Amiodarone effectively blocks both the sodium and calcium channels and ẞ-adrenoceptors as well as the IKr, IKs, Ik1, IKACh and IKNa potassium channels (IKr: rapidly activating component of delayed rectifier K+ current; IKs: slowly activating component of delayed rectifier K+ current; Ik1: transient outward K+ current; IKr: inward rectifier K+ current; IKNa: Na+-activated K+ current; IKACh: muscarinic acetylcholine receptor-operated K+ current).1–9 Amiodarone is now considered one of the most promising drugs for the treatment of life-threatening ventricular tachyarrhythmias in patients with structural heart disease.10–14 Nevertheless, as with other antiarrhythmic agents, torsade de pointes (TdP) following QT interval prolongation has been a concern during amiodarone therapy in the setting of bradyarrhythmias and hypokalemia, although the incidence of TdP associated with amiodarone therapy is reportedly low.15–19 However, the extent of the in vivo proarrhythmic potential of amiodarone remains incompletely understood because of the scarcity of adequate experimental model systems.

The present study was designed to simultaneously assess the acute electrophysiological and proarrhythmic profiles of amiodarone using a canine chronic atrioventricular block model.20,21 The model is a suitable large-animal model for the study of TdP because its hemodynamic status is stable despite an extreme bradycardia of 20–40 beats/min.20–26 Previous studies have revealed the functional adaptations that predispose the canine AV block heart to acquired TdP;21–23,26 namely, the repolarization period is prolonged not only by the bradycardia but also by the reduction of the delayed rectifier potassium currents (IKr and IKs). We administered amiodarone orally without anesthesia under continuous ECG monitoring to better mimic the clinical therapy, and compared the results with those of a selective IKr channel blocker, sematilide.27–29

**Methods**

All experiments were carried out according to the Guidelines for Animal Experiments of Yamanashi Medical University. Beagle dogs of either sex weighing approximately 10 kg were obtained through the animal Laboratory for Research of Yamanashi Medical University. Production of Complete AV Block

AV block was induced as previously described.20 Briefly, the dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv) and artificially ventilated with room air (Shinano, SN-480-3, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. Heparin calcium (200IU/kg, iv) was administered to prevent blood clotting. The surface lead II ECG and systemic blood pressure at the right femoral artery were continuously monitored using a polygraph system (Nihon-Kohden, RM-6000, Tokyo, Japan) A quad-polar electrode catheter with a large tip of 4 mm (Cordis-
Webster, D7-DL-252, Baldwin Park, CA, USA) was inserted through the right femoral vein using a standard percutaneous technique\textsuperscript{30} under sterile conditions and positioned across the tricuspid valve under the guide of bipolar electrograms from the distal electrode pair. The optimal site for AV node ablation, namely the compact AV node, was determined on the basis of the intracardiac electrogram, on which a very small His deflection was recorded and the atrial/ventricular voltage ratio was greater than 2. This site was usually found 1–2 cm proximal to where the largest His bundle electrogram was recorded. The power source for the AV node ablation was obtained from an electrosurgical generator (Mera, MS-1500, Tokyo, Japan), which delivered continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After adequate localization, 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, and then continued for 30 s if a junctional rhythm was induced. The endpoint of this procedure was the development of complete AV block with the onset of a stable idioventricular escaped rhythm (VR). More than 4 weeks after the induction of complete AV block, the following experiments were carried out.

**Continuous ECG Monitoring and Analysis**

A Holter recording system (Model 456A, Del Mar Avionics, Irvine, CA, USA) was used to monitor the ECG over 24 h\textsuperscript{20} and the ECG was analyzed with the Holter analyzing software (Protrack Model 563, Del Mar Avionics). The effects of the drugs on the VR, sinoatrial rate (SAR) and QT interval, as well as the proarrhythmic effects, were assessed. The SAR was calculated from the P–P interval, and the VR from the R–R interval. These values were expressed as the mean of 3 consecutive complexes. Ventricular premature contractions (PVCs) were defined as a premature depolarization of coupling interval $\leq 600$ ms with prolonged, bizarre QRS complexes. Non-sustained ventricular tachycardia (VT) was defined as that which lasted more than 30 s and less than 30 s, whereas sustained VT was that which lasted 30 s or more. TdP was defined as polymorphic VT, consisting of the QRS complexes of 6 beats or more twisting around the baseline\textsuperscript{31}.

**Experimental Protocol**

The 16 animals were randomly assigned to 4 groups: 3 mg/kg (n=4) and 30 mg/kg (n=4) of sematilide, and 3 mg/kg (n=4) and 30 mg/kg (n=4) of amiodarone. The doses of sematilide and amiodarone used in this protocol were determined from previous reports to produce same degree of QT prolongation\textsuperscript{32–35} Approximately 2h after the start of ECG monitoring, sematilide or amiodarone in gelatin capsules was administered orally and the ECG was recorded for at least 22 h. The ECG values at 1 h before the drug administration were defined as the control (C) for each group. A volume of 2 ml of venous blood was drawn...
to measure the plasma concentration of amiodarone at 5 h after administration. The blood samples were centrifuged at 1,500 g for 30 min at 4°C and the plasma stored at −80°C until the drug concentration was measured. Sensitive and specific determinations of the concentrations of amiodarone were performed at SRL Co, Ltd (Tokyo, Japan) using a standard high-performance liquid chromatography method. The limit of quantification was 50 ng/ml.

**Drugs and Statistics**

Sematilide was generously provided by Nippon Roussel (Tokyo, Japan) and the other drugs were purchased (amiodarone: Sigma, Tokyo, Japan; pentobarbital sodium: Tokyo Kasei, Tokyo, Japan; heparin calcium: Mitsui, Tokyo, Japan). Data are presented as the mean±SE. Differences within a parameter were assessed using one-way, repeated-measures ANOVA followed by Contrasts for the comparison of mean values. Meanwhile, those of unpaired data between the groups were evaluated by one-way, factorial ANOVA. A p value less than 0.05 was considered significant.

**Results**

**Comparison of the Proarrhythmic Effects (Fig 1)**

In the sematilide groups, PVCs were induced in 3 of 4 animals with the 3 mg/kg dose and in all animals with the 30 mg/kg dosage. Non-sustained VT was induced in 1 of 4 animals by the low dose and 3 of 4 by the high dose. Sustained VT was not observed in any animals with either dose, but TdP (>300 beats/min) was induced in 3 of 4 animals by the high dose. The number of episodes of TdP was no significant difference in the control values of the 4 groups.

In the low-dose sematilide group, PVCs tended to increase for 5–6 h, but the change did not achieve statistical significance. In the high-dose sematilide group, the number of PVCs increased significantly for 5–6 h and both TdP and non-sustained VT were observed during this time. We often could not count the precise number of PVCs during TdP and ventricular fibrillation because of the absence of clearly defined QRS complexes and in those cases we tentatively defined the number of PVCs as 5 beats/s. The initial TdP episode was observed 5.3±0.2 h (n=3) after high-dose sematilide administration, and the last episode led to ventricular fibrillation and the animal’s death at 5.4±0.2 h (n=3) after drug administration.

There was no change in the number of PVCs after low- or high-dose amiodarone. Each VT episode was observed during the 2–6 h after the high dose, and the peak number of PVCs was 5–6 h after administration for both doses. The plasma drug concentration at 5 h after 3 mg/kg and 30 mg/kg of amiodarone was <50 ng/ml and 1,083±188 ng/ml, respectively.

**Time Course of the Effects on ECG Parameters (Figs 3–5)**

The QT interval (ms) at the pre-drug control (C) was 315±6 in the low-dose sematilide group and 309±5 in the high-dose group, compared with 319±2 in the low-dose amiodarone group and 309±5 in the high-dose amiodarone group. VR (beats/min) and SAR (beats/min) at the pre-drug control (C) were 36±2 and 108±20, respectively, in the low-dose sematilide group, and 38±2 and 115±32, respectively, in the high-dose sematilide group. There was no significant difference in the control values of the 4 groups (Fig 3).

After sematilide administration, the QT interval was significantly prolonged at 4th and 11th after the low dose and for 3–5 h after the high dose (Fig 4). VR was decreased by the low dose and a significant change was detected at 2h and 17h after administration; however, no significant change in VR was detected after the high dose, possibly
because of the limited number of animals that survived (Fig 5). No significant change in SAR was observed after the low or high.

In the amiodarone group, the QT interval was gradually but significantly prolonged only after the high dose and a significant change was detected for 8–20 h (Fig 4). VR was decreased by the low and high dose: a significant decrease was detected at 9, 14 and 17 h after the low dose and at 14, 16 and 20 h after the high dose. No significant change in SAR was observed after the low dose, but it was decreased by the high dose. Significant changes were detected for 1–3 h, at 5 h, for 7–8 h, 11–14 h and 16–20 h after drug administration.

Discussion

We characterized the in vivo electrophysiological and proarrhythmic effects of amiodarone in comparison with those of sematilide, a selective IKr channel blocker, using a canine chonic AV block model. The chonic bradycardia of this model facilitates the induction of lethal ventricular tachyarrhythmias, including TDP, through electrophysiological and morphological remodeling of the heart.2,23,26 As clearly shown in the present results, the high dose of sematilide induced TDP following QT prolongation and led to the death of 3 of 4 animals (75%). In contrast, amiodarone induced sustained VT in only one animal (25%) and although it prolonged the QT interval, no lethal arrhythmias were observed. These results are essentially in accordance with most of the previous knowledge from animal and clinical studies.15–18,36–39

The plasma drug concentration at 5 h after 30 mg/kg of amiodarone was approximately 1 μg/ml in this study, suggesting that a clinically, and experimentally, expected antiarrhythmic plasma concentration can be obtained with oral administration.1 Thus, the currently observed effects of amiodarone may in part reflect the cardiovascular profile at sub-therapeutic to therapeutic levels of plasma drug concentration.

We demonstrated that a single oral dose of sematilide or amiodarone can prolong the QT interval, with a faster onset speed for sematilide than for amiodarone, possibly reflecting the difference in the pharmacokinetic properties of these drugs. The prolongation of the QT interval can be largely explained by their previously demonstrated IKr-channel inhibition28 because IKr is one of the major outward currents that determine the duration of the repolarization phase of the ventricle. In addition, amiodarone inhibits the other outward potassium currents, including IK1, IKr, IKNa, IKCaL and Ito. Thus, further in vitro studies are needed to analyze the role of these repolarizing currents for QT interval prolongation in the currently used chonic AV block model.

Amiodarone exerted a negative chonotrophic effect, whereas sematilide hardly affected the SAR. The lack of negative chonotropic effects of sematilide suggests that IKr inhibition itself may not be enough to induce bradycardia in the present canine chonic AV block model. On the other hand, amiodarone has been reported to possess a calcium channel blocking effect and a non-competitive weak β-blocking action at the plasma concentration reached in the present experiment.1,3–5,7 which would be a possible mechanism of the observed negative chonotropic effect.

The most important finding in this study is that sematilide induced TDP, but amiodarone did not. It has been shown that IKr and IKs are down-regulated in the ventricles of the canine chonic AV block model, leading to less repolarization reserve.25 In the presence of such a labile repolarization process, amiodarone induced only 1 episode of sustained VT, which did not degenerate into TDP (Fig 1C), although it prolonged QT interval in a similar fashion to sematilide. These results support in part our recent hypothesis regarding the antiarrhythmic mechanism of acute amiodarone3 and the proarrhythmic effects of sematilide;40 that is, the antiarrhythmic effects of acute amiodarone depends on the combination of use-dependent sodium channel inhibition and reverse use-dependent potassium channel blockade, which shortens the electrically vulnerable period of the ventricle to provide post-repolarization refractoriness during tachycardia, leading to termination of re-entry arrhythmia? Prolongation of the terminal repolarization process (phase 3 repolarization) by sematilide would enhance the chance of conduction slowing at less complete repolarization levels and may be associated with its high incidence of TDP induction.40 However, further in vivo electrophysiological analysis is required, including the QT interval dispersion as well as transmural dispersion, to better understand the difference in the proarrhythmic potential of these drugs.

In summary, amiodarone did not induce lethal ventricular arrhythmias in the canine chonic AV block model, making it a more desirable choice than sematilide. IKr-channel inhibition with additional ion channel blocking actions of amiodarone may contribute to its less proarrhythmic profile.

Acknowledgments

The authors thank Miss E. Tatsuzawa and Ms Y. Nakamura for their skillful technical assistance.

References


24. YOSHIDA H et al.


