rug-induced long QT syndrome is currently a hot topic of concern for the pharmaceutical companies as well as for clinicians.1,2 We have previously assessed the effects of class II, III and IV antiarrhythmic agents and some non-cardiovascular drugs on the repolarization process using the halothane-anesthetized canine in vivo model, and found that the effects of the drugs on phase 3 repolarization in this model are quite useful for predicting drug-induced QT prolongation that might trigger torsades de pointes in clinical practice.3–11

Class I drugs are known to affect the QT interval,12,13 but information regarding the effects of class I antiarrhythmic drugs in this type of model is still limited.14–17 In this study, we simultaneously assessed the in vivo electrophysiological and cardiohemodynamic effects of typical class I antiarrhythmic drugs, disopyramide and mexiletine, using the halothane-anesthetized canine model. To better analyze the electrophysiological effects on the depolarization/repolarization process, we recorded His bundle electrograms and monophasic action potentials (MAPs), respectively, in addition to the standard lead II surface ECG. Moreover, a MAP recording/pacing combination catheter was used to simultaneously measure both MAP and effective refractory period (ERP) at the same site to assess the drug effects on the terminal repolarization period (phase 3) of the action potential.3–11,14–17

Methods

All experiments were performed in accordance with the Guidelines for Animal Experiments, Yamanashi Medical University. Experiments were carried out using beagle dogs weighing approximately 10 kg. Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University. Dogs were anesthetized initially with thiopental sodium (30 mg/kg, iv) and after intubation with a cuffed endotracheal tube, 1.0% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 stroke/min, respectively. To prevent blood clotting, heparin calcium (100 units/kg, iv) was administered.

Cardiohemodynamic and Electrophysiological Parameters

The surface lead II ECG was obtained from the limb electrodes and the corrected QT interval (QTc) was calculated using Bazett’s formula.18 The systemic blood pressure (BP) was measured at the left femoral artery, and a pig-tail catheter was positioned at the left ventricle through the left femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure (LVdp/dtmax) and the left ventricular end-diastolic pressure (LVEDP) were obtained to estimate the contractility and
pacing cycle lengths of 400 ms (MAP 90(CL400)) and 300 ms
electrical vulnerability of the heart.3–11,14–17,19

The animals were randomized into 2 groups; namely, disopyramide-administered group (n=6) and mexiletine (right panels). Data are presented as the mean±SE. The closed symbols represent the significant differences from each control value (C) by p<0.05.

Fig 2. Time courses of the maximum upstroke velocity of the left ventricular pressure (LVdP/dtmax), left ventricular end-diastolic pressure (LVEDP), cardiac output (CO) and total peripheral resistance (TPR) after the administration of disopyramide (left panels) and mexiletine (right panels). Data are presented as the mean±SE. The closed symbols represent the significant differences from each control value (C) by p<0.05.

Experimental Protocol

The animals were randomized into 2 groups; namely, disopyramide-administered group (n=6) and mexiletine-administered group (n=6). The systemic BP, left ventricular pressure, ECG, His bundle electrogram and MAP signals were continuously monitored using a polygraph system (RM-6000, Nihon Kohden), and analyzed using a real time fully automatic data analysis system (MP/VAS 3 for Macintosh V.1.0; Physio-Tech, Tokyo, Japan). The cardiovascular variables were assessed in the following order at each time point. CO was measured twice; the ECG, His bundle electrogram, systemic BP and left ventricular pressure and MAP signal were analyzed during sinus rhythm; then the MAP signals were analyzed during ventricular pacing at the cycle lengths of 400 and 300 ms; finally, the ERP of the ventricle was measured. All data were usually obtained within 1 min at each time point.

In the disopyramide-administered group, after each basal control cardiovascular value was assessed, a low dose of 0.3 mg/kg was intravenously administered over 10 min and the effects of the drug on each cardiovascular parameter were assessed at 5, 10, 15, 20 and 30 min after the start of infusion. Disopyramide at a high dose of 3.0 mg/kg, which has been demonstrated to exert an antiarrhythmic effect in canine ventricular arrhythmia models20 was then administered over 10 min and the effect on each parameter was assessed in the same manner.

In the mexiletine-administered group, after each basal control value was assessed, a low dose of 0.3 mg/kg was intravenously administered over 30 s and the effects of the drug on each cardiovascular parameter were assessed at 1, 3, 5, 7, 10, 15, 20 and 30 min after the start of injection. Mexiletine in a high dose of 3.0 mg/kg, which also has an antiarrhythmic effect20 was subsequently administered over 30 s and its effect on each parameter assessed in the same manner.

A volume of 3 ml of blood was drawn from the right femoral artery at each time point to measure the plasma drug concentration. The blood samples were centrifuged at 1,500G for 30 min at 4°C and the plasma was stored at −80°C until the drug concentration was measured. Sensitive and specific determinations of the concentrations of disopyramide and mexiletine were performed at SRL Co, Ltd (Tokyo, Japan) using a standard high-performance liquid chromatographic method. The limit of quantification of disopyramide and mexiletine was 0.2μg/ml and 0.02μg/ml.
respectively.

**Drugs**
The following drugs were purchased: disopyramide phosphate (Chugai, Tokyo, Japan), mexiletine hydrochloride (Boehringer, Hyogo, Japan), thiopental sodium (Tanabe, Osaka, Japan), halothane (Takeda, Tokyo, Japan) and heparin calcium (Mitsui, Tokyo, Japan).

**Statistics**
Data are presented as the mean ± SE. The statistical difference within a parameter was evaluated by paired t-test or one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison, while that of unpaired data between the groups was evaluated by unpaired t-test. A p value of less than 0.05 was considered significant.

**Results**
There was no statistically significant difference in each pre-drug control value between the disopyramide- and mexiletine-administered groups except for QTc. Neither spontaneously occurring ventricular premature contraction nor cardiovascular collapse was observed in any dog during the whole experimental period.

**Plasma Drug Concentrations**
The time courses of the plasma concentrations of disopyramide (n=6) and mexiletine (n=6) are summarized in Fig 1. The peak plasma drug concentrations after 0.3 and 3.0 mg/kg of disopyramide administration were 0.90±0.37 and 7.65±0.61 μg/ml, respectively, and 0.59±0.10 and 7.37±0.37 μg/ml, respectively, for 0.3 and 3.0 mg/kg of mexiletine. The decrease in the plasma concentration of both drugs followed a pattern that could be predicted by the two-component theory of pharmacokinetics.

**Effects on the HR and Mean BP**
The time courses of the HR and mean BP are summarized in Fig 1. At the pre-drug control, the HR (beats/min) and mean BP (mmHg) were 119±7 and 121±5 in the disopyramide group (n=6), and 114±5 and 123±5 in the mexiletine group (n=6), respectively. In the disopyramide group, no significant change was observed in HR, but the mean BP increased, and significant changes were detected for 5–30 min after the high dose. In the mexiletine group, no significant change was observed in HR, but the mean BP decreased and significant changes were detected for 1–10 min after the high dose.

**Effects on the LVdP/dtmax and LVEDP**
The time courses of the LVdP/dtmax and LVEDP are summarized in Fig 2. The LVdP/dtmax (mmHg/s) and LVEDP (mmHg) at the pre-drug control were, respectively, 2,275±133 and 12.0±2.3 in the disopyramide group (n=6), and 2,594±240 and 11.4±0.6 in the mexiletine group (n=6). In the disopyramide group, the LVdP/dtmax decreased and significant changes were detected for 5–10 min after the high dose; the LVEDP increased and significant changes were detected for 10–15 min after the high dose. In the mexiletine group, the LVdP/dtmax also decreased and significant changes were detected for 1–10 min after the high dose administration, but no significant change was observed in the LVEDP.

**Effects on the CO and TPR**
The time courses of the CO and TPR are summarized in Fig 2. The CO (L/min) and TPR (mmHg·min/L) at the pre-drug control were, respectively, 1.65±0.19 and 77±8 in the disopyramide group (n=6), and 1.56±0.15 and 81±6 in the mexiletine group (n=6). In the disopyramide group, the CO decreased and significant changes were detected for 5–10 min and at 30 min after the high dose; the TPR increased and significant changes were detected for
Effects on the ECG During Sinus Rhythm

Typical ECG traces after the administration of disopyramide and mexiletine are shown in Fig. 3, and the time courses of the parameters of ECG are summarized in Fig 4. The PR interval, QRS width, QT interval and QTc (ms) at the pre-drug control were, respectively, 106±6, 66±5, 274±18 and 383±21 in the disopyramide group (n=6), and 110±3, 64±5, 324±13 and 446±14 in the mexiletine group (n=6). In the disopyramide group, no significant change was observed in the PR interval, but the QRS width, QT interval and QTc were prolonged. Significant prolongation was observed in the QT interval for 1–5 min after the high dose and for 5–10 min after the high dose. In the mexiletine group, no significant change was observed in the QT interval for 10–15 min after the low dose, but the QTc was prolonged and both the QT interval and QTc were shortened. Significant prolongation was observed in the QTc for 1–20 min after the high dose. In addition, the time courses of the absolute change in MAP90(CL400) and MAP90(CL300) from their respective pre-drug control values were calculated (data not shown) to examine the reverse frequency-dependent change of the repolarization process. In the disopyramide group, the increment tended to be greater in the MAP90(CL400) than in the MAP90(CL300), but did not achieve the statistical significance. Meanwhile, in the mexiletine group, no significant difference was detected between the decrements in the MAP90(CL400) and MAP90(CL300).

Discussion

The present study used the halothane-anesthetized canine model to simultaneously assess the acute in vivo electrophysiological and cardiohemodynamic actions of typical class I antiarrhythmic drugs, disopyramide and mexiletine, to better understand their clinical utility and adverse effects.

Plasma Drug Concentrations

In our previous studies using digitalis-, epinephrine- and coronary ligation-induced canine ventricular arrhythmia models, the minimum effective plasma concentrations of disopyramide and mexiletine were 1.7–4.2 g/ml and 1.8–3.7 g/ml, respectively. In addition, previous clinical studies have shown that therapeutic drug concentrations of disopyramide and mexiletine were 2–5 g/ml and 0.7–1.6 g/ml, respectively. Thus, the plasma drug concentrations achieved in the present study indicate that experimental and clinical antiarrhythmic concentrations of both drugs can be obtained by intravenous administration during the experimental period, but the HV interval and MAP90(sinus) were prolonged. Significant prolongation was observed in the HV interval for 5–30 min after the high dose, and in the MAP90(sinus) for 10–15 min after the low dose and for 5–30 min after the high dose. The peak prolongation of MAP90(sinus) was observed at 10 min after the high dose and was 322±28 ms. In the mexiletine group, no significant change was observed in the AH interval, but the HV interval was prolonged and the MAP90(sinus) was shortened. Significant prolongation was observed in the HV interval for 1–20 min and as well as significant shortening in the MAP90(sinus) for 1–7 min after the high dose.

Effects on the MAP90(CL400), MAP90(CL300), ERP and TRP During Ventricular Pacing

The time courses of the MAP90(CL400), MAP90(CL300), ERP and TRP are summarized in Fig 5. The pre-drug control values (ms) were, respectively, 246±9, 230±9, 214±9 and 32±2 in the disopyramide group (n=6), and 243±3, 230±4, 207±4 and 37±3 in the mexiletine group (n=6). In the disopyramide group, the MAP90(CL400), MAP90(CL300) and ERP were prolonged, but no significant change was detected in the TRP. Significant prolongation in MAP90(CL400), MAP90(CL300) and ERP was observed for 5–30 min after the high dose and the peak prolongation of MAP90(CL400) occurred at 10 min after the high dose at 305±21 ms. No significant difference was detected between the peak values for MAP90(sinus) and MAP90(CL400). In the mexiletine group, MAP90(CL400), MAP90(CL300) and TRP were shortened, and the ERP was prolonged. Significant shortening in the MAP90(CL400) and MAP90(CL300) was observed for 1–20 min after the high dose, and significant change in the ERP and TRP was detected for 1–30 min after the high dose. In addition, the time courses of the absolute change in MAP90(CL400) and MAP90(CL300) from their respective pre-drug control values were calculated (data not shown) to examine the reverse frequency-dependent change of the repolarization process. In the disopyramide group, the increment tended to be greater in the MAP90(CL400) than in the MAP90(CL300), but did not achieve the statistical significance. Meanwhile, in the mexiletine group, no significant difference was detected between the decrements in the MAP90(CL400) and MAP90(CL300).
of 3.0 mg/kg in this canine model.

**Cardiohemodynamic Effects**

The high dose of disopyramide increased the meanBP and preload to the left ventricle, suppressed ventricular contraction, but hardly affected sinus automaticity. In contrast, the high dose of mexiletine slightly decreased the mean BP, suppressed ventricular contraction, but hardly affected either the preload to the left ventricle or sinus automaticity. These cardiohemodynamic effects were essentially in accordance with previous reports\(^{12-14}\). More importantly, it should be noted that mexiletine as well as disopyramide suppressed ventricular contraction, because the negative inotropic effect of class Ib antiarrhythmic drugs, such as lidocaine, is commonly thought to be clinically insignificant\(^{13}\).

**In Vivo Electrophysiological Effects**

Disopyramide and mexiletine both inhibited intraventricular conduction, which depends solely on the sodium current\(^2\), confirming their well-known inhibitory effect on the sodium channels\(^{23,24}\). On the other hand, disopyramide prolonged the ventricular repolarization phase during ventricular pacing as well as during sinus rhythm, whereas mexiletine shortened it. Disopyramide has been reported to inhibit several potassium currents, including \(I_k\) (rapidly activating component of delayed rectifier K\(^+\) current), \(I_o\) (transient outward K\(^+\) current), \(I_{Ki}\) (inward rectifier K\(^+\) current) \(I_{KATP}\) (muscarinic acetylcholine receptor-operated K\(^+\) current) and \(I_{KATP}\) (ATP-sensitive K\(^+\) current)\(^{25-28}\). Although we could not detect significant differences between the disopyramide-induced increments in MAP\(_{90}\) at CL400 and MAP\(_{90}\) at CL300, it has been reported that disopyramide may exert a reverse frequency-dependent prolongation of the repolarization period at a slow HR\(^29\). On the other hand, mexiletine is known to reduce the sodium window current and calcium current in addition to activating the \(I_{KATP}\) currents\(^{30,31}\). Thus, the previous knowledge from the in vitro studies may at least in part explain the currently observed in vivo effects of the drugs on the ventricular repolarization phase. Moreover, the in vivo canine model can be considered to bridge the gap between basic research reports and the clinically observed electrophysiological profile of disopyramide and mexiletine\(^{12,13,25-31}\).

**Effects on the Terminal Repolarization Phase**

In our recent study using the canine isolated, blood-perfused ventricular tissue preparation\(^9\), we demonstrated that prolongation and backward shift of the TRP enhanced the chance of conduction slowing at a less complete repolarization level, which is associated with a high incidence of torsades de pointes. In the present study, disopyramide prolonged the MAP\(_{90}\) and ERP to a similar extent at a HR of 150 beats/min, thus displacing the TRP backward in the cardiac cycle, potentially increasing the likelihood of the R on T phenomenon. As the effects of disopyramide on the ERP might be attenuated and those on the MAP\(_{90}\) enhanced at a slower HR\(^29\), one can speculate that disopyramide might prolong the TRP during bradycardia, and that the reverse would be true in the case of tachyarrhythmia. Experiments are now on-going to demonstrate that hypothesis using canine complete atrioventricular conduction block models in which HR can be controlled by ventricular pacing over 40–200 beats/min\(^32\). On the other hand, mexiletine shortened the MAP\(_{90}\), but prolonged the ERP, resulting in the disappearance of TRP, which could prevent premature excitation with its associated conduction slowing\(^14\). These differences in the electrophysiological profiles of these drugs might explain their clinically described antiarrhythmic and proarrhythmic effects\(^1\,\,2\,\,12,13\).

**Conclusions**

Antiarrhythmic concentrations of mexiletine as well as disopyramide exert a negative inotropic effect. As well as their well-known electrophysiological properties, disopyramide displaces the TRP backward in the cardiac cycle, whereas mexiletine significantly shortens it, which could be their antiarrhythmic and proarrhythmic mechanisms. Thus, the currently used in vivo canine model is useful for better understanding the relationship between the basic electrophysiological observations in vitro and the clinical experience of these drugs.

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**References**


