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# Long-term bradycardia caused by atrioventricular block can remodel the canine heart to detect the histamine $H_1$ blocker terfenadine-induced torsades de pointes arrhythmias

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**1** Although a second-generation histamine $H_1$ blocker terfenadine induced torsades de pointes (TdP) arrhythmias in patients *via* the blockade of a rapid component of delayed rectifier $K^+$ current ($I_{Kr}$), such action of terfenadine has not been detected in previous animal models.

**2** We analysed the potential of the canine persistent atrioventricular block heart, a new *in vivo* proarrhythmia model, to detect a torsadogenic effect of terfenadine of an oral dose of 3 or 30 mg kg$^{-1}$. The doses can provide therapeutic to supra-therapeutic plasma concentrations as an anti-histamine.

**3** In 2 weeks of bradycardiac heart model, there were no significant changes in any of the electrocardiogram parameters after the administration of both doses of terfenadine.

**4** In 4–6 weeks of bradycardiac heart model, the low dose of terfenadine hardly affected any of the electrocardiogram parameters except that it induced TdP in one out of six animals. The high dose significantly decreased the atrial rate and ventricular rate, prolonged the QT interval, and induced TdP in five out of six animals. Moreover, temporal variability of repolarization increased after the high-dose administration.

**5** These results suggest that long-term bradycardia caused by atrioventricular block can remodel the canine heart to detect terfenadine-induced TdP.
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**Keywords:** Terfenadine; QT interval prolongation; torsades de pointes; chronic atrioventricular block dog

**Abbreviations:** ECG, electrocardiogram; ICH, the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use; $I_{Kr}$, a rapid component of delayed rectifier $K^+$ currents; $I_{Ks}$, a slow component of delayed rectifier $K^+$ currents; TdP, torsades de pointes; QTc, corrected QT interval

## Introduction

Drug-induced QT interval prolongation is often associated with the onset of torsades de pointes (TdP) resulting in a life-threatening ventricular arrhythmia (Belardinelli *et al*., 2003; Redfern *et al*., 2003). To avoid the occurrence of such dangerous events, new drug candidates are being carefully evaluated according to the guideline ICH S7B for safety pharmacology studies (The ICH Steering Committee, 2005). While most of the drugs that induced TdP have been shown to inhibit a rapid component of delayed rectifier $K^+$ currents ($I_{Kr}$) (Belardinelli *et al*., 2003; Redfern *et al*., 2003), some drugs possessing $I_{Kr}$-blocking property *in vitro*, such as sildenafil and verapamil, did not induce QT interval prolongation or TdP *in vivo* (Zhang *et al*., 1999; Geelen *et al*., 2000; Shiina *et al*., 2000; Sugiyama *et al*., 2001). Therefore, it is important to develop a model that can predict proarrhythmic potential of drug candidates with high sensitivity, specificity and reproducibility.

α-Chloralose-anesthetized rabbits have been widely used as an *in vivo* proarrhythmia model for assessing the QT prolonging drugs, including class III antiarrhythmics, quinolones and prokinetics (Carlsson *et al*., 1990; 1997; Chiba *et al*., 2004). A second-generation histamine $H_1$ blocker, terfenadine, has induced TdP clinically *via* the $I_{Kr}$ blockade after its overdose or by coadministration with a cytochrome P-450 inhibitor (Monahan *et al*., 1990; Tarantino *et al*., 2005); however, the torsadogenic action of the drug has not been detected in the rabbit model (Lu *et al*., 2000; Batey and Coker, 2002). In addition, previous studies have indicated that terfenadine hardly affects the repolarization interval of the isolated heart tissues (Pinney *et al*., 1995; Gintant *et al*., 2001; Masumiya *et al*., 2004) due to its multichannel blocking property including $K^+$, $Na^+$ and L-type $Ca^{2+}$ channels (Ming and Nordin, 1995; Liu *et al*., 1997; Tarantino *et al*., 2005).

Recently, chronic atrioventricular block dogs have been developed as a new *in vivo* proarrhythmic model for assessing the risks of the QT prolonging drugs, including class III antiarrhythmics, psychotropics, quinolones and prokinetics (Vos *et al*., 1998; Sugiyama *et al*., 2002a, b; Thomsen *et al*., 2003; 2004; Chiba *et al*., 2004; Satoh *et al*., 2004). In this model, electrical, mechanical and structural adaptations are known to be induced after the onset of bradycardia (Verduyn *et al*., 2001; Sugiyama *et al*., 2002a). Furthermore, recent electrophysiological studies have demonstrated that the QT interval was prolonged on the 7–14th day, and that $K^+$

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channel was downregulated on the 3rd day after creation of atrioventricular block (Schoenmakers *et al*., 2003; Stengl *et al*., 2004). However, information is still limited regarding the time course of changes in its potential to detect the drug-induced TdP after the onset of the bradycardia (Sugiyama *et al*., 2002a), which was assessed in this study. We propose that persistent bradycardia of 2 weeks will remodel the canine heart moderately, whereas that of 4–6 weeks can do it severely (Sugiyama *et al*., 2002a).

## Methods

All experiments were performed according to Guidelines for Animal Experiments, University of Yamanashi. A total of 10 beagle dogs of either sex weighing about 10 kg were used in this study.

### *Production of complete atrioventricular block*

The catheter ablation technique of atrioventricular node was employed as previously described (Sugiyama *et al*., 2002a; Takahara *et al*., 2004). The dogs were anaesthetized with pentobarbital sodium (30 mg kg$^{-1}$, i.v.). After intubation with a cuffed endotracheal tube, the dogs were artificially ventilated with a room air using a volume-limited ventilator (SN-408-3; Shinano, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml kg$^{-1}$ and 15 strokes min$^{-1}$, respectively. To prevent blood clotting, heparin calcium (100 IU kg$^{-1}$) was intravenously administered. The surface lead II electrocardiogram (ECG) was continuously monitored using a polygraph system (RM-6000; Nihon-Kohden, Tokyo, Japan). A quadpolar electrodes catheter with a large tip of 4 mm (D7-DL-252; Cordis-Webster, Baldwin Park, CA, U.S.A.) was inserted through the right femoral vein using the standard percutaneous technique under sterile condition and positioned around the tricuspid valve 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 and atrium/ventricular voltage ratio was $>2$. The site was usually found at 1–2 cm proximal from the position where the largest His bundle electrogram was recorded. The power source for atrioventricular node ablation was an electrosurgical generator (MS-1500; Mera, 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 an indifferent patch electrode positioned on the animal's back, which was continued then for 30 s if junctional rhythm was induced. The end point of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm.

### *Holter ECG recording*

A Holter recording and analysis system (QR2100 and HS1000, Fukuda ME Kogyo, Tokyo, Japan) was used to record and analyse ECG over 24 h. The effects of terfenadine on the atrial rate, ventricular rate, QT interval and corrected QT interval (QTc) as well as the proarrhythmic effects were assessed without anaesthesia. These values were expressed as the mean of three consecutive complexes. QTc was calculated using the Van de Water's formula (Van de Water *et al*., 1989). TdP was defined as a polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting $\geqslant 6$ consecutive beats (Satoh & Zipes, 1996).

### *Experimental protocol*

Experiment 1: ECG was recorded without anaesthesia at 24 h before and 0.5, 24 h, 1, 2, 4 and 8 weeks after the atrioventricular node ablation ($n=4$).

Experiment 2: At 2 weeks after the induction of complete atrioventricular block ($n=6$), 3 or 30 mg kg$^{-1}$ of terfenadine was orally administered as a powder using a gelatin capsule, which will provide therapeutic to supra-therapeutic plasma concentrations as an anti-histamine (Ferguson *et al*., 1985; Usui *et al*., 1998) 2 h after the start of ECG monitoring. Initially, the low-dose of terfenadine was orally administered, and then 2 days later, 10 times higher dose was orally administered. At 4–6 weeks after the induction of complete atrioventricular block, the same protocol was applied to the same animal group ($n=6$). Since the time at maximum plasma concentration ($T_{max}$) and $t_{1/2}$ of orally administered terfenadine are reported to be 1.3 and 15.1 h, respectively (Lalonde *et al*., 1996), plasma concentration of terfenadine at 2 weeks after the oral administration can be estimated to be 1/100,000 (0.01%) of its maximum plasma concentration ($C_{max}$).

### *Beat-to beat analysis*

ECG of 51 consecutive beats under stable idioventricular automaticity was recorded before and after the drug administration. Poincaré plots with $QT_n$ *versus* $QT_{n+1}$ were prepared for each of two analysis time points. The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability ($=\sum|QT_{n+1}-QT_n|/[50\times\sqrt{2}]$). 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 ($=\sum|QT_{n+1}+QT_n-2QT_{mean}|/[50\times\sqrt{2}]$). These nomenclatures are adopted from heart rate variability investigations using Holter monitoring in humans (Brennan *et al*., 2001), which have been applied to the QT interval of normal dogs and chronic atrioventricular block dogs (Thomsen *et al*., 2004; Schneider *et al*., 2005).

### *Drugs*

The following drugs were purchased: terfenadine (Sigma, St Louis, MO, U.S.A.), pentobarbital sodium (Tokyo Kasei, Tokyo, Japan) and heparin calcium (Mitsui, Tokyo, Japan).

### *Statistics*

Data are presented as the mean$\pm$s.e.m. The statistical comparisons within a parameter were evaluated by one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison. A $P$-value $<0.05$ was considered statistically significant.

## Results

### *Time course of change in the QT interval after the induction of complete atrioventricular block (experiment 1)*

Figure 1a shows typical tracings of ECG from a dog before and 1 min after the atrioventricular node ablation, whereas Figure 1b summarized the time courses of the heart rate, QT interval and QTc after the induction of complete atrioventricular block ($n=4$). Before the surgery, the ventricular rate, QT interval and QTc were $86\pm2$ beats min$^{-1}$, $239\pm6$ and $265\pm7$ ms, respectively. At 24 h after the atrioventricular node ablation, these were $33\pm2$ beats min$^{-1}$, $289\pm8$ and $209\pm14$ ms, respectively. This bradycardia of $<40$ beats min$^{-1}$ continued throughout the experiment. The QT interval was gradually prolonged after the induction of complete atrioventricular block, and significant changes were observed for 2–8 weeks. On the other hand, significant prolongation of the QTc was detected only at 4 weeks.

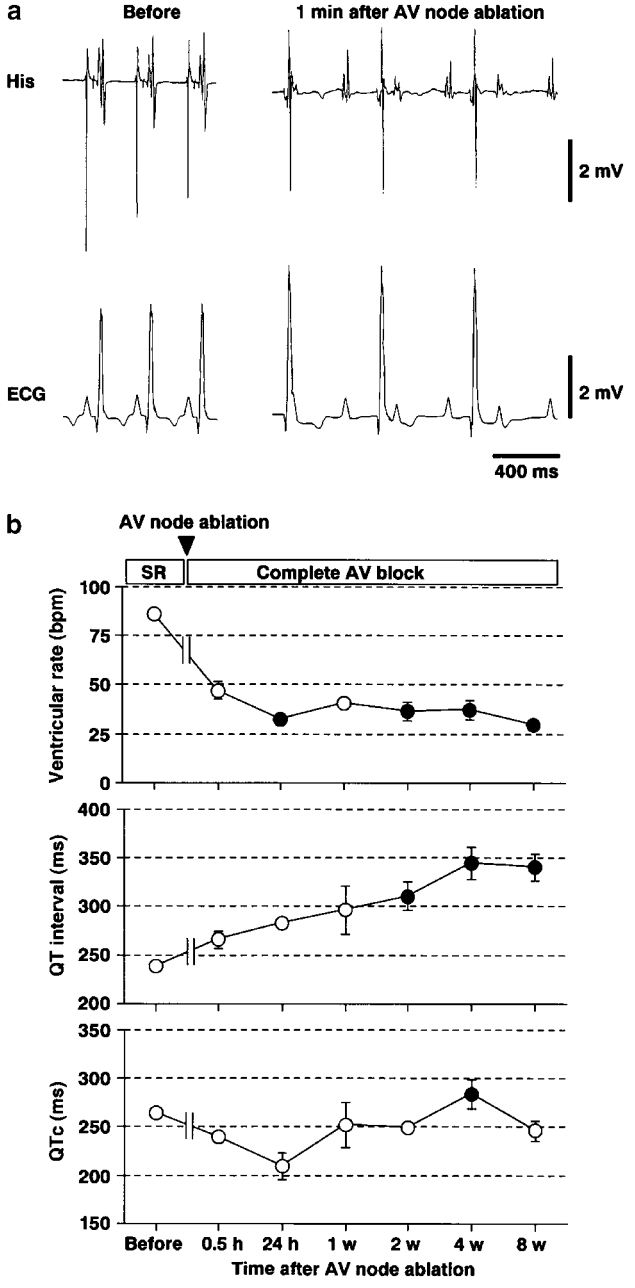


**Figure 1** Electrocardiogram of complete atrioventricular block dogs. (a) Typical tracings of His bundle electrogram (His) and lead II electrocardiogram (ECG) before and after the atrioventricular (AV) node ablation. (b) Time course of the heart rate, QT interval and corrected QT (QTc) after the AV node ablation ($n=4$). Data are presented as mean$\pm$s.e.m. SR: sinus rhythm. Closed symbols represent statistically significant differences from each value at 0.5 h after the AV node ablation by $P<0.05$.

### *Torsadogenic action of terfenadine (experiment 2)*

The number of animals showing TdP is summarized in Figure 2a, whereas a typical tracing of terfenadine-induced TdP is depicted in Figure 2b. In 2 weeks of bradycardiac heart model (2 weeks model), no TdP was detected after administration of both doses of terfenadine. In 4–6 weeks of bradycardiac heart model (4–6 weeks model), the number of episodes of TdP arrhythmias increased in a dose-dependent manner. At 80 min after the administration of the low dose, one episode of TdP was detected in one animal out of six, which lasted for 2.3 s and was spontaneously terminated. In this animal, the QT interval was 440 ms just before the onset of TdP, and the morphology of ECG during TdP was similar to that observed after the high dose. After the administration of the high dose, $2\pm1$ episodes of short duration of TdP ($2.7\pm0.2$ s) were detected in five out of six animals, and the dogs lost consciousness after the latest TdP that degenerated into ventricular fibrillation, leading to death. The initial TdP was observed at $11.2\pm3.3$ h after the drug administration, whereas the latest TdP leading to the animal's death was induced at $14.6\pm2.3$ h. Onset of TdP followed the R on T phenomenon. The QT interval was $464\pm10$ ms just before the onset of TdP.

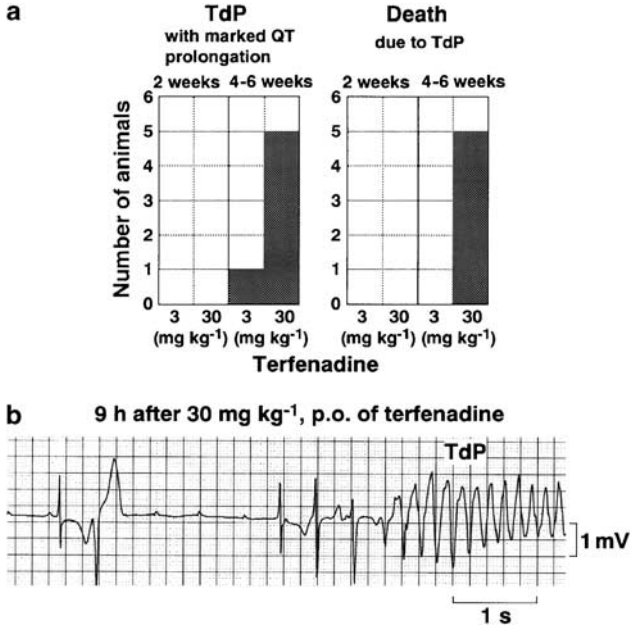


**Figure 2** Terfenadine-induced TdP. (a) Summary of proarrhythmic effects of terfenadine in the 2 and 4–6 weeks of bradycardiac heart models. (b) A typical tracing of torsades de pointes (TdP) observed at 9 h after the oral administration of 30 mg kg$^{-1}$ of terfenadine in a dog of the 4–6 weeks model.

*Effects of terfenadine on the electrocardiogram parameters (experiment 2)*

The time courses of the changes in the ECG parameters are summarized in Figure 3. In the 2 weeks model, the pre drug control values of the atrial rate, ventricular rate, QT interval and QTc were $146 \pm 8$, $39 \pm 3$ beats min$^{-1}$, $227 \pm 6$ and $183 \pm 8$ ms in the low-dose group ($n = 6$), and $149 \pm 13$, $39 \pm 3$ beats min$^{-1}$, $227 \pm 8$ and $178 \pm 11$ ms in the high-dose group ($n = 6$), respectively. In the 4–6 weeks model, those were $141 \pm 12$, $36 \pm 2$ beats min$^{-1}$, $276 \pm 10$ and $214 \pm 9$ ms in the low-dose group ($n = 6$), and $133 \pm 8$, $31 \pm 2$ beats min$^{-1}$, $306 \pm 10$ and $224 \pm 15$ ms in the high-dose group ($n = 6$), respectively. The pre drug control values of the QT interval and QTc in

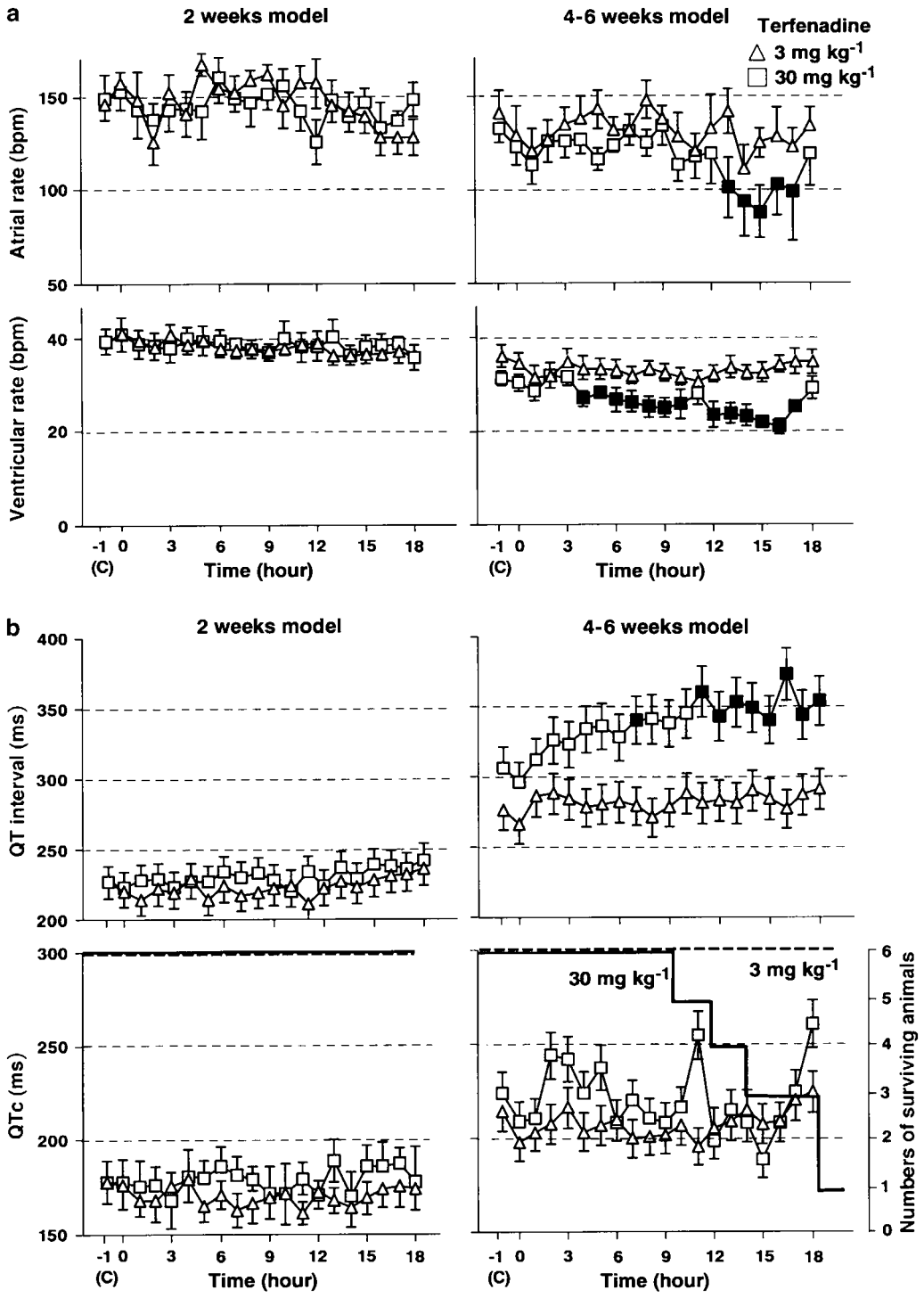


**Figure 3** Effects of terfenadine on the electrocardiogram. (a) Time courses of the effects of terfenadine on the atrial rate and ventricular rate in the 2 weeks and 4–6 weeks of bradycardiac heart models ($n = 6$). (b) Time courses of the effects of terfenadine on the QT interval, corrected QT (QTc) and number of surviving animals ($n = 6$). Data are presented as mean $\pm$ s.e.m. Closed symbols represent statistically significant differences from each pre drug control (c) value by $P < 0.05$.

the 4–6 weeks model were significantly longer than those in the 2 weeks model. Meanwhile, no significant difference was detected in the pre drug control values of the atrial rate or ventricular rate between the 2 weeks and 4–6 weeks model. In the 2 weeks model, the administration of both doses of terfenadine did not affect any of these parameters. In the 4–6 weeks model, the administration of the low dose did not affect any of these parameters, whereas the high dose significantly decreased the atrial rate for 13–17 h and ventricular rate for 4–10 and 12–17 h, and increased the QT interval at 7 and for 11–18 h. Significant changes were not detected in QTc.

*Beat-to-beat analysis (experiment 2)*

Beat-to-beat analysis was employed for dogs receiving 30 mg kg$^{-1}$ of terfenadine to assess relationship between the progression of remodelling process and torsadogenic potential of the model. ECG of 51 consecutive beats under stable idioventricular rhythm was recorded from six dogs of the 2 weeks model before and 12.7 ± 1.8 h after terfenadine administration and from six dogs of the 4–6 weeks model before and 14.0 ± 1.1 h after drug administration. As shown in Figure 4, the QT interval, short-term variability and long-term variability of each dog increased. Table 1 summarizes the short-term variability and long-term variability in the 2 and 4–6 weeks models. Although terfenadine hardly affected the short-term variability or long-term variability in the 2 weeks model, the drug significantly increased these parameters in the 4–6 weeks model. It should be noted that #5 dog of the 4–6 weeks model, which did not complicate TdP, showed the smallest increment in the long-term variability (+0.4 ms, Figure 4).

## Discussion

In the present study, TdP was detected with high reproducibility after terfenadine administration in the 4–6 weeks

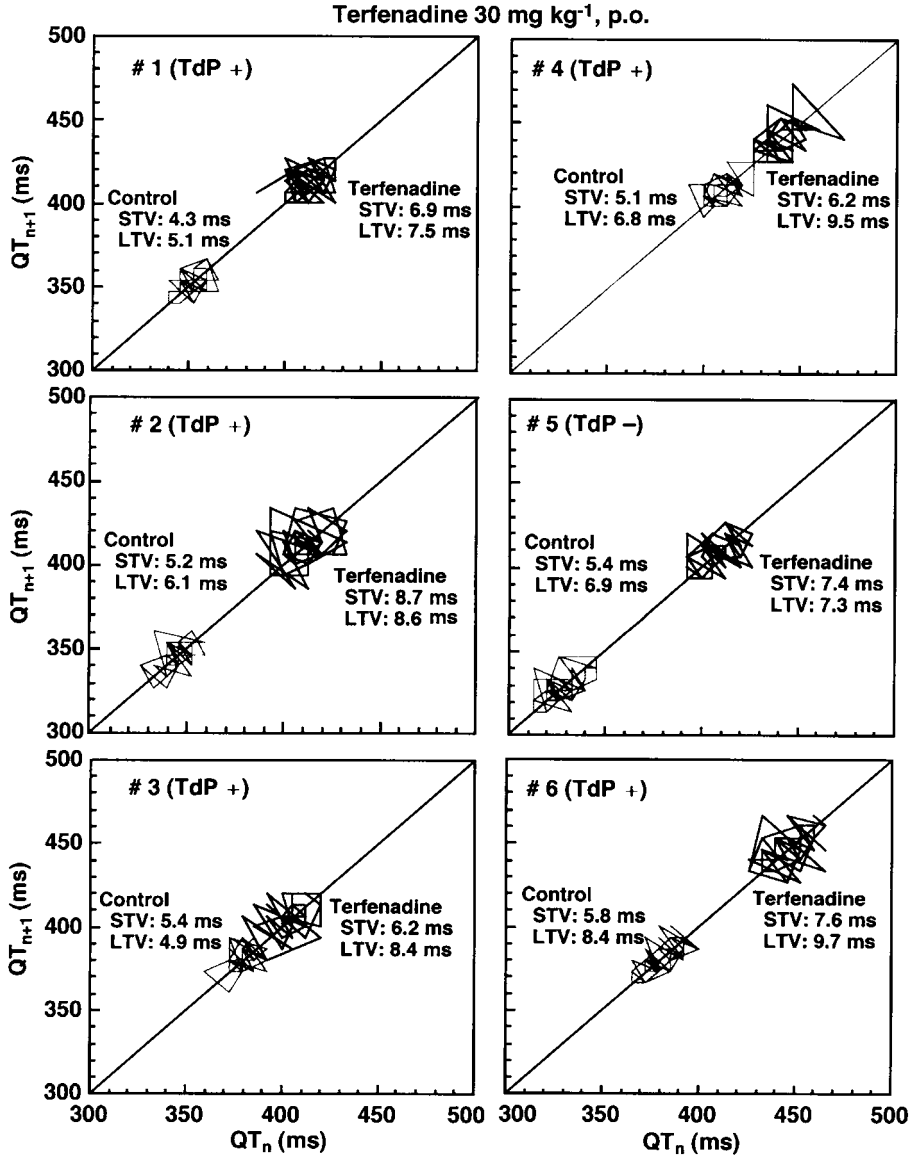


**Figure 4** Poincaré plots of the QT interval assessed in the 4–6 weeks of bradycardiac heart model. A total of 51 beats were plotted for each of the two analysis time points; before and after 30 mg kg$^{-1}$, p.o. of terfenadine administration. Torsades de pointes (TdP) was induced in five out of six dogs (#1–4 and 6). STV: short-term variability, LTV: long-term variability.

**Table 1** Effects of a torsadogenic dose of terfenadine (30 mg kg$^{-1}$, p.o.) on the QT interval variability in the atrioventricular (AV) block heart

| *Time after the onset of AV block* | *Short-term variability* (ms) | | | *Long-term variability* (ms) | | |
|---|---|---|---|---|---|---|
| | *Baseline* | *Terfenadine* | *Change* | *Baseline* | *Terfenadine* | *Change* |
| 2 weeks ($n=6$) | 5.4±0.4 | 5.1±0.2 | −0.3±0.4 | 6.2±0.4 | 6.7±0.7 | +0.5±0.8 |
| 4–6 weeks ($n=6$) | 5.2±0.2 | 7.2±0.4** | +2.0±0.4$^{\dagger\dagger}$ | 6.4±0.5 | 8.5±0.4** | +2.2±0.5 |

Data are presented as mean±s.e.m. **$P<0.01$, compared with the respective baseline values; $^{\dagger\dagger}P<0.01$, compared with change in variability of the 2 weeks model.

model, whereas it was not induced in the 2 weeks model. This was in good accordance with our observation using a 5-HT$_4$ agonist cisapride in the canine chronic atrioventricular block model (Sugiyama *et al*., 2002a). The $K^+$ currents, including a slow component of delayed rectifier $K^+$ currents ($I_{Ks}$) and $I_{Kr}$, generally compensate each other to secure the repolarization process; namely, the repolarization reserve (Roden, 1998), and the intact canine heart has been shown to possess wider safety margin for pharmacological $I_{Kr}$ blockade than human (Biliczki *et al*., 2002; Satoh *et al*., 2005). Since the significant downregulation of the $I_{Ks}$ and $I_{Kr}$ has been demonstrated in the cardiomyocytes of dogs at least 3 days after the onset of complete atrioventricular node block (Volders *et al*., 1999; Schoenmakers *et al*., 2003; Stengl *et al*., 2004), similar electrophysiological changes may have occurred in our atrioventricular block model. Furthermore, as shown in Figure 1b, the QT interval at 4 weeks was longer than that at 2 weeks, which may suggest the development of remodelling process of the heart related to the decreased repolarization reserve, resulting in enhancement of the drug-induced QT interval prolongation and onset of TdP. Also, experimental conditions may alter sensitivity to detect the drug-induced TdP, since a class III antiarrhythmic drug dofetilide has been shown to induce such arrhythmias in anaesthetized mongrel dogs at both 2 and 5 weeks after the onset of atrioventricular block (Schoenmakers *et al*., 2003).

In our previous studies with the chronic atrioventricular block dogs, torsadogenic doses of cisapride, sulpiride or nifekalant hardly affected the atrial and/or idioventricular rhythm (Sugiyama *et al*., 2002a, b; Satoh *et al*., 2004). Meanwhile as shown in the results, the supra-therapeutic dose of terfenadine prolonged the QT interval leading to induction of TdP along with marked reduction of the atrial and ventricular rate in the 4–6 weeks model. The inhibition of the ventricular automaticity can be explained by $Na^+$ channel-blocking property and $I_{Kr}$-inhibitory action of terfenadine (Sugiyama *et al*., 1994; Ming and Nordin, 1995; Liu *et al*., 1997; Usui *et al*., 1998; Takahara *et al*., 2005). The fact that terfenadine decreased the idioventricular rate only in the 4–6 weeks model indicates that the longer-term bradycardia may have remodelled the Purkinje, resulting in the increased sensitivity of phase 4 depolarization. Furthermore, since slow ventricular rhythm has been demonstrated to enhance electrical vulnerability in the ventricular muscle (Sugiyama & Hashimoto, 2002; Sugiyama *et al*., 2002a), the enhanced bradycardiac effect of terfenadine can increase its torsadogenicity associated with $I_{Kr}$-inhibitory action, which may partly explain strong proarrhythmic effects of terfenadine in the 4–6 weeks model as well as the clinical case reports (Monahan *et al*., 1990).

In previous reports, terfenadine did not cause TdP in the α-chloralose-anaesthetized rabbit model (Lu *et al*., 2000; Batey and Coker, 2002). In a recent study using fluoroquinolone antibacterial drugs, sensitivity of the rabbit model for detecting TdP was significantly less than that of the canine chronic atrioventricular block model (Chiba *et al*., 2004). Since an α-adrenoceptor agonist, such as methoxiamine or phenylephrine, was administered to enhance the induction of TdP in the rabbit model (Carlsson *et al*., 1990; Lu *et al*., 2000; Batey and Coker, 2002), the multiple ion channel-blocking effects of terfenadine (Ming & Nordin, 1995; Liu *et al*., 1997) might have counteracted the effects of pharmacological cardiac α-adrenoceptor stimulation of the heart, resulting in the decrease of the sensitivity.

It was clearly demonstrated that the interventricular dispersion of repolarization plays a key role in the induction of acquired type of TdP using the chronic atrioventricular block dogs (Vos *et al*., 1998; Schoenmakers *et al*., 2003). In addition to spatial heterogeneity of cardiac repolarization, its temporal heterogeneity has been analysed in the *in vivo* canine models (Eckardt *et al*., 2002; Thomsen *et al*., 2004; Schneider *et al*., 2005), since class III antiarrhythmic or $I_{Kr}$ blocking drugs indeed produce a repolarization instability (Hondeghem & Hoffmann, 2003). Furthermore, a recent study using chronic atrioventricular block dogs has shown the utility of short-term variability in predicting the drug-induced TdP (Thomsen *et al*., 2004). In this study, a torsadogenic dose of terfenadine significantly increased both short-term and long-term variability of repolarization in the 4–6 weeks model. However, there was no change in baseline values of the short-term variability of repolarization between the 2 and 4–6 weeks models, which may suggest that the bradycardiac effect of terfenadine may also have promoted the induction of TdP. More importantly, change in the long-term variability was the smallest in a dog that did not complicate TdP in the 4–6 weeks group. Therefore, analysis of long-term as well as short-term variability of repolarization may be reliable in predicting the proarrhythmic potential of a drug before the onset of TdP.

In conclusion, long-term bradycardia caused by atrioventricular block can remodel the heart severely to detect terfenadine-induced TdP. Therefore, the current canine model can be useful for detecting proarrhythmic potential of the drug candidates with unknown multifarious pharmacological actions in addition to $I_{Kr}$ blockade.

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