since *d*-isomer lacks ß-adrenoceptor blocking action [8].

In order to better understand how the difference of the *dl*-sotalol-induced changes in J–T_{peak}c between the intact hearts of beagle dogs and *microminipigs* will be reflected in their pathologic hearts, we assessed the proarrhythmic effects of the drug by using well-established proarrhythmia model animals; namely, the chronic atrioventricular block beagle dogs and *microminipigs* [13, 16]. In our preliminary study using a limited number of *microminipigs* at 2–12 months after the induction of atrioventricular block [16], we could not detect the episode of *dl*-sotalol-induced torsade de pointes possibly due to physiologically full adaptation against bradycardia-induced cardio-mechanical volume overload. Accordingly, we performed the current experiment using *microminipigs* at 8 to 9 weeks after the induction of atrioventricular block when the sensitivity would be enhanced for detecting drug-induced torsade de pointes [16].

Materials and Methods

Experiments were performed using beagle dogs and *microminipigs*, which were obtained from Kitayama Labes Co., Ltd. (Nagano, Japan) and Fuji Micra, Inc. (Shizuoka, Japan), respectively. The animals were kept in individual cages on a 12 h light (6:00–18:00) to dark (18:00–6:00) cycle. The ventilation provided a total air exchange rate of 10–15 times per hour. The room temperature was maintained at 23 ± 2 °C, and relative humidity was 50 ± 30%.

Production of Chronic Atrioventricular Block Animals

The catheter ablation technique of atrioventricular node was employed as previously described [17, 18]. Beagle dogs were anesthetized with thiopental sodium (30 mg/kg, i.v.) [13]. Meanwhile, *microminipigs* were pre-anesthetized by an intramuscular injection of ketamine (16 mg/kg)/xylazine (1.6 mg/kg); and then, propofol (1 mg/kg) was intravenously injected through a superficial auricular vein for inducing anesthetic condition. After intubation with a cuffed endotracheal tube, the respiration was controlled using a volume-limited ventilator (SN-480-3; Shinano Manufacturing Co., Ltd., Tokyo, Japan) with room air. Tidal volume was set at 20 mL/kg for beagle dogs and 10 mL/kg for *microminipigs*, whereas respiratory rate was done at 15 breaths/min for both animals. To prevent blood clotting, heparin calcium (100 IU/kg, i.v.) was administered. The surface lead II electrocardiogram was continuously monitored.

A quad-polar electrodes catheter with a large tip of 4 mm (D7-DL-252; Cordis-Webster, CA, USA) was inserted through the right femoral vein under sterile condition and 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, of which a very small His deflection was recorded and atrium/ventricular voltage ratio was > 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. Proper care was taken for the animals until the proarrhythmic properties of a drug were studied [13, 17, 18].

Experimental Protocol

Experiments were performed using four beagle dogs of either sex weighing 10.8 ± 0.8 kg and four male *microminipigs* weighing 9.8 ± 1.3 kg, of which atrioventricular node had been ablated > 2 months and 8–9 weeks before, respectively, since sexual difference has not been confirmed in the sensitivity for detecting the drug-induced torsade de pointes in the animal studies [13, 16]. The electrocardiogram over 24 h without anesthesia was monitored using Holter electrocardiograph with analysis system (QR2100 and HS1000; Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan). Ten recordings of consecutive complexes were used to calculate the mean for the electrocardiographic indices. The QTc was calculated with Fridericia's formula: $QTc = QT/(RR/1000)^{1/3}$ [19]. Torsade de pointes was defined as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting ≥ 6 consecutive beats [20]. After the basal assessment, *dl*-sotalol in a dose of 10 mg/kg was orally administrated to each animal. The effects of the drug on the ventricular rate and QT intervals were assessed at 1, 2, 3, 4, 6, 8, 12 and 21 h after the start of administration, whereas the onset of arrhythmias was monitored for > 21 h.

Drugs

The following drugs were purchased: *dl*-sotalol (Sotacor®, Bristol-Myers Squibb Company, Tokyo, Japan), ketamine (Ketalar®, Daiichi Sankyo Company Ltd., Tokyo, Japan),

xylazine (Seractal®, Bayer Yakuhin Ltd., Osaka, Japan), thiopental sodium (Ravonal® for injection, Mitsubishi Tanabe Pharma Co., Osaka, Japan) and heparin calcium (Caprocin®, Sawai Pharmaceutical Co. Ltd., Osaka, Japan).

Statistical Analyses

Data are expressed as mean ± SEM. The statistical significances within a variable were evaluated by one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts as a post hoc test for mean values comparison. A p value < 0.05 was considered to be statistically significant.

Results

The time courses of changes in the ventricular automaticity rate (top), QT interval (middle), QTc (bottom), and the number of surviving animals (bottom) are summarized in Fig. 1 for beagle dogs (left panels, $n = 4$) and *microminipigs* (right panels, $n = 4$). The pre-drug control values of

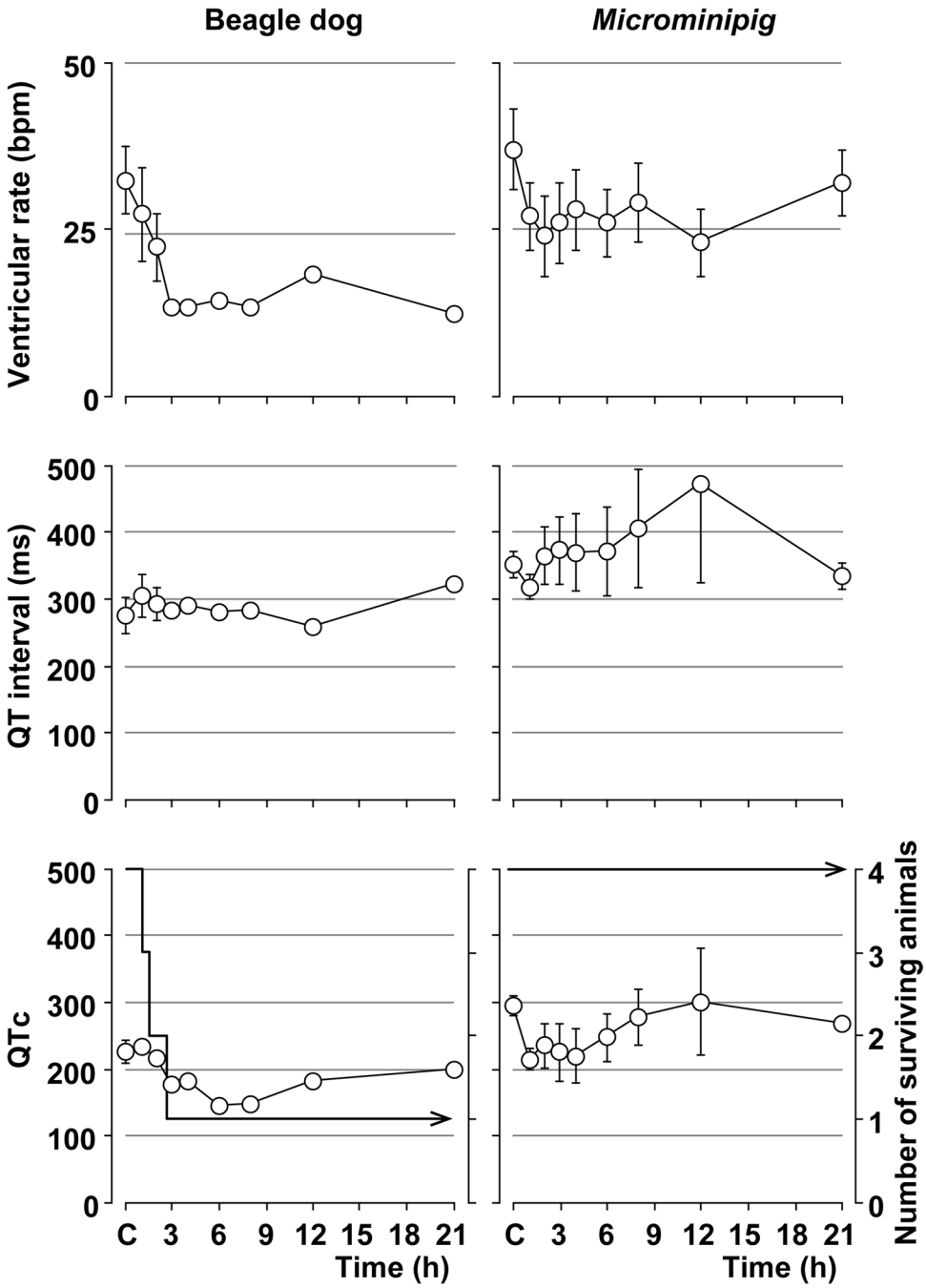
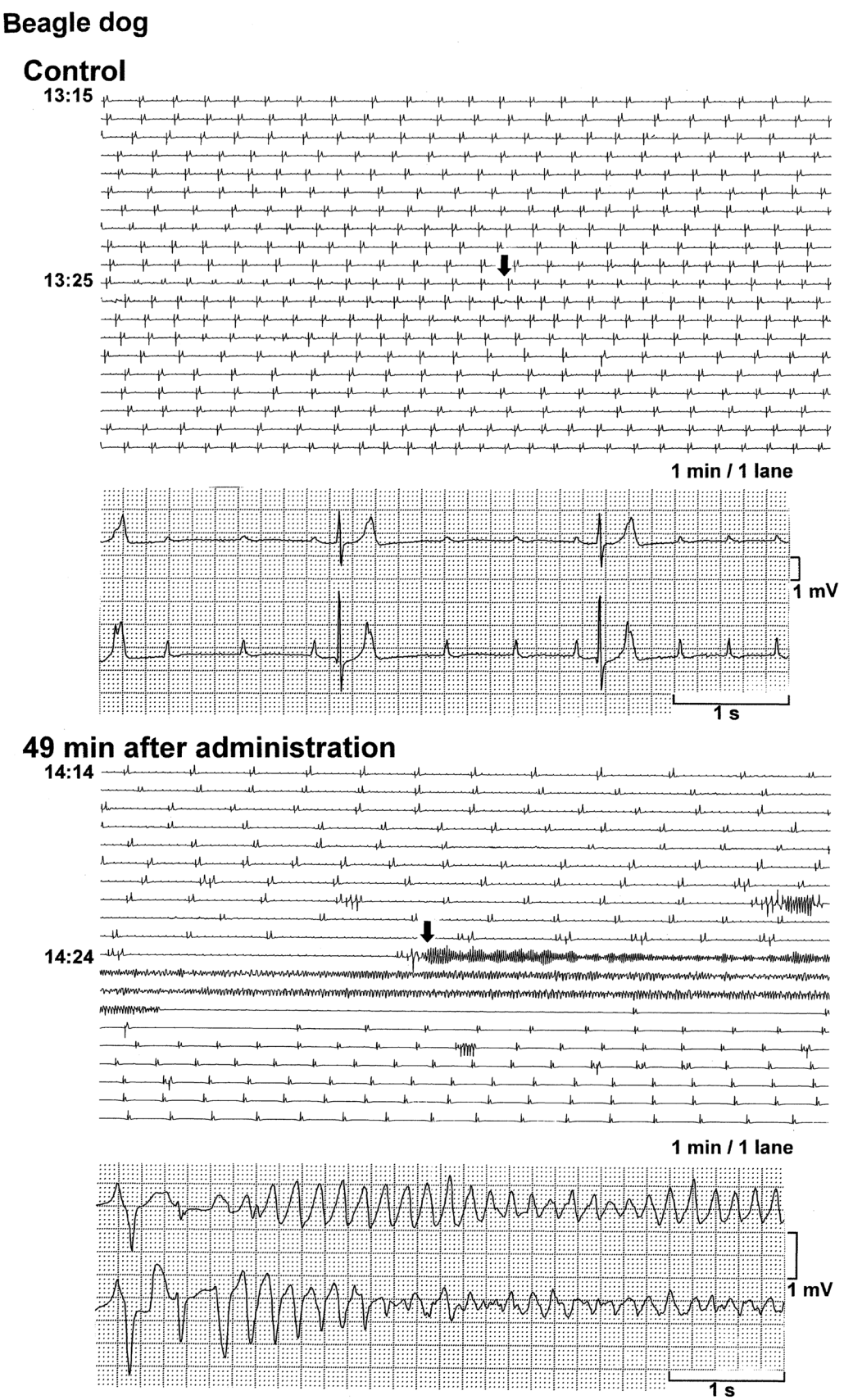


Fig. 1 Time courses of the ventricular automaticity rate (Ventricular rate, top), QT interval (middle), QT interval corrected by Fridericia's formula (QTc, bottom) and the number of surviving animals (Number of surviving animals, bottom) in the chronic atrioventricular block beagle dogs and *microminipigs* after the oral administration of 10 mg/kg of *dl*-sotalol. Since *dl*-sotalol induced torsade de pointes in three out of four beagle dogs degenerating into ventricular fibrillation, the number of surviving animals decreased to one (bottom, left). On the other hand, *dl*-sotalol evoked no episode of torsade de pointes in *microminipigs*; thus, no animal died during the experimental period (bottom, right). Data are presented as mean ± SEM ($n = 4$ for each species)


Beagle dog
Control
13:15
13:25
1 min / 1 lane
1 mV
1 s
49 min after administration
14:14
14:24
1 min / 1 lane
1 mV
1 s

◂**Fig. 2** Typical tracings of compressed and standard electrocardiograms at 10 min before (Control; upper traces) and at 49 min after the start of administration of 10 mg/kg of *dl*-sotalol (49 min after administration; lower traces) in a chronic atrioventricular block beagle dog. Arrow part of compressed electrocardiogram (NASA lead) was enlarged as standard ones below (NASA lead: upper; and CM5 lead: lower). Note the onset of torsade de pointes at 14:24, which degenerated into ventricular fibrillation followed by cardiac standstill. Although regular ventricular automaticity gradually developed following the onset of cardiac standstill, it gradually slowed and finally stopped within 20 min

the ventricular automaticity rate, QT interval, QTc obtained ≥ 10 min before the administration of *dl*-sotalol were 33 ± 5 beats/min, 275 ± 27 ms and 226 ± 17 in beagle dogs, whereas those were 37 ± 6 beats/min, 352 ± 19 ms and 295 ± 14 in *microminipigs*, respectively. *dl*-Sotalol did not significantly alter any of these variables in either beagle dogs or *microminipigs*.

In beagle dogs, *dl*-sotalol induced torsade de pointes in three out of four animals, which degenerated into ventricular fibrillation leading to the animals' death. Thus, the number of surviving animals was one at the end of the experiment as shown in Fig. 1 (bottom left). Typical tracings of electrocardiogram before and after the oral administration of *dl*-sotalol are depicted in Fig. 2. In the first animal, the ventricular fibrillation was induced by the initial attack of torsade de pointes at 80 min after the drug administration. In the second animal, the ventricular fibrillation was induced by the second attack of torsade de pointes at 49 min after the initial attack of torsade de pointes was spontaneously terminated at 46 min. In the third animal, the ventricular fibrillation was induced by the third attack of torsade de pointes at 155 min after the initial and second attacks were spontaneously terminated at 140 and 152 min, respectively. In the fourth animal, torsade de pointes was not induced, although ventricular premature beats were observed.

In *microminipigs*, neither episode of torsade de pointes nor lethal ventricular tachycardia was observed following the *dl*-sotalol administration, although it induced intermittent ventricular pauses. Thus, the number of surviving animals was 4 out of 4 animals at the end of the experiment as shown in Fig. 1 (bottom right). Typical tracings of electrocardiogram before and after the administration of *dl*-sotalol are depicted in Fig. 3. *dl*-Sotalol induced the ventricular pauses in each *microminipig* lasting up to 13 ± 3 s at a frequency of 7 ± 0 episodes/min followed by the ventricular contractions consisting of up to 4 ± 1 beats at a rate of 103 ± 6 bpm, which was observed at 53 ± 10 to 476 ± 153 min after the drug administration ($n = 4$). It should be noted that the shape of the initial QRS complex after each pause was essentially the same to those before the *dl*-sotalol administration (Fig. 3), indicating that the initial beat was induced by idioventricular automaticity. Meanwhile, the QRS-complex morphologies following the initial beat were largely different from that of the initial beat or those before the *dl*-sotalol administration (Fig. 3), showing that those beats after the initial one depended on ectopic ventricular automaticity.

Discussion

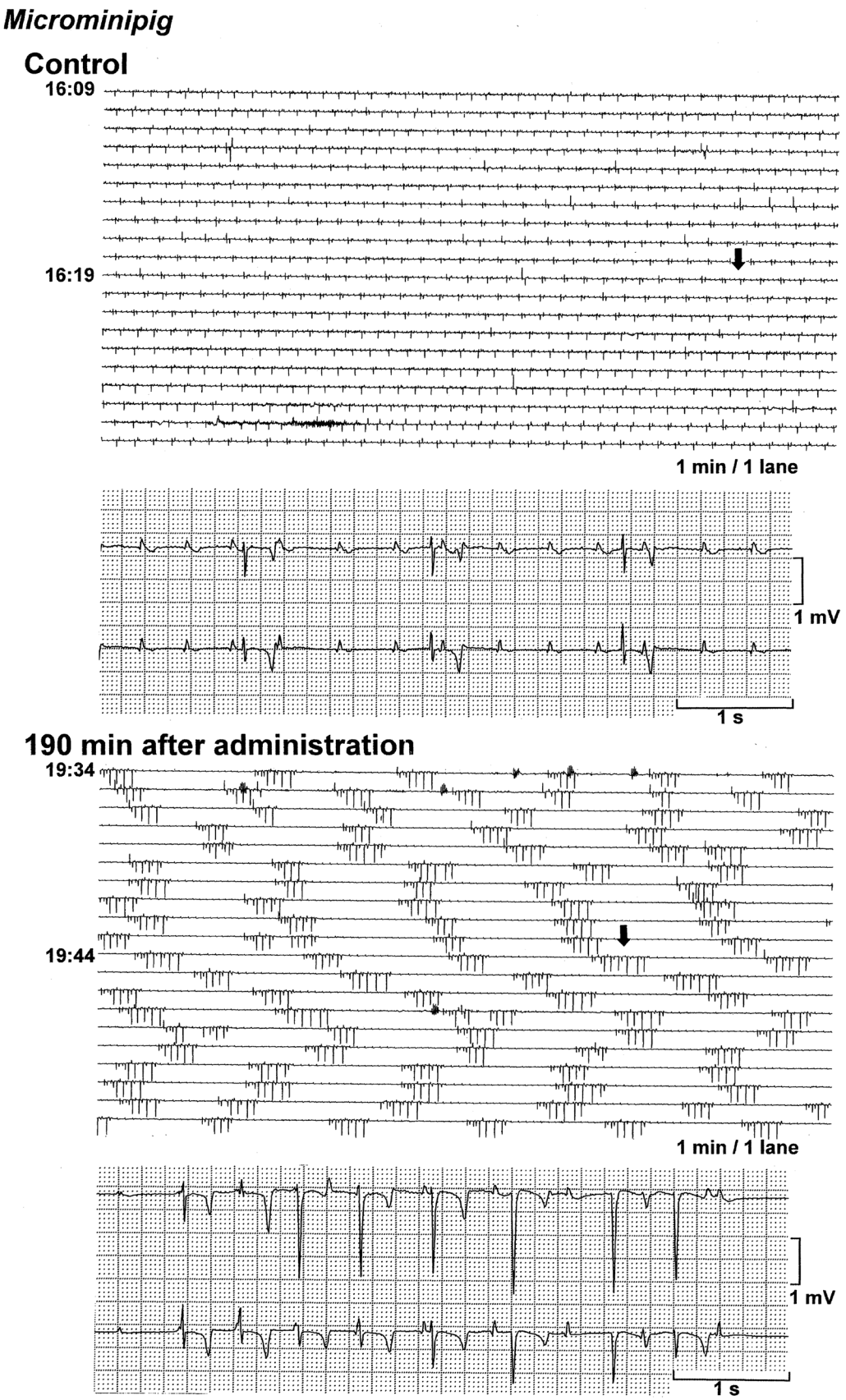
Proarrhythmic effects of *dl*-sotalol were analyzed by using the chronic atrioventricular block beagle dogs and *microminipigs* to estimate how the difference in the *dl*-sotalol-induced J–T_{peak}c changes between the intact hearts of both animals would be reflected in pathologic hearts, respectively. Orally administered 10 mg/kg of *dl*-sotalol induced torsade de pointes in three beagle dogs out of four, whereas it brought about intermittent ventricular pauses followed by idioventricular automaticity contractions without triggering torsade de pointes in any of *microminipigs*.

Rationale for the Drug Dose and Experimental Protocol

Clinically recommended initial daily dose of *dl*-sotalol is 80 mg, p.o. according to the interview form from the manufacturer (7th edition; April, 2016). Since *dl*-sotalol is well absorbed orally with bioavailability of nearly 100%, which is neither metabolized in the liver nor bound to plasma proteins [8], currently administered dose of 10 mg/kg, p.o. would provide toxic concentration in both beagle dogs and *microminipigs* enough to induce QT-interval prolongation and torsade de pointes [13–15]. In order to avoid excessively increasing the sympathetic tone of the animals during observation period, which may modify the proarrhythmic effects of *dl*-sotalol, we chose the following experimental protocol. First, we orally administered *dl*-sotalol instead of venous injection to assess its proarrhythmic effects at T_{max} in the absence of the operators. Second, we did not sample the blood for assaying the plasma drug concentration, although the lack of pharmacokinetic information may become a limitation of this study.

Electrophysiological Effects

Administration of *dl*-sotalol tended to decrease the ventricular rate but prolong the QT interval in both animals, although they did not achieve statistical significance. The former may be related to its ß-adrenoceptor blocking property, since selective I_{Kr} blocker E-4031 rather increased the ventricular automaticity rate of the chronic atrioventricular block dogs [21]. The latter might be partly associated with its I_{Kr} blocking action; however, it should be noted that changes in the QT interval of the atrioventricular block heart may not necessarily reflect the extent of K^+ channel inhibition,


Microminipig
Control
16:09
16:19
1 min / 1 lane
1 mV
1 s
190 min after administration
19:34
19:44
1 min / 1 lane
1 mV
1 s

◂**Fig. 3** Typical tracings of compressed and standard electrocardiograms at 14 min before (Control; upper traces) and at 190 min after the start of the administration of 10 mg/kg of *dl*-sotalol (190 min after administration; lower traces) in a chronic atrioventricular block *microminipig*. Arrow part of compressed electrocardiogram (NASA lead) was enlarged as standard ones below (NASA lead: upper; and CM5 lead: lower). As shown in compressed electrocardiogram, intermittent ventricular pauses were induced lasting 1.7–10.2 s at a rate of 5–7 episodes/min. Note a group of ventricular contractions largely consisting of 6–8 beats at a rate of 69–124 bpm

since the order of ventricular depolarization is different from that in the normal heart under sinus rhythm, giving rise to secondary ST–T morphological changes.

Proarrhythmic Effects

Since the J–T_{peak}c can reflect the net balance of inward and outward currents during an early repolarization phase [1], assessment of J–T_{peak}c can differentiate whether a drug may have balanced blockade on the inward late I_{Na} and/or I_{CaL} channels and the outward I_{Kr} channel. When the inward currents became greater than the outward one during the early repolarization phase, the J–T_{peak}c would be prolonged, which could induce intracellular Ca^{2+} overload and enhance the beat-to-beat variability of QT interval, resulting in the development of early afterdepolarization that may trigger torsade de pointes in the presence of spatial dispersion of repolarization [1–7, 14, 18, 21]. *dl*-Sotalol induced torsade de pointes in the beagle dogs, which degenerated into ventricular fibrillation, whereas in *microminipigs*, it did not trigger torsade de pointes. Accordingly, previously analyzed proarrhythmic potential of *dl*-sotalol quantified by the change in J–T_{peak}c along with the QT-interval prolongation in normal beagle dogs [10] and *microminipgs* [9] can be well reflected in their pathologically modified hearts in this study, respectively. Thus, the measurement of the J–T_{peak}c along with the QT-interval prolongation in the normal hearts can contribute to the improvement of the assay sensitivity and specificity for predicting the onset of drug-induced torsade de pointes.

Onset mechanisms of intermittent pauses in *microminipigs* may also deserve comments. Previous in vivo electropharmacological comparison of the animals under the physiologically maintained experimental condition has shown that *microminipigs* may have smaller effective volume of distribution of a drug and greater basal sympathetic tone than beagle dogs, and that inward Na^+ and Ca^{2+} channel currents in the ventricular myocytes compared with outward K^+ channel current could be more easily inhibited by ionic channel modulators in *microminipigs* than in beagle dogs [3, 9, 22, 23]. Thus, the extent of net ß-adrenoceptor blocking effect of *dl*-sotalol would be greater in *microminipigs* than in beagle dogs, more suppressing the ventricular automaticity rate in *microminipigs* possibly via I_f channel current inhibition [24, 25], which may explain the onset of intermittent ventricular pauses in *microminipigs*. Also, the discordance in the QRS-complex morphology between the initial beat and those following the initial one may suggest that the pause-induced excessive ventricular volume overload might have risen up the stretch-activated ionic channel current after the initial beat via mechano-electrical feedback [26].

Conclusion

Current study demonstrated that utilization of the J–T_{peak}c assessment under physiologically maintained condition can improve the assay sensitivity and specificity for predicting the onset of drug-induced torsade de pointes in pathologically modified hearts. Thus, assessment of the J–T_{peak}c along with the QT-interval prolongation in healthy subjects may provide reliable information of risk prediction for patients susceptible to the drug-induced torsade de pointes.

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Compliance with Ethical Standards

Conflict of interest The authors indicated no potential conflict of interest.

Ethical Approval All experiments were planned based upon the rules and regulations of the Committee for Research at Yamanashi Research Center of Clinical Pharmacology (#2009-04) and Toho University Animal Care and User Committee (#17-52-323), and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of both facilities.

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