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Effects of azimilide, a $K_{V(r)}$ and $K_{V(s)}$ blocker, on canine ventricular arrhythmia models

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Abstract

Using canine coronary artery ligation/reperfusion and adrenaline arrhythmia models, we determined the effects of azimilide, a class III antiarrhythmic agent, *E*-1-{[(5-(4-chlorophenyl)-2-furanyl) methylene]-amino}-3-[4-(4-methyl-1-piperazinyl)butyl]-2,4-imidazolidinedione dihydrochloride. The coronary ligation/reperfusion arrhythmia experiments were divided into two groups, one using low heart rate halothane-anesthetized and the other using high heart rate pentobarbital-anesthetized dogs. Azimilide (6 mg kg^{-1} + 0.1 mg kg^{-1} min^{-1} i.v.) prolonged the corrected QT interval (QTc), decreased the heart rate and suppressed the premature ventricular complexes during ligation (35 ± 17 beats/30 min as compared with 909 ± 246 in the control group), and also suppressed ventricular fibrillation induced by coronary ligation/reperfusion in the two groups (1/8 halothane-anesthetized dogs as compared with 7/8 dogs in the control group and 2/8 pentobarbital-anesthetized dogs as compared with 8/8 dogs in the control group). In adrenaline arrhythmia, azimilide hastened the onset of adrenaline arrhythmias and also aggravated the arrhythmias, showing proarrhythmic effects.

Keywords: Azimilide; Ventricular arrhythmia; Ligation arrhythmia; Reperfusion arrhythmia; Proarrhythmia; Heart

1. Introduction

Azimilide, *E*-1-{[(5-(4-chlorophenyl)-2-furanyl)methylene]-amino}-3-[4-(4-methyl-1-piperazinyl)butyl]-2,4-imidazolidinedione dihydrochloride, is chemically different from class III drugs that selectively block the rapid component of the delayed rectifier K^+ channel ($K_{V(r)}$), in particular, those which belong to the methylsulfonamide group such as dofetilide and sotalol. However, the main electrophysiological effects of azimilide are common ones, similar to those of other class III drugs, i.e., to prolong action potential duration in cardiac fibers of calf, dog, ferret, guinea pig, sheep and human (Tatla et al., 1993; Gintant, 1994; McIntosh et al., 1994; Fermini et al., 1995; Lamorgese et al., 1995; Zhang et al., 1995). Azimilide decreases the $K_{V(r)}$ current (20% inhibition in guinea pig ventricular myocytes at 0.2–0.4 mM), and also decreases the slow ($K_{V(s)}$) component at higher concentrations (20% inhibition at 1–2 mM) (Conder et al., 1994a; Fermini et al., 1995; Zhang et al., 1995). The virtual rate-independence of increasing refractoriness, at least in in vitro studies, can be attributed to this property (Salata and Brooks, 1997). In addition, azimilide does not affect the transient outward current of rat (Zhang et al., 1995) or canine ventricular myocytes (Conder et al., 1994a). However, at 3–10 mM, azimilide inhibits the inward rectifier of guinea pig and canine myocardial cells (Conder et al., 1994a; Fermini et al., 1995; Zhang et al., 1995), and as at higher concentrations, it also inhibits the L-type Ca^{2+} current of rat and guinea pig (Fermini et al., 1995; Zhang et al., 1995) and the Na^+ current of dog and guinea pig ventricular cells (Conder et al., 1994b). These electrophysiological characteristics could explain the antiarrhythmic efficacy of azimilide in coronary ligation-induced ventricular arrhythmias in rats (Brooks et al., 1996) and digitalis-induced arrhythmias in guinea pigs (Miller et al., 1994). Although there are reports indicating azimilide's effectiveness on ventricular arrhythmias and sudden cardiac death induced by programmed electrical stimulation in dogs with old myocardial infarction (Black et al., 1993; Drexler et al., 1996), there have been no reports of effects of azimilide on acute coronary ligation and reperfusion or adrenaline-induced arrhythmias.

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We have been studying antiarrhythmic and proarrhythmic effects of class III drugs, using canine ventricular arrhythmia models produced by coronary artery ligation/reperfusion and adrenaline. Of the drugs we have used, i.e., D-sotalol, MS-551 (1,3-dimethyl-6-{2-[*N*-(2-hydroxyethyl)-3-(4-nitrophenyl) propylamino] ethylamino}-2, 4(1*H*,3*H*)-pyrimidinedione hydrochloride), E-4031 (1-[2-(6-methyl)-2-pyridil] ethyl)-4-(4-methylsulfonyl-aminobenzoyl) piperidine hydrochloride), intravenous (i.v.) amiodarone, sematilide, dofetilide and KCB-328 (1-(2-mino-4-methanesulfonamidophenoxy)-2-[*N*-(3,4-dimthylphenethyl)-*N*-methylamino] ethane), all except sematilide and dofetilide were effective on the ventricular arrhythmias produced by coronary ligation and reperfusion in dog hearts (Hashimoto et al., 1991, 1995; Awaji et al., 1995; Chen et al., 1996; Xue et al., 1998a). All drugs suppressed programmed electrical stimulation-induced ventricular arrhythmias. However, many drugs aggravated the adrenaline-induced ventricular arrhythmias in halothane-anesthetized dogs and induced ventricular tachycardia in halothane-anesthetized open-chest dogs, demonstrating proarrhythmic effects of these class III drugs (Chen et al., 1996; Xue et al., 1996, 1998b). In order to characterize azimilide as compared to other class III drugs regarding its antiarrhythmic, as well as its proarrhythmic potency, we used in vivo canine arrhythmia models for the present study.

2. Materials and methods

The animal experiments were approved by the Yamanashi Medical University Animal Experimentation Committee and the dogs were obtained through the Animal Laboratory for Research of Yamanashi Medical University.

2.1. Production of coronary ligation and reperfusion arrhythmia

Thirty-two beagle dogs of either sex, weighing 6.0–13.0 kg, were used. The experimental groups were divided into two. Dogs of group 1 were anesthetized with halothane, while dogs of group 2 were anesthetized with pentobarbital. For investigating the maximal effect against coronary ligation and reperfusion, the dose of azimilide chosen was a bolus injection of 6 mg kg^{-1} i.v. followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion for up to the end of 30 min of reperfusion, the same dose used in the study of Black et al. (1993). Control dogs of both groups 1 and 2 were given the same volume of 5% glucose. Dogs of group 1 were anesthetized initially with i.v. thiopental sodium, 30 mg kg^{-1}, and intubated. Anesthesia was maintained with 1.0% halothane, vaporized with 100% oxygen using a volume-limited ventilator (20 ml kg^{-1}, 15 strokes min^{-1}). Dogs of group 2 were anesthetized with i.v. pentobarbital sodium 30 mg kg^{-1} followed by an infusion of 5 mg kg^{-1} h^{-1}.

In both groups, the chest was opened and the left anterior descending coronary artery was isolated just proximal to the first diagonal branch. Since the incidence of coronary ligation/reperfusion ventricular fibrillation is known to be quite variable, experiments were randomized using a pair of beagles [selected by coin-flip]; one received the drug and the other received 5% glucose. After 30 min from the start of either azimilide or 5% glucose, the left anterior descending coronary artery was ligated using a silk thread and, 30 min later, was released to examine the reperfusion responses.

A pair of epicardial electrodes was sutured on the border zone of the ischemic area of the left ventricle for continuous recording of the ventricular electrograms. The QT interval was assessed from the lead II electrocardiogram and the ventricular surface electrogram. The corrected QT interval (QTc) was calculated using Bazett's formula, $QTc = QT/\sqrt{RR}$. The heart rate was measured from the lead II electrocardiogram and the blood pressure was continuously monitored through a double-lumen arterial cannula in the femoral artery. Arterial blood samples were obtained through another lumen of the cannula at times 0, 1, 5, 10, 15, 29 (just before artery ligation) and 59 min (just before reperfusion).

2.2. Production of adrenaline-induced arrhythmias

Seven beagle dogs of either sex, weighing 7.5–13.5 kg, were anesthetized initially with thiopental sodium. After tracheal intubation, 1.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator (20 ml kg^{-1}, 15 strokes min^{-1}, Shinano, SN-480-4, Tokyo, Japan). Both vagi were cut at the mid-cervical region. The lead II electrogram and atrial electrocardiogram from catheter tip electrodes in the right atrium were continuously monitored. A femoral artery catheter was inserted for blood pressure monitoring. The electrocardiogram, atrial electrogram and blood pressure were recorded with a polygraph system (Nihon Kohden, Tokyo, Japan). A femoral vein was also cannulated for administering drugs and adrenaline.

After surgical preparation, 30–45 min was allowed for stabilization, and then adrenaline diluted in 20 ml saline was intravenously infused for 50 s, according to the method of Hashimoto et al. (Hashimoto and Hashimoto, 1972; Matsubara et al., 1976). The starting dose of adrenaline was 0.5 μg kg^{-1}. If 0.5 μg kg^{-1} adrenaline did not produce arrhythmia, the dose of adrenaline was increased by increments of 0.25 μg kg^{-1} until ventricular arrhythmias were induced. The maximum adrenaline dose in the control period was 2.5 μg kg^{-1}, since this dose usually produces severe ventricular tachycardia or occasionally fatal ventricular fibrillation. If 0.5 μg kg^{-1} adrenaline produced arrhythmia, a lower dose of 0.25 μg kg^{-1} adrenaline was infused. Between the challenges with adrenaline infusion, a recovery period of about 10 min was

allowed, during which time the hemodynamic parameters, e.g., heart rate and blood pressure, became stable. We defined the arrhythmia-inducing dose of adrenaline as the lowest dose which produced either premature ventricular complexes, bigeminy or ventricular tachycardia defined as more than three consecutive premature ventricular complexes. The non-inducing dose of adrenaline was defined as the highest dose which did not induce any arrhythmia.

The time (in seconds) between the onset of adrenaline infusion and the appearance of arrhythmia was defined as the latent period. Using the same model, we previously tested other class III drugs, MS-551 and KCB-328 using doses to prolong QTc by 20–33%. To compare proarrhythmic effects of azimilide with those of MS-551 and KCB-328, we used the same equipotent dose as used in the coronary ligation and reperfusion experiment, a bolus of 6 mg kg^{-1} i.v. followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion for 30 min. In preliminary experiments, this dose of azimilide prolonged QTc by approximately 30% ($n = 3$). Non-arrhythmia-inducing and arrhythmia-inducing doses of adrenaline were determined before and after azimilide administration.

2.3. Determination of azimilide plasma levels

Plasma samples were distributed into tubes to which an internal standard was added. Proteins were precipitated out and removed by centrifugation. The supernatant was evaporated, and the remaining material was reconstituted in 350 μl of mobile phase, 70% acetate buffer pH 4.5 and 30% acetonitrile, and injected on a high-performance liquid chromatograph with UV detection at 340 nm. Quantification was obtained from the ratio of peak height to an internal standard (azimilide), utilizing a dog plasma spiked standard curve and weighed ($1/x^2$) regression analysis.

2.4. Drugs

Drugs used were azimilide dihydrochloride (Procter and Gamble Pharmaceuticals), thiopental sodium (Tanabe Seiyaku, Tokyo, Japan), halothane (Takeda Chemical Industries, Osaka, Japan), pentobarbital sodium (Tokyo Kasei Kogyo, Tokyo, Japan) and adrenaline hydrochloride (Daiichiseiyaku, Tokyo, Japan).

2.5. Evaluation of antiarrhythmic and proarrhythmic effects

In the coronary ligation and reperfusion arrhythmia model, the number of premature ventricular complexes during coronary ligation and the incidence of ventricular fibrillation by the ligation and/or reperfusion were counted. If both the number and the incidence of ventricular fibrillation were decreased significantly after azimilide administration, azimilide was judged to have an antiarrhythmic effect. In the adrenaline-induced arrhythmia model, the arrhythmic ratio was calculated by dividing the number of premature ventricular complexes, identified on the basis of the difference in the ventricular complex and the normal QRS complex, by the total heart rate, i.e., the number of premature ventricular complexes plus the number of conducted beats. If the arrhythmic ratio and the number of the ranks expressing the severity of adrenaline arrhythmia after drug administration were increased significantly from that of the control period, or if the latent period was shortened, or the non-inducing dose of adrenaline induced new arrhythmias, the drug was judged to aggravate arrhythmia.

2.6. Statistics

For the analysis of hemodynamic and electrocardiographic parameters and arrhythmic ratio, analysis of variance (ANOVA) was performed; when a statistically significant difference was detected, Dunnett's multiple comparison test was used to determine the difference between the 0 time value and the other values. The severity of arrhythmias was compared by Wilcoxon signed-ranks test. Differences in the incidence of ventricular fibrillation between the azimilide-treated group and 5% glucose-treated group were analyzed by Fisher's exact probability test. All data are expressed as means ± S.E.M. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Coronary ligation and reperfusion arrhythmias

3.1.1. Group 1: Azimilide, 6 mg kg^{-1} i.v. bolus followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion in halothane-anesthetized beagles

The heart rate and mean blood pressure before the drug treatment were 129 ± 3 beats min^{-1} and 100 ± 5 mmHg ($n = 8$) in the 5% glucose-treated group, and 120 ± 6 beats min^{-1} and 102 ± 4 mmHg ($n = 8$) in the azimilide-treated group (difference not statistically significant). As shown in Fig. 1 and Table 1, after 30 min of administration, azimilide significantly prolonged the QTc interval from 391 to 522 ms $s^{-1/2}$ (34% increase) just before the left anterior descending coronary artery ligation, as compared with that of the 5% glucose-treated group, from 403 to 404 ms $s^{-1/2}$. Azimilide decreased the heart rate by 19% (from 120 to 97 beats min^{-1}) just before coronary ligation and by 14% (to 103 beats min^{-1}) just before reperfusion. There was no change in the heart rate of the 5% glucose-treated group (129 to 123 beats min^{-1} just before coronary ligation). During the 30 min of ligation, azimilide significantly reduced the number of premature ventricular complexes counted each minute from the 18th to the 28th min after coronary ligation (Fig. 2), and the number of total premature ventricular complexes during the 30 min of

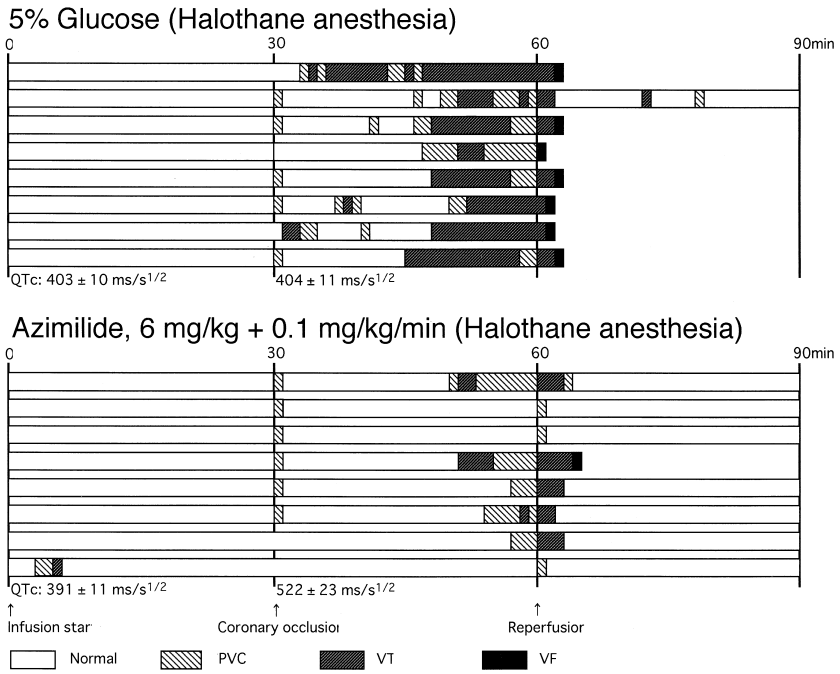

Fig. 1. Summary of the effects of azimilide, 6 mg kg^{-1} followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion on the coronary ligation/reperfusion experiments in halothane-anesthetized beagles. Each column indicates the responses of each dog. PVC: premature ventricular complexes, VT: ventricular tachycardia, VF: ventricular fibrillation.

ligation (35 ± 17 beats/30 min during the ligation period as compared to 909 ± 246 beats/30 min of the 5% glucose-treated group, $P < 0.01$) (Table 1). Azimilide had antifibrillatory effects after reperfusion (one ventricular fibrillation death out of eight dogs in the azimilide-treated group as compared to seven deaths out of eight dogs in the 5% glucose-treated group, $P < 0.01$). In addition, during the 30 min of infusion of azimilide and before coronary ligation, only one out of eight dogs showed non-sustained ventricular tachycardia, but no dogs showed other types of ventricular arrhythmias including those resembling torsades de pointes. Thirty minutes after reperfusion, the heart rate and QTc interval were 91 ± 4 beats min^{-1} and 548 ± 18 ms $s^{-1/2}$ in the azimilide-treated group ($n = 7$). The plasma concentrations of azimilide at 0, 5, 10, 15, 29 and 59 min after administration were 0, 2141 ± 166, 1775

Table 1
Effects of azimilide on coronary artery ligation and reperfusion arrhythmias in dogs. VPC: ventricular premature complexes, VF: ventricular fibrillation

	Group 1		Group 2	
	5% Glucose ($n = 8$)	Azimilide ($n = 8$)	5% Glucose ($n = 8$)	Azimilide ($n = 8$)
Before treatment				
HR (beats min^{-1})	129 ± 3	120 ± 6	161 ± 3	162 ± 4
PR (ms)	89 ± 2	91 ± 4	76 ± 6	77 ± 2
QRS (ms)	68 ± 3	68 ± 5	56 ± 1	58 ± 1
QTc (ms $s^{-1/2}$)	403 ± 10	391 ± 11	407 ± 5	397 ± 10
JTc (ms)	300 ± 10	295 ± 14	316 ± 4	302 ± 10
SBP (mmHg)	126 ± 6	129 ± 3	136 ± 13	133 ± 4
DBP (mmHg)	87 ± 5	88 ± 5	94 ± 5	98 ± 5
30 min after treatment				
HR (beats min^{-1})	123 ± 4	97 ± 4^{b}	158 ± 1	133 ± 4^{b}
PR (ms)	89 ± 2	92 ± 4	76 ± 2	78 ± 2
QRS (ms)	66 ± 2	68 ± 5	56 ± 1	58 ± 1
QTc (ms $s^{-1/2}$)	404 ± 11	522 ± 23^{b}	406 ± 3	488 ± 16^{b}
JTc (ms)	310 ± 12	436 ± 22^{b}	315 ± 4	402 ± 16^{b}
SBP (mmHg)	123 ± 5	121 ± 5	138 ± 4	136 ± 5
DBP (mmHg)	83 ± 4	73 ± 3^{a}	95 ± 5	95 ± 4
VPC (during ischemia)	909 ± 246	35 ± 17^{b}		
VF (during ischemia/reperfusion)	7/8	$1/8^{b}$	8/8	$2/8^{b}$

[a] $P < 0.05$, [b] $P < 0.01$ when compared with pretreatment values or 5% glucose-treated group.

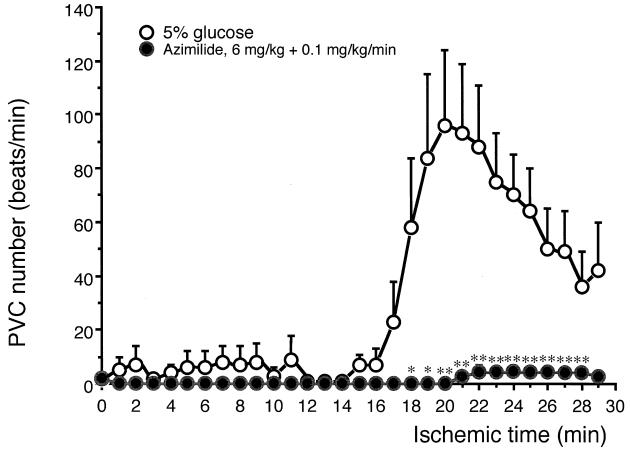

Fig. 2. Effects of azimilide, 6 mg kg^{-1} followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion on the number of premature ventricular complexes (PVC) during coronary artery ligation in halothane-anesthetized beagles. $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant difference from 5% glucose-treated group.

$\pm$ 188, 1516 $\pm$ 74, 1474 $\pm$ 106 and 1612 $\pm$ 80 ng ml^{-1} ($n = 7$).

3.1.2. Group 2: Azimilide, 6 mg kg^{-1} i.v. bolus followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion in pentobarbital-anesthetized beagles

Changing the anesthesia to i.v. pentobarbital, we repeated the experiment with azimilide, using the same dose as group 1. The baseline heart rate and mean blood pressure were 161 $\pm$ 3 beats min^{-1} and 108 $\pm$ 5 mmHg ($n = 8$) in the 5% glucose-treated group, and 162 $\pm$ 4 beats min^{-1} and 110 $\pm$ 5 mmHg ($n = 8$) in the azimilide-treated group. As shown in Table 1, azimilide prolonged the QTc interval significantly from 397 to 488 ms $s^{-1/2}$ (23% increase) just before left anterior descending coronary artery ligation, as compared with that of the 5% glucose-treated group, from 407 to 406 ms $s^{-1/2}$. Azimilide decreased the heart rate by 18% (from 162 to 133 beats min^{-1}) just before ligation and by 21% (to 128 beats min^{-1}) just before reperfusion. In the 5% glucose-treated group, five out of eight dogs fibrillated during the 30 min of ligation, and the surviving three dogs fibrillated immediately after reperfusion. Azimilide had antifibrillatory effects during coronary ligation and reperfusion, e.g., only one out of eight dogs fibrillated during the 30 min of ligation and one out of seven dogs fibrillated after reperfusion (two out of eight dogs in the azimilide-treated group as compared to all eight dogs in the 5% glucose-treated group, $P < 0.01$) (Table 1). The number of total premature ventricular complexes during ligation tended to decrease in the azimilide-treated group, but could not be analyzed due to the decreased number of surviving dogs — only three dogs in the 5% glucose-treated group. In addition, during the 30 min of infusion of azimilide and before coronary occlusion, no dogs showed premature ventricular complexes. Thirty minutes after reperfusion, the heart rate and QTc intervals were 103 $\pm$ 9 beats min^{-1} and 499 $\pm$ 33 ms $s^{-1/2}$ in the azimilide-treated group ($n = 6$). The plasma concentrations of azimilide at 0, 5, 10, 15, 29 and 59 min after administration were 0, 1940 $\pm$ 198, 1565 $\pm$ 140, 1469 $\pm$ 119, 1496 $\pm$ 122 and 1663 $\pm$ 103 ng ml^{-1} ($n = 8$).

3.2. Adrenaline-induced arrhythmia

Azimilide, 6 mg kg^{-1} i.v. bolus followed by 30 min of 0.1 mg kg^{-1} min^{-1} i.v. infusion, significantly decreased

Table 2
Effect of azimilide on hemodynamic and electrophysiologic parameters in adrenaline-induced arrhythmias in dogs. HR: heart rate, BP: blood pressure, QTc: corrected QT interval, Adr: adrenaline, i.v.: intravenously. Date are mean $\pm$ S.E.M.

Time	n	HR (beats min^{-1})	BP (mmHg)	QTc interval (ms $s^{-1/2}$)
Control	7	138 $\pm$ 3	118 $\pm$ 5	405 $\pm$ 16
Azimilide (0 min)	7	141 $\pm$ 3	125 $\pm$ 3	402 $\pm$ 16
Azimilide (5 min)	7	117 $\pm$ 2[b]	110 $\pm$ 5[a]	496 $\pm$ 20[b]
Azimilide (10 min)	7	114 $\pm$ 2[b]	107 $\pm$ 5[a]	515 $\pm$ 23[b]
Azimilide (15 min)	7	115 $\pm$ 3[b]	105 $\pm$ 5[b]	520 $\pm$ 14[b]
Azimilide (20 min)	7	114 $\pm$ 3[b]	105 $\pm$ 5[b]	518 $\pm$ 15[b]
Azimilide (25 min)	7	113 $\pm$ 3[b]	104 $\pm$ 6[b]	531 $\pm$ 12[b]
Azimilide (30 min)	7	112 $\pm$ 3[b]	106 $\pm$ 5[b]	524 $\pm$ 16[b]
Before Adr (0.5 μg kg^{-1})	7	112 $\pm$ 3[b]	107 $\pm$ 5[b]	527 $\pm$ 16[b]
After Adr (0.5 μg kg^{-1})	7	116 $\pm$ 4[b]	112 $\pm$ 5	523 $\pm$ 11[b]
Before Adr (0.75 μg kg^{-1})	7	118 $\pm$ 4[b]	112 $\pm$ 5	523 $\pm$ 11[b]
After Adr (0.75 μg kg^{-1})	7	118 $\pm$ 4[b]	114 $\pm$ 5	523 $\pm$ 12[b]
Before Adr (1.0 μg kg^{-1})	7	118 $\pm$ 4[b]	114 $\pm$ 5	523 $\pm$ 12[b]
After Adr (1.0 μg kg^{-1})	7	120 $\pm$ 5[b]	114 $\pm$ 5	518 $\pm$ 14[b]
Before Adr (1.5 μg kg^{-1})	7	120 $\pm$ 5[b]	117 $\pm$ 5	509 $\pm$ 13[b]
After Adr (1.5 μg kg^{-1})	5	127 $\pm$ 6[a]	118 $\pm$ 5	494 $\pm$ 10[b]
Before Adr (2.0 μg kg^{-1})	5	127 $\pm$ 6[a]	118 $\pm$ 5	494 $\pm$ 10[b]
After Adr (2.0 μg kg^{-1})	4	132 $\pm$ 4	123 $\pm$ 7	455 $\pm$ 15[a]

[a] $P < 0.05$, [b] $P < 0.01$ relative to azimilide administration at 0 time value.

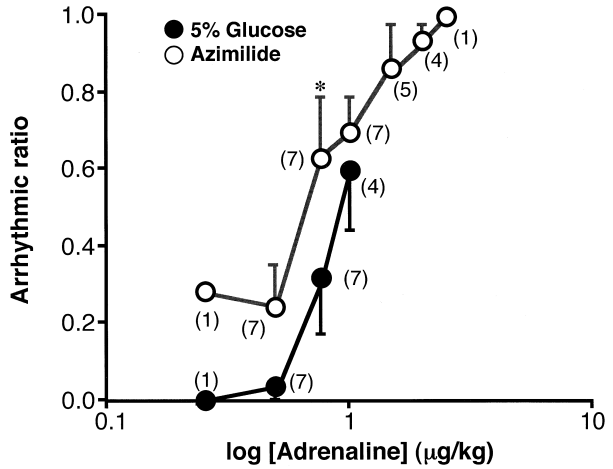

Fig. 3. Effects of azimilide, 6 mg kg^{-1} followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion on the arrhythmic ratio. The arrhythmic ratio was calculated by dividing the number of premature ventricular complexes by the total heart rate, i.e., the number of premature ventricular complexes plus the number of conducted beats. The arrhythmic ratios in the azimilide group were compared with those of the control group at corresponding adrenaline doses. Each number in parenthesis is the number of experiments. Each datapoint represents the mean, and the vertical bars show S.E.M. of each experiment. (○) Control group treated with 5% glucose. (●) Azimilide-treated group. $^{*}P < 0.05$, statistically significant difference from control.

the heart rate from 138 ± 3 to 112 ± 3 beats min^{-1} and mean blood pressure from 118 ± 5 to 106 ± 5 mmHg, and prolonged QTc interval from 402 to 524 ms $s^{-1/2}$ (30% increase) as shown in Table 2. Azimilide hastened the occurrence of arrhythmias, i.e., decreased the latent period of arrhythmias produced by inducing doses of adrenaline (0.71 ± 0.04 μg kg^{-1}), 29 ± 1 s as compared with 49 ± 5

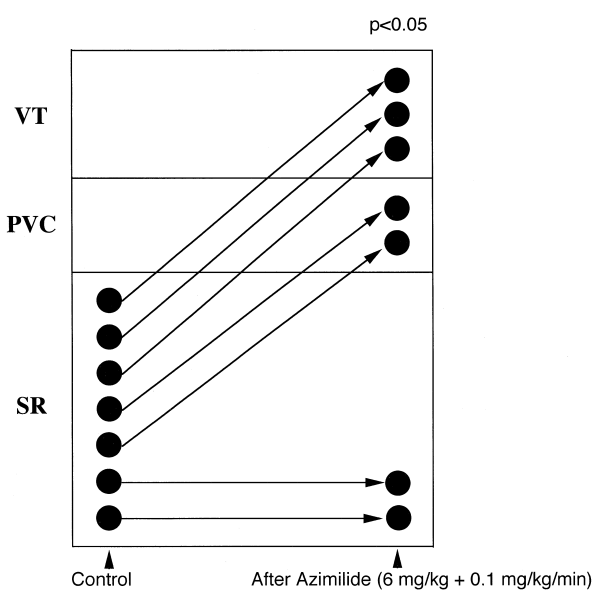

Fig. 4. Effects of azimilide, 6 mg kg^{-1} followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion on the changes in severity of the arrhythmia caused by non-inducing doses of adrenaline. VT: ventricular tachycardia, PVC: premature ventricular complexes, SR: sinus rate.

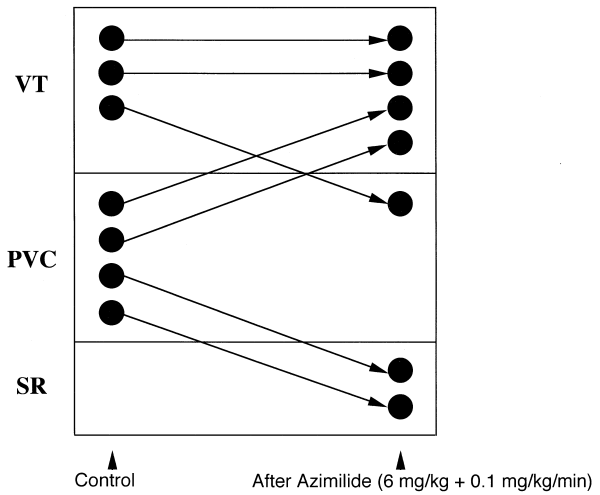

Fig. 5. Effects of azimilide, 6 mg kg^{-1} followed by 0.1 mg kg^{-1} min^{-1} i.v. infusion on the changes in severity of arrhythmia caused by inducing doses of adrenaline. VT: ventricular tachycardia, PVC: premature ventricular complexes, SR: sinus rate.

s of the control period ($P < 0.05$). Azimilide also aggravated adrenaline arrhythmias, i.e., increased the arrhythmic ratio attained with 0.75 μg/kg/50 s adrenaline (Fig. 3), and altered non-inducing doses of adrenaline (0.46 ± 0.04 μg kg^{-1}) to induce ventricular arrhythmias ($P < 0.05$) (Fig. 4). However, azimilide did not affect the arrhythmias produced by inducing doses of adrenaline ($P < 0.05$) (Fig. 5). During the 30 min of azimilide infusion alone, ventricular arrhythmias were induced in four out of seven dogs, i.e., premature ventricular complexes ($n = 3$) and ventricular tachycardia ($n = 1$). No dogs showed torsades de pointes-like ventricular tachycardia. The plasma concentrations of azimilide at 0, 3, 5, 10, 15 and 30 min after administration were 0, 2567 ± 371, 1968 ± 272, 1575 ± 185, 1509 ± 179 and 1455 ± 165 ng ml^{-1} ($n = 7$).

4. Discussion

In the present study, azimilide significantly decreased the heart rate, and prolonged the QTc interval. Azimilide significantly suppressed the number of premature ventricular complexes and the incidence of fatal ventricular fibrillation induced by coronary artery ligation/reperfusion in both the low heart rate halothane-anesthetized dogs and the high heart rate pentobarbital-anesthetized dogs. Though azimilide is reported to have no reverse use-dependent prolongation of the QT interval (Brandt and Maynard, 1994), the prolongation of QTc by azimilide with a high heart rate in pentobarbital-anesthetized dogs, a 23% increase, was less than that of the low heart rate halothane-anesthetized dogs, 34% increase. The plasma concentrations of azimilide producing the above QTc prolongation were the same, 1.5 μg ml^{-1}, for both the pentobarbital- and the halothane-anesthetized group. Azimilide showed minor proarrhythmic effects, only one out of eight dogs

showed non-sustained ventricular tachycardia by the direct action of azimilide in these halothane-anesthetized dogs before applying coronary artery ligation. In the adrenaline-induced arrhythmia model, azimilide decreased the latent period of adrenaline arrhythmias produced by the inducing dose, and increased the arrhythmic ratio and shifted the dose–response curve of adrenaline to the left, showing some proarrhythmic effects.

The generation of the coronary ligation/reperfusion ventricular fibrillation may be due to re-entry (Akiyama, 1981; Karagueuzia and Mandel, 1987), but certainly, abnormal automaticity may be an initiating factor (Pogwizd and Corr, 1978, 1987). So class III antiarrhythmic drugs, theoretically being effective on narrow excitable gap re-entry arrhythmias, may not necessarily be effective on this arrhythmia as compared to that induced by programmed electrical stimulation in dogs with old myocardial infarction. As compared with our results for class III drugs on reperfusion ventricular fibrillation in pentobarbital-anesthetized dogs (Hashimoto et al., 1991, 1995; Chen et al., 1996; Xue et al., 1996), effective antifibrillatory drugs were KCB-328 and D-sotalol. Similarly, in the halothane-anesthetized dogs, antifibrillatory drugs were dofetilide and E-4031. Azimilide is the only class III antiarrhythmic drug that suppressed ventricular fibrillation whether dogs were low heart rate halothane-anesthetized or high heart rate pentobarbital-anesthetized.

In contrast to those for other class III drugs, our present results showed that azimilide decreased the number of premature ventricular complexes during coronary ligation. Such significant decreases during ligation were not observed for other class III drugs we have tested. Similar to our results, azimilide has been reported to suppress the ventricular arrhythmias induced by coronary artery ligation and reperfusion in rats (Brooks et al., 1996), and to suppress programmed electrical stimulation-induced ventricular arrhythmias in infarcted dogs (Black et al., 1993; Drexler et al., 1996). It is also effective in a sudden cardiac death model with old myocardial infarction in dogs (Black et al., 1993). The antiarrhythmic effects of azimilide against ligation and reperfusion appeared in the present study at plasma concentrations of about 1–2 $\mu g\ ml^{-1}$ (2–4 μM disregarding plasma protein binding and calculated from a molecular weight of 458 for azimilide), thus the effect may be induced by the inhibition of $K_{V(r)}$ and $K_{V(s)}$ because IC_{50} values for inhibition of $K_{V(r)}$ and $K_{V(s)}$ in canine ventricular myocytes are 0.3 and 2.3 μM, which is lower than those inhibiting inward rectifier K^+, Ca^{2+} and Na^+ currents ($>$ 50, 17.8 and 12 μM) (Yao and Tseng, 1997).

We investigated whether azimilide has a proarrhythmic effect under the low heart rate halothane-anesthetized conditions, and whether azimilide showed reverse rate-dependent QT prolongation in vivo in the low and high heart rate dogs, as compared with those of other class III drugs. Under low heart rate halothane-anesthetized conditions, azimilide produced premature ventricular complexes and non-sustained ventricular tachycardia (only one out of eight dogs), but did not induce torsades de pointes-type ventricular tachycardia before the application of coronary ligation. Under the same conditions, MS-551 induced torsades de pointes in one out of six dogs (Hashimoto et al., 1995), E-4031 in three out of six dogs and dofetilide in three out of eight dogs (Hashimoto et al., 1991; Chen et al., 1996).

After 30 min of administration of azimilide, the QTc interval was prolonged 34% in halothane-anesthetized dogs, and 23% in pentobarbital-anesthetized dogs at the same plasma concentrations. This result shows that azimilide prolongs the QTc interval in a reverse rate-dependent manner (48% increase of QTc prolongation under the low heart rate halothane-anesthetized conditions). However, the difference observed by azimilide in the QTc prolongation in halothane-and pentobarbital-anesthetized dogs was less than those of MS-551 and dofetilide. Under the same conditions, MS-551, 3.6 $mg\ kg^{-1}\ h^{-1}$ prolonged the QTc interval by 48% and by only 13% in halothane- and pentobarbital-anesthetized dogs, respectively (Hashimoto et al., 1995), thus showing a 270% increase with low heart rate, while dofetilide, 100 $\mu g\ kg^{-1}\ h^{-1}$, prolonged the QTc interval by 43% and 19% under the same conditions (Chen et al., 1996), a 126% increase with the low heart rate. The reverse rate-dependent QTc-prolonging effect of class III drugs has been hypothesized to be partly due to the predominant block of $K_{V(r)}$ channels (Jurkiewicz and Sanguinetti, 1993; Gintant, 1996), and as azimilide blocks not only $K_{V(r)}$ but also $K_{V(s)}$ (Busch et al., 1994; Gintant, 1994; Herzer et al., 1995; Groh et al., 1997), a lesser degree of QTc prolongation might now have been observed in the low heart rate dogs than with MS-551, sematilide or dofetilide. The reverse use-dependent effect has already been confirmed by others, i.e., a greater effect of azimilide on action potential duration at 90% repolarization at slower (1 Hz) pacing rates than at faster (3 Hz) rates in guinea pig papillary muscle (Fermini et al., 1995; Bril et al., 1996), and with greater increases in the effective refractory period by 3 μM azimilide at 1 Hz than at 3 Hz in ferret papillary muscles (Fermini et al., 1995). Also, a greater increase in action potential duration by 1 μM azimilide at 0.33 Hz than at 1 Hz was seen in canine ventricular myocytes (Yao and Tseng, 1997). However, different results are reported. In normal dogs, both left and right ventricular effective refractory periods increased by 15% after an oral dose of 12.5 $mg\ kg^{-1}$, regardless of whether slower (500 ms cycle length) or faster (300 ms) pacing rates (Brandt and Maynard, 1994) were used. In dogs with old myocardial infarction, increases in the ventricular effective refractory period were similar with the slow (600 ms) and the fast (350 ms) rates in a dose range of 10 to 30 $mg\ kg^{-1}$ i.v. (Restivo et al., 1996). The difference between our results and these reports may be related to the different protocol, dose administered and route, and the anesthetics used.

The mechanism of adrenaline-induced ventricular arrhythmia models is thought to be abnormal automaticity and triggered activity (Hashimoto and Hashimoto, 1972; Hashimoto et al., 1982; Wit and Rosen, 1992), and halothane is known to interfere with cell-to-cell coupling. This model mimics the clinical situation of sympathetic overactivity. In the present experiment, azimilide prolonged the QTc interval by 30% and the drug alone induced arrhythmias and worsened adrenaline-induced arrhythmias after i.v. azimilide, so it surely has proarrhythmic effects. However, no torsades de pointes-type ventricular tachycardia was induced by azimilide. When the same model (Xue et al., 1998b) was used, the other two $K_{V(r)}$ blockers, MS-551 (prolonged QTc by 20%) and KCB-328 (prolonged QTc by 25%), induced torsades de pointes-type ventricular tachycardia even before adrenaline administration, but also significantly aggravated adrenaline-induced arrhythmias and induced fatal ventricular fibrillation in two of seven MS-551-treated dogs and one of seven KCB-328-treated dogs. Under the same experimental conditions, azimilide never aggravated adrenaline-induced ventricular arrhythmias to ventricular fibrillation. This suggests, though the effect is difficult to analyze, that the proarrhythmic effects of azimilide are less than those of MS-551 and KCB-328. Azimilide has not been reported to produce excessive prolongation of action potential duration that may lead to early after-depolarizations, but adrenaline may intensify azimilide-induced action potential duration and effective refractory period prolongation. Indeed, adrenaline has been reported to significantly prolong the monophasic action potential at 90% repolarization level and increase the dispersion of action potential duration in patients (Shimizu et al., 1995). Therefore, the prolongation of action potential duration was probably intensified when azimilide was combined with adrenaline administration and this prolongation of action potential duration may be related to proarrhythmic effects.

5. Conclusion

Azimilide prolonged the QTc interval in the low heart rate halothane-anesthetized dogs, showing that its QT-prolonging effect shows reverse use dependence. However, azimilide significantly suppressed the number of premature ventricular complexes during ligation and coronary artery ligation and/or reperfusion ventricular fibrillation regardless of the low heart rate halothane-anesthetized dogs and high heart rate pentobarbital-anesthetized dogs. Azimilide alone induced arrhythmias, but never induced torsades de pointes, and the aggravation of adrenaline-induced arrhythmias was less than with other class III drugs. Thus, azimilide can be said to have antiarrhythmic effects common to class III drugs with fewer arrhythmogenic potencies.

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