Efficacy of the Class III Antiarrhythmic Agent Azimilide in Rodent Models of Ventricular Arrhythmia (43995)

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Abstract. **Azimilide exhibited antiarrhythmic activity in several rodent models of ventricular arrhythmias. In the mouse chloroform model, azimilide provided limited efficacy by the ip route (50% at 100 mg/kg versus 20% by vehicle), and no efficacy by the oral route (300 mg/kg). In a rat model in which arrhythmias are induced by ligation and reperfusion of the left descending coronary artery (CALR model), azimilide provided dose-dependent (1–18 mg/kg) efficacy by the intravenous route. The estimated dose that suppressed ventricular fibrillation (VF) was 5.0 mg/kg iv. At 18 mg/kg iv azimilide also partially suppressed ventricular tachyarrhythmia (VT) and extrasystoles (VES). Rats dosed orally (100 mg/kg) were fully protected from VF. In isolated guinea pig hearts exposed to 1 μ*M* ouabain, azimilide at 10 μ*M* prevented the VT and VF seen in 69% and 23%, respectively, of control hearts. In anesthetized guinea pigs, azimilide at 10 and 30 mg/kg iv increased the dose of ouabain required to induce VES. While sematilide, dofetilide, and E-4031 significantly increased sensitivity to the arrhythmogenic actions of ouabain (by lowering the dose that caused VF), azimilide did not. Azimilide's antiarrhythmic profile in these rodent models differs from that of other class III agents, since azimilide had less efficacy in the mouse chloroform model, could suppress VT and VES as well as VF in the CALR rat model, and protected from or did not aggravate cardiac glycoside-induced arrhythmias in guinea pigs. These results demonstrating the antiarrhythmic efficacy of azimilide in the intact animal suggest that the compound has a different profile than other class III agents.**
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Azimilide dihydrochloride (NE-10064, (E)-1-[[[5-(4-chlorophenyl)-2-furanyl]methylene]-amino-3-[4-(4-methyl-1-piperazinyl)butyl]-2,4-imidazolidinedione dihydrochloride, Fig. 1), a class III antiarrhythmic agent first described in several abstracts in 1993, prolongs action potential duration in Purkinje and ventricular muscle fibers of seven species, including humans (1–9). The compound increased action potential duration as a function of concentration in the micromolar range and effects were slowly reversible on washout. Prolongation was due to selective block, as a function of concentration, of both the rapid I_{Kr} and slow I_{Ks} components of the delayed rectifier potassium current. Concentrations required to inhibit I_{Kr} and I_{Ks} by 20% have been estimated in guinea pig ventricular myocytes as 0.2–0.4 μ*M* and 1–2 μ*M*, respectively (3, 5, 6). Several other currents underlying the cardiac action potential have been examined. Azimilide had no effects on the transient outward current (I_{to}) of rat (5) or dog ventricular myocytes (3), but at 3–10 μ*M* inhibited the inward rectifier I_{K1} of guinea pig and dog cells (3) and at a higher concentration the L-type calcium current (I_{CaL}) of rat (EC_{50} = 43.6 μ*M* [5]) and guinea pigs (6) myocytes. Azimilide is chemically different from other blockers of the delayed rectifier, lacking, in particular, the methylsulfonamide group found in dofetilide and sotalol.

A report on the antifibrillatory activity of azimilide in a canine model of sudden cardiac death has ap-

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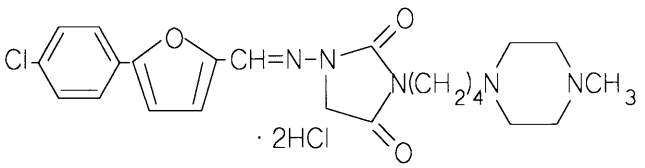

Figure 1. Azimilide dihydrochloride (NE-10064).

peared (10). At 10 mg/kg iv azimilide significantly reduced the 24-hr mortality compared with that in dogs treated with saline vehicle. Effects on arrhythmias other than VF have not been fully reported. Azimilide was discovered in a program that used rodent arrhythmia models to evaluate compounds that had a desirable electrophysiological profile, since it was required to verify that action potential prolongation translated into an antiarrhythmic property prior to evaluation in larger species. Only those compounds that demonstrated good efficacy and tolerance in rodents were further studied in more difficult canine arrhythmia models. We have described these rodent test systems and their response to a variety of reference antiarrhythmic drugs of all Vaughan Williams classes (11). Experiments conducted with azimilide and comparator class III antiarrhythmic agents provide an extensive demonstration of azimilide's antiarrhythmic efficacy, determine which types of arrhythmias are affected by the new agent, and identify differences between this compound and other drugs of the class. The azimilide results have appeared in abstract form (12, 13)

Materials and Methods

Materials. Sterile water, 0.9% sodium chloride, and pentobarbital sodium (Nembutal) were obtained from Abbott Laboratories (North Chicago, IL). Propranolol HCl, ouabain octahydrate, Evans Blue stain, and Patent Blue Violet dye were from Sigma Chemical Co. (St. Louis, MO). Azimilide 2HCl, sematilide HCl, and the free bases of dofetilide and E-4031 were synthesized by Procter & Gamble chemists. The reference antiarrhythmic agents, bretylium tosylate (American Critical Care, McGraw, IL), clofilium PO_4 (Eli Lilly & Co., Indianapolis, IN) and *d*- and *dl*-sotalol HCl (Bristol-Myers Co., New York, NY), were gifts from their manufacturers. All other chemicals were obtained commercially and were usually of reagent grade. Solutions of test articles in the indicated vehicle were prepared on the day of use and stored at room temperature.

Animals. Male Sprague Dawley rats (TAC:SD/NfBr), 350–450 g, were purchased from Taconic Farms (Germantown, NY). Male CD-1 mice, 18–34 g, and male Hartley guinea pigs (Crl:(HA)BR), 300–600 g, were purchased from Charles River Laboratories (Wilmington, MA). Animals were maintained under standard laboratory conditions and fed standard commercial feeds. Water was provided *ad libitum*. No contaminants of the feed or water were expected to affect the results of these experiments, so analysis was not done. Experiments were done in compliance with animal welfare regulations under protocols approved by the Institutional Animal Use and Care Committee.

Mouse Chloroform Model. By the method of Lawson (14), mice were placed on a wire mesh in a chamber whose atmosphere was saturated with chloroform vapors. Anesthesia occurred in 2–3 min and apnea in 3–4 min. Upon apnea the mice were removed from the chloroform and the chest was opened within 5 sec. The exposed heart was rated visually by a trained observed as either being in normal sinus rhythm or arrhythmia. For ip testing, groups of 20 mice were given 100 mg/kg azimilide 2HCl or 40 mg/kg propranolol HCl, or vehicle (0.5% w/v methylcellulose) alone in a volume of 10 ml/kg. These mice were exposed to chloroform 30 min later. For oral testing, groups of 20 mice were given 300 mg/kg azimilide 2HCl, 40 mg/kg propranolol HCl, or vehicle (0.5% w/v methylcellulose) alone in a volume of 10 ml/kg. At 60, 120, and 180 min (azimilide) or at 60 min (propranolol) after dosing, separate groups of mice were exposed to chloroform. Efficacy was assessed as the percentage of animals showing normal sinus rhythm.

Coronary Artery Ligation/Reperfusion (CALR) Rat Model. A method of Manning *et al.* (15) and Kane *et al.* (16) was modified as previously reported (11) to assess antiarrhythmic activity in anesthetized rats. Rats were anesthetized with pentobarbital sodium (60 mg/kg), intubated, and respired with room air. The left carotid artery and the right jugular vein were exteriorized and cannulated with PE-50 tubing for the recording of blood pressure (BP) and for iv administration of drug, respectively. The thoracic cavity was opened and the left anterior descending coronary artery (LAD) was located and ligated with 6-0 surgical silk (17). The suture ends were threaded through a small length of PE-280 tubing. A lead II electrocardiogram (ECG) and BP tracings were continuously recorded (Model 79D ECG and Polygraph Data Recording System; Grass Instruments Co; Quincy, MA).

Compound dissolved in vehicle (sterile distilled water, Abbott) or vehicle alone was administered iv at a volume of 1–3 ml/kg delivered over 1–3 min just before occlusion of the left anterior descending (LAD) coronary artery (see below). Volume and time was varied to accommodate compound solubility. In a few rats vehicle or azimilide 2HCl (100 mg/kg) was administered orally 1 hr before the LAD was ligated by clamping its suture/tubing tight against the heart surface using a 25-mm Schwartz forceps. For iv adminis-

tration, the LAD was occluded 1 min after compound administration. After 5 min of occlusion, the left ventricle was reperfused by releasing the clamp. Successful ligation of the LAD artery was determined by measuring the decrease (>1°C) in surface temperature of the left ventricle after ligation with a Sensotek Model TH-8 temperature monitor and type MT-4 needle sensor (Sensotek, Inc., Clifton, NJ). Additionally, in those animals where fibrillation was not observed during the reperfusion, the occluder apparatus was reapplied and Evans Blue stain was injected into the right jugular vein catheter. Discoloration of surrounding tissue with no change in the left ventricle indicated successful LAD occlusion. Release of the apparatus allowed immediate perfusion of dye into the uncolored area, thereby indicating restoration of blood flow.

Mean arterial blood pressure (MABP) and heart rate (HR) were determined just prior to treatment (baseline) and just prior to occlusion of the LAD. Maximum percentage change in MABP and HR was determined from these measurements. The minimum acceptable MABP during the occlusion was 50 mm Hg. The incidence and onset time of ventricular extrasystoles (VES), ventricular tachyarrhythmia (VT), and ventricular fibrillation (VF) were noted during the 5-min reperfusion period. VES was defined as ventricular contractions without atrial depolarization, VT was six or more consecutive VES, and VF was characterized by a loss of synchronicity of the ECG with decreased amplitude and a precipitous fall in blood pressure for >1 sec. These definitions are consistent with the Lambeth Conventions (18), except for our slightly more stringent definition of VT (six instead of four consecutive premature beats). Literature studies in rats either do not define VT (16) or use minimum numbers of consecutive VES of three (19), four (20), five (15), and seven (21). For screening purposes, the arbitrary choice of six consecutive beats reliably identified a transition to more serious arrhythmias and showed differences among class III drugs (11).

Guinea Pig Models. The *in vitro* model using isolated perfused guinea pig hearts was as described by Tanz *et al.* (22). Guinea pigs were anesthetized with urethane (1.5 g/kg ip) and heparinized (200 units/kg iv). The chest was opened and the heart was quickly excised into a petri dish containing modified pH 7.4 Krebs-Henseleit solution (118 m*M* NaCl, 11 m*M* glucose, 25 m*M* $NaHCO_3$, 4.69 m*M* KCl, 2.52 m*M* $CaCl_2$, 1.18 m*M* KH_2PO_4, and 1.16 m*M* $MgSO_4$) saturated with 95% oxygen/5% CO_2 at 35°–37°C. The aorta was freed of adipose tissue and a blunt, 17-gauge, stainless steel cannula was inserted and tied in place. Within a minute of excision, the heart was retrogradely perfused with oxygenated modified Krebs-Henseleit solution at 37° ± 1°C in a Langendorff apparatus. Perfusion adequacy was checked by brief staining with 0.1–0.2 ml of 0.1% Patent Blue Violet dye (23). Pre-load pressure in a latex, water-filled balloon in the left ventricle was adjusted to produce the greatest dp/dt_{max} (largest value of first derivative of ventricular pressure with respect to time). Ventricular pressure was monitored as an index of cardiac performance (data not shown). Right atrial temperature was monitored via a thermistor probe connected to a digital temperature monitor. Platinum, plunge electrodes were fixed in the apex, the anterior aspect of the left ventricle, and at the base on the posterior aspect of the right ventricle to provide electrocardiograms. After a 60-min perfusion period, the perfusate was changed to Krebs-Henseleit with 1 μ*M* ouabain, an arrhythmogenic concentration based on experiments conducted with 0.1–10 μ*M* ouabain. To test for efficacy, the antiarrhythmic agent was included in the ouabain perfusion fluid. The incidence and onset of ouabain-induced VES (characterized as premature ventricular beats lacking a P-wave, and generally appearing as sequential episodes of bigeminy and trigeminy), and VF (characterized as a lack of synchronicity of the ECG with a concomitant loss of the left ventricular pressure waveform) were noted. At the end of the experiment, adequacy of perfusion was checked by reinjection of dye and data from hearts (1%) with incomplete perfusion were excluded.

The *in vivo* model of Sekiya and Vaughan Williams (24) was modified and used as described by Brooks *et al.* (11). Anesthetized (1.25 g urethane/kg ip), respired guinea pigs were cannulated for drug administration (jugular vein, PE-50) and blood pressure measurements (carotid artery, Statham P23ID transducer). A lead II electrocardiogram was recorded from limb electrodes. After 10–15 min of equilibration, a bolus (less than 1-min infusion time) iv injection of the tested antiarrhythmic agent was given, followed immediately by start of an infusion of 75 μg/ml ouabain octahydrate in saline at 0.1 ml/kg/min. Onset time of VES, VT (characterized as greater than four sequential VES), and VF was noted. As with rats, the literature for *in vitro* and *in vivo* arrhythmia models in guinea pigs either lacks, or uses various, definitions of VT. When defined, VT is often considered four or more consecutive VES (25–27). We have chosen a slightly more stringent definition of five or more VES.

Calculations and Statistical Methods. Because these data derived from a program of screening compounds with a desirable *in vitro* electrophysiological profile to verify activity prior to more extensive testing in advanced arrhythmia models, group sizes were small and often varied. Nevertheless, assessments of significance could be done. For the mouse chloroform test, the statistical significance of differences in per-

centage protection from fibrillation compared with the vehicle-treated groups was assessed by Fisher's exact two-tailed chi-square analysis. For the CALR rat test, arrhythmia scores as previously described (11) were calculated based on the sum of factors for the occurrence of ventricular arrhythmias (i.e., VES, VT, and VF) and the onset time (min) of the first occurrence of each type of arrhythmia during the 5-min period following reperfusion. The occurrence of VES, VT, and VF were arbitrarily assigned values of 2, 6, and 12, respectively, reflecting the severity of the event. These values were multiplied by the time (full minutes) free of that type of arrhythmia. Subtraction from 100 resulted in a scale from 0 to 100. This score reflecting the severity and onset time of the rhythm disturbance can also be expressed as a single equation:

$$\text{Score} = 100 - (2 \times \text{min before VES}) - (6 \times \text{min before VT}) - (12 \times \text{min before VF})$$

Development of VES, VT, and VF within the first minute of reperfusion would result in an arrhythmia score of 100, while lack of arrhythmias within 5 min of reperfusion would result in a score of 0. Early occurrence of VES and VT, but protection from VF, would produce a score of 40. This score is analogous to that described by Johnston *et al.* (20) in that both use ar-

Table I. Efficacy of Intravenous Class III Antiarrhythmic Drugs in the Rat Coronary Artery Ligation-Reperfusion Model of Ventricular Arrhythmias

Treatment	Dose (mg/kg)	*n*	Percent change		Percent protection			Arrhythmia score
			MABP	HR	VES	VT	VF	
Vehicle	1 ml/kg	66	−2 ± 2	−2 ± 1	0	0	15	71 ± 2
Bretylium tosylate	10	5	−13 ± 9[a]	−13 ± 7[a]	20	40	60	35 ± 12[b]
Clofilium PO_4	0.33	2	14 ± 3[a]	−6 ± 1[a]	0	0	0	64 ± 8
	1	3	15 ± 7[a]	−7 ± 3[a]	0	0	67	60 ± 16
dl-Sotalol HCl	0.01	3	−9 ± 8[a]	−5 ± 0[a]	0	0	0	72 ± 12
	0.1	3	1 ± 7[a]	−9 ± 1[a]	0	0	33	56 ± 6
	1	3	7 ± 3[a]	−7 ± 5[a]	0	0	67	44 ± 3[b]
	3.1	3	−12 ± 3[a]	−24 ± 1[a]	0	0	100	40 ± 0[b]
	10	6	−19 ± 6[a]	−22 ± 3[a]	17	33	100	25 ± 6[b]
d-Sotalol HCl	1	3	18 ± 7[a]	−10 ± 1[a]	0	0	0	80 ± 3
	3	3	−11 ± 8[a]	−13 ± 4[a]	0	0	33	56 ± 6
	10	5	ND	ND	0	0	60	64 ± 13
	32	5	−38 ± 3[a]	−23 ± 3[a]	0	0	100	40 ± 0[b]
	56	3	−47 ± 0[a]	−30 ± 2[a]	0	0	100	38 ± 2[b]
Sematilide HCl	0.3	3	1 ± 3	−1 ± 2	0	0	0	72 ± 9
	1	4	7 ± 8[a]	−6 ± 2[a]	0	0	25	67 ± 9
	3	3	5 ± 5[a]	−11 ± 3[a]	0	0	33	60 ± 12
	5	3	−7 ± 0[a]	−13 ± 0[a]	0	0	33	56 ± 9
	10	3	−9 ± 5[a]	−13 ± 0[a]	0	0	67	52 ± 10
	18	3	−13 ± 6[a]	−18 ± 1[a]	0	0	33	68 ± 12
	30	3	−33 ± 8[a]	−16 ± 0[a]	0	0	100	40 ± 0[b]
	56	3	−33 ± 4[a]	−17 ± 2[a]	0	0	67	52 ± 10
Dofetilide	0.3	2	6 ± 1[a]	10 ± 1[a]	0	0	50	46 ± 4
	0.6	9	26 ± 8[a]	−5 ± 2[a]	0	0	44	60 ± 6
	1	3	25 ± 3[a]	−3 ± 2	0	0	100	60 ± 9
	3	3	46 ± 13[a]	−9 ± 2[a]	0	0	0	68 ± 6
	5.6	3	17 ± 7[a]	−8 ± 2[a]	0	0	100	40 ± 0[b]
	10	3	10 ± 9[a]	−19 ± 4[a]	0	0	33	58 ± 10
	18	3	20 ± 14[a]	−18 ± 5[a]	0	0	100	36 ± 4[b]
E-4031	0.3	4	14 ± 1[a]	−9 ± 3[a]	0	0	0	82 ± 12
	0.56	4	27 ± 7[a]	−7 ± 3[a]	0	0	100	36 ± 4[b]
	1	1	−6	−10	0	0	100	40
	5	4	7 ± 12	−4 ± 6	0	0	100	40 ± 0[b]

Note. Values are group means ± SEM for *n* rats. Vehicle was saline. Mean arterial blood pressure (MABP) and heart rate (HR) percentage changes from baseline values were determined 1 min after compound administration. VES, ventricular extrasystole, VT, ventricular tachyarrhythmia, VF, ventricular fibrillation. Arrhythmia score is defined in the text and ranges from 0 (full protection) to 100 (no protection).

[a] Significant ($P > 0.05$) change from baseline control value by Student's *t* test.

[b] Significant ($P > 0.05$) difference in score versus the vehicle-treated group of rats by the Mann-Whitney comparisons of medians.

TABLE II. Summary of Antiarrhythmia Efficacy of Azimilide and Reference Antiarrhythmic Agents in the CALR Rat Model of Ventricular Arrhythmias

Compound	Antifibrillatory dose ED_{40} (mg/kg iv)
Azimilide 2HCl	4.99
dl-Sotalol HCl	1.54
E-4031	ca. 0.56[a]
d-Sotalol HCL	46.7
Sematilide HCl	413
Dofetilide	ca. 5.6[a]

[a] Not dose dependent; visual estimate from Table I data.

rhythmia severity and time of occurrence factors. Although the present score has been used successfully to demonstrate a concentration-response relationship for efficacy in rats by drugs of each Vaughan Williams class (11), no assumption of a Gaussian distribution is made and significance of differences in arrhythmia scores was assessed by the nonparametric Mann-Whitney two-sample sum of ranks test. Significance of effects on blood pressure and heart rate was evaluated by Student's two-sample pooled t-test. Arrhythmia incidences are also expressed as percentage protection from VES, VT, and VF after treatment. An estimate of the dose required for a score of 40 (ED_{40} value) was obtained from a plot of log iv dose versus arrhythmia score fitted to a straight line by least square regression analysis. The ED_{40}, rather than the ED_{50}, was used, since this is an arbitrary score scale rather than a percentage effect, and the value of 40 often indicated a compound that completely suppressed fibrillation. For the guinea pig models, incidence and onset times of arrhythmias were compared between treatment groups by Student's *t* test. Probabilities less than 0.05 were considered significant.

Results

Mouse Chloroform Model. Intraperitoneal administration of vehicle to two groups of 20 mice did not prevent VF (5% and 20% protection). Propranolol at 40 mg/kg ip protected 90% ($P < 0.001$). Azimilide had a moderate antiarrhythmic effect (50% protection) at 100 mg/kg ip ($P < 0.1$). Oral administration of vehicle did not prevent VF (5%–20% protection). Propranolol at 40 mg/kg po dose given 60 min before exposure to chloroform gave 85% protection ($P < 0.001$). Azimilide at 300 mg/kg po was ineffective at 60, 120, or 180 min after dosing (data not shown).

CALR Rat Model. For validation purposes, reference class III antiarrhythmic compounds were tested with prophylactic administration by the intravenous route. All of 66 control rats dosed with vehicle exhibited VES and VT and 85% developed VF during the 5-min reperfusion period. The arrhythmia score for control rats was 71 ± 2 (Table I). All tested class III agents, including bretylium at 10 and clofilium at 1 mg/kg, showed antiarrhythmic activity (Table I). *dl*-Sotalol was more effective and more potent than *d*-sotalol in suppressing arrhythmias. At the highest tested dose (10 mg/kg), *dl*-sotalol suppressed not only VF, but VT in 2 of 6 rats and VES in 1 of 6 rats. *d*-Sotalol only suppressed VF, even at the highest 56 mg/kg iv dose. Estimated ED_{40} values were 1.5 and 47, respectively (Table II). E-4031 (ED_{40} = 0.6 mg/kg) were 10-fold more potent than dofetilide (ED_{40} = 5.6 mg/kg), and neither compound suppressed VES or VT. Sematilide lacked potency (extrapolated ED_{40} estimate of 413 mg/kg). A plot of log iv dose versus arrhythmia

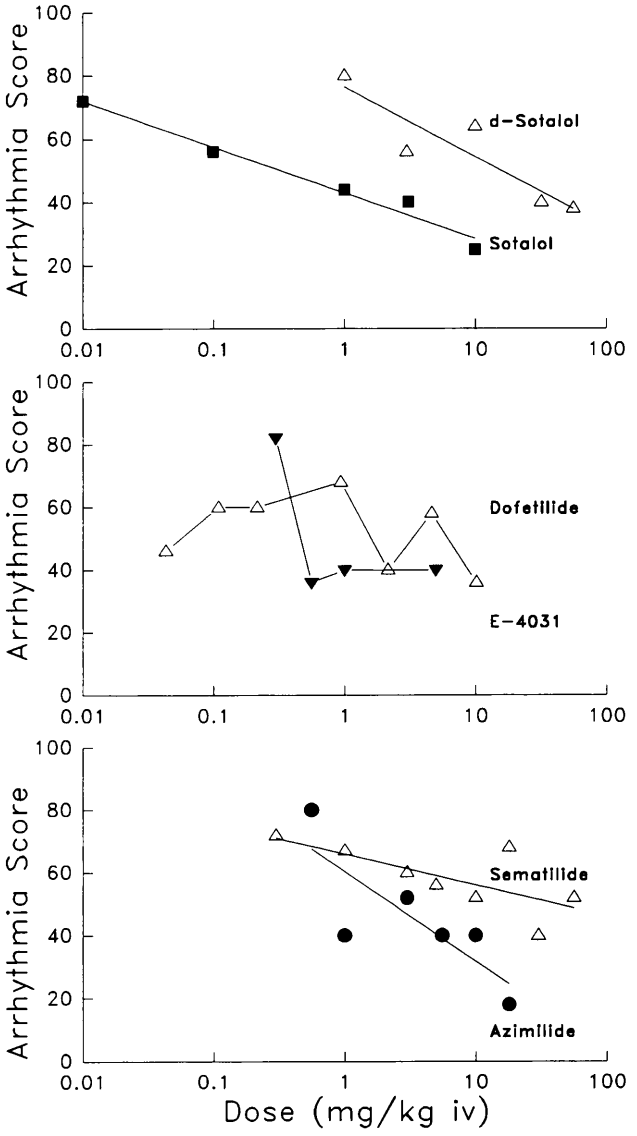

Figure 2. Effect of class III antiarrhythmic agents in the CALR rat model ventricular arrhythmias. Regression lines are shown for *dl*-sotalol HCl, *d*-sotalol HCl, sematilide HCl, and azimilide 2HCl. Lines connecting the points are shown for E-4031 and dofetilide. Results are plotted from the data of Table I and III, and each point represents a mean score of one to nine rats. (See tables for SEM.)

Table III. Efficacy of Azimilide in the Rat Coronary Artery Ligation-Reperfusion Model of Ventricular Arrhythmias

Treatment	Dose (mg/kg)	*n*	Percent change		Percent protection			Arrhythmia score
			MABP	HR	VES	VT	VF	
Intravenous								
Water	1 ml/kg	5	−2 ± 3	−2 ± 1	0	0	20	64 ± 11
Azimilide 2HCl	0.56	3	10 ± 2[a]	−1 ± 2[a]	0	0	0	80 ± 4
	1	3	27 ± 8[a]	−5 ± 1[a]	0	0	100	40 ± 0[c]
	3	3	3 ± 4	−10 ± 2[a]	0	0	67	52 ± 10
	5.6	3	5 ± 6	−18 ± 3[a]	0	0	100	40 ± 0[c]
	10	3	−1 ± 4[a]	−18 ± 1[a]	0	0	100	40 ± 0[c]
	18	4	−6 ± 6[a]	−28 ± 4[a]	25	75	75	18 ± 10[c]
Oral								
Water	0	3	118 ± 14[b]	437 ± 12[b]	0	0	0	68 ± 8
Azimilide 2HCl	100	4	104 ± 13[b]	390 ± 25[b]	0	0	100	40 ± 0[c]

Note. Values are group means ± SEM for *n* rats. Ligation of the coronary artery was done 60 min after oral administration. VES, ventricular extrasystole, VT, ventricular tachyarrhythmia; VF, ventricular fibrillation. Arrhythmia score is defined in the text and ranges from 0 (full protection) to 100 (no protection).

[a] Significant ($P > 0.05$) change from baseline control value by Student's *t* test.

[b] Mean arterial blood pressure (MABP) and heart rate (HR) values after oral administration are actual values in mm Hg and beats per min, respectively, recorded 55 min after dosing.

[c] Significant ($P > 0.05$) difference in score versus the vehicle-treated group of rats by the Mann-Whitney comparisons of medians.

score illustrates the lack of a strong dose-response effect of dofetilide and E-4031 (Fig. 2).

Azimilide provided dose-dependent antiarrhythmic efficacy between 1 and 18 mg/kg (Table III) and partially or fully suppressed VF at all doses in this range. At the highest tested dose, azimilide suppressed VES in one of four rats and VT in three of four rats. The only other class III agent to have an effect on VES and VT was *dl*-sotalol at the highest tested dose. *dl*-Sotalol at 10 mg/kg iv prevented VES in one of six rats and VT in two of six rats. At this highest tested dose *dl*-sotalol significantly depressed MABP and HR, while azimilide only decreased HR. The potency of azimilide (ED_{40} = 5 mg/kg) was comparable to other class III agents in the CALR rat model (Table II).

Hemodynamic effects were assessed in terms of mean arterial blood pressure and heart rate. E-4031 and dofetilide significantly increased MABP (Table I). At doses with a significant antiarrhythmic effect, other reference compounds decreased MABP. Azimilide increased MABP a significant 27% at the least effective antiarrhythmic dose of 1 mg/kg iv, was without a significant effect in the center of the effective antiarrhythmic dose range, and decreased MABP less than 10% at the highest tested dose of 18 mg/kg iv (Table III). All tested compounds lowered HR (Table I). Azimilide decreased HR a maximum of 28% at the highest (18 mg/kg iv) tested dose (Table III).

In three rats dosed orally with 100 mg/kg azimilide one h before ligation and reperfusion, no VF occurred compared with VF in three of three rats given vehicle (water) orally (Table III). Extrasystoles and VT occurred in both vehicle-treated and azimilide-treated rats.

Guinea Pig Models. Ouabain induced arrhythmias in isolated guinea pig hearts as evidenced by both the percent incidence of VES, VT, and VF, and in the decreased time of onset of a given arrhythmia with increasing ouabain concentration (Table IV). Since tested antiarrhythmic drugs could potentially produce either a pro- or antiarrhythmic effect during perfusion with ouabain, evaluation of such drugs was done with a 1 μ*M* ouabain infusion. In 13 vehicle-treated control hearts, 1 μ*M* ouabain caused VES in 100%, VT in

Table IV. Effects of Ouabain on Arrhythmia Onset and Incidence in the Isolated Perfused Guinea Pig Heart

Ouabain concentration (μ*M*)	*n*	Onset time (mean ± SEM, min)			Percent incidence		
		VES	VT	VF	VES	VT	VF
0.3	3	4.2	4.6	>30	33	33	0
1	13	10.0 ± 1.1	11.6 ± 1.7	20.6 ± 3.7	100	69	23
3	3	5.0 ± 0.4	6.8 ± 1.5	11.1 ± 0.8	100	100	100
10	3	2.4 ± 0.5	2.9 ± 0.6	5.7 ± 0.6	100	100	100

Note. After 60 min equilibration, the fluid bathing the heart was changed to Krebs-Henseleit containing the indicated concentration of ouabain octahydrate.

Table V. Effects of Propranolol and Class III Antiarrhythmic Drugs on Ouabain-Induced Arrhythmias in the Isolated Perfused Guinea Pig Heart

Treatment	Conc (μM)	*n*	VES onset time (mean ± SEM, min)	Percentage incidence	
				VT	VF
Control		13	10.0 ± 1.1	69	23
Propranolol HCl	1	3	21.4 ± 2.5	67	0
	10	3	18.2 ± 5.1	33	0
Clofilium PO_4	0.3	3	11.0 ± 1.7	100	0
	10	3	5.2 ± 1.2	100	0
dl-Sotalol HCl	10	3	3.4 ± 1.8[a]	100	33
d-Sotalol HCl	10	4	10.9 ± 3.9	100	0
Sematilide HCl	1	3	15.2 ± 2.4	100	33
	10	4	11.0 ± 2.4	100	0
Dofetilide	0.3	3	13.8 ± 1.4	100	33
	10	3	13.0 ± 3.0	100	33
E-4031	0.3	4	6.5 ± 1.6	100	0
	10	4	3.2 ± 0.6[a]	75	0
Azimilide 2HCl	1	3	9.1 ± 1.5	100	67
	3	4	11.0 ± 2.0	75	0
	10	4	12.2 ± 0.5	0[b]	0

Note. Conc, concentration; n, number of hearts; VES, ventricular extrasystoles; VT, ventricular tachyarrhythmia; VF, ventricular fibrillation.
[a] Significantly different from control onset time ($P < 0.05$, pooled *t* test).
[b] Significantly different from control incidence ($P < 0.05$, chi-square test).

69%, and VF in 23%. Mean onset times were 10.0 min for VES, 11.6 min for VT, and 20.6 min for VF (Table IV).

Effects of azimilide, propranolol, and selected class III agents are shown in Table V. All hearts showed extrasystoles and the effect on VES is therefore expressed as the mean onset time. For VT and VF, percentage incidence is given. Only two treatments significantly changed VES onset time. *dl*-Sotalol and E-4031 at 10 μM significantly reduced the control onset time of 10.0 min to 3.4 and 3.2 min, respectively. VT was present in all hearts treated with clofilium, *dl*- and *d*-sotalol, sematilide, and dofetilide. The incidence of VT was reduced by 10 μM E-4031, propranolol, and azimilide, but only azimilide completely prevented VT. VF was prevented by clofilium, *d*-Sotalol, E-4031, propranolol, and azimilide.

In 11 anesthetized guinea pigs infused with ouabain octahydrate, the first arrhythmia (VES) occurred at a mean ± SEM ouabain dose of 117 ± 7 μg/kg and VF occurred at 208 ± 8 μg/kg (Table VI). The threshold dose for VES was unchanged by several dose levels of *d*-sotalol, E-4031, sematilide, dofetilide, or propranolol. In contrast the VES onset dose was significantly increased in animals treated with 1 ml/kg *dl*-sotalol or 10 and 30 mg/kg azimilide. Azimilide caused dose-dependent elevation of the amount of ouabain that caused VES.

Propranolol dose-dependently and significantly raised the VF threshold dose of ouabain (Table VI). E-4031, sematilide, and dofetilide at selected doses actually reduced the dose of ouabain required to create VF. Azimilide, *dl*-sotalol, and *d*-sotalol did not significantly change the VF onset dose. However, the few animals tested at the higher *dl*-sotalol dose required over twice the ouabain dose for VF as did control animals.

Discussion

Azimilide's *in vitro* electrophysiological profile of prolonging action potential duration in cardiac tissue would predict activity as an antiarrhythmic agent in animals, if the drug were able to reach the heart in a sustained concentration after enteral or parenteral administration. The antiarrhythmic discovery program that produced azimilide tested this expectation in several standard screening rodent models of ventricular arrhythmias as a prerequisite to further evaluation in arrhythmia models in larger animals.

Azimilide showed slight activity in the mouse chloroform model by the intraperitoneal and no activity by the oral route. There is no data in the mouse on bioavailability of azimilide after administration by these routes, so poor absorption cannot be ruled out as the reason for the poor activity. This seems unlikely, however, because good (60%–100%) oral bioavailabil-

Table VI. Effects of Propranolol and Class III Antiarrhythmic Drugs on Ouabain-Induced Arrhythmias in the Anesthetized Guinea Pig

Treatment	Dose (mg/kg iv)	*n*	Arrhythmia onset dose (mean ± SEM, μg ouabain/kg)	
			VES	VF
Control		11	117 ± 7	208 ± 8
Propranolol HCl	0.03	5	126 ± 7	226 ± 10
	0.3	5	121 ± 10	246 ± 20[a]
	1	5	141 ± 12	358 ± 45[a]
dl-Sotalol HCl	0.1	5	138 ± 12	224 ± 28
	0.3	5	133 ± 12	210 ± 18
	1	2	230 ± 51[a]	>450
d-Sotalol HCl	1	5	105 ± 5	185 ± 14
	3	5	138 ± 7	235 ± 21
	10	5	155 ± 24	247 ± 35
Sematilide HCl	3	5	116 ± 8	165 ± 8[a]
	10	5	131 ± 8	170 ± 16[a]
	30	5	126 ± 10	183 ± 7
Dofetilide	0.3	5	112 ± 5	169 ± 11[a]
	1	5	122 ± 4	163 ± 7[a]
	3	5	106 ± 4	148 ± 12[a]
E-4031	0.3	5	115 ± 12	172 ± 19
	1	5	111 ± 11	141 ± 11[a]
	3	5	110 ± 2	170 ± 9[a]
Azimilide 2HCl	1	5	112 ± 12	196 ± 15
	3	5	110 ± 10	220 ± 22
	10	5	150 ± 10[a]	214 ± 23
	30	5	161 ± 8[a]	211 ± 10

Note. Ouabain octahydrate was used. *n*, number of animals; VES, ventricular extrasystoles; VF, ventricular fibrillation.
[a] Significantly different from control value ($P < 0.05$, pooled *t* test)

ity has been seen in rat, dog, monkey, and humans. Antiarrhythmic activity in rats is shown by data reported here, and a class III action has been seen in dogs after oral administration (28). Limited efficacy of azimilide contrasts with the previously reported efficacy of reference class III antiarrhythmics in the mouse chloroform model. By the ip route the class III agents, bretylium, clofilium, and sotalol were effective at doses of 4, 8, and 4 mg/kg, respectively, in protecting half the mice (11). The relative ineffectiveness of azimilide in this model suggests a different mechanism is at play than that found with these other class III agents. The mouse chloroform model is particularly sensitive to β-blockers (29, 30), which is confirmed by the efficacy of propranolol. The inactivity of azimilide may imply that the compound's β-adrenergic antagonism, suggested by radioligand displacement assay (31) and an *in vitro* test (32), is not pharmacologically relevant here. Neither is cardiodepression a major effect of azimilide, since that action would also be revealed as arrhythmia suppression in the chloroform model (33).

We have previously demonstrated that antiarrhythmic agents of all four Vaughan Williams classes suppress arrhythmias in the CALR rat model when administered by the intraperitoneal route (11). Data reported here are consistent with the broad sensitivity of the model, since class III agents were active by the intravenous route. Azimilide's efficacy in this model was chiefly directed at suppression of VF, although an effect on VES and VT was noted at the highest tested dose. Given the unique configuration of various potassium currents in the rat heart (34, 35) and lack of data showing that azimilide increases refractoriness in the rat heart, azimilide's antiarrhythmic efficacy in this species may be due to something other than inhibition of the delayed rectifier. The importance of the delayed rectifier in adult rat ventricle is small compared with other species, although genes for delayed rectifier K channels are expressed in rat ventricle (34, 36, 37). The transient outward current I_{to} is very important in the rat (38) and the I_{to}-blocker tedisamil has been shown to suppress VT and VF in the rat (39), but azimilide does not inhibit this current (5). Although azimilide, at high *in vitro* concentrations, has been shown to block I_{K1} (3) and I_{K1} has been identified in adult rat ventricle (40), this action is unlikely to be effective in the rat, which was not protected from ventricular arrhythmias by the I_{K1}-blocker UK 66,914 (41). Since an L-type calcium current I_{CaL} contributes to repolarization of the rat ventricular action portential

(42) and azimilide inhibits this current (5), it is reasonable to posit that I_{CaL} block contributes to azimilide's efficacy in the rat CALR model.

Class III agents have a lesser efficacy for VES and VT prevention than for VF prevention in the CALR rat model. The purer class III agents, *d*-sotalol, sematilide, and dofetilide, did not even partially prevent VES or VT even at the highest doses tested. *dl*-Sotalol gave partial suppression at 10 mg/kg iv, which may be a manifestation of its class II properties. The greater efficacy and potency of *dl*-sotalol versus *d*-sotalol suggest the importance of β-adrenergic blockade in the model. It is noteworthy that azimilide was able to partially suppress VES and VT, perhaps reflecting a different mechanistic profile as compared to the pure I_{Kr}-blocking *d*-sotalol, sematilide, dofetilide, and E-4031.

The cause of ouabain-induced arrhythmias is not well defined, but is generally attributed to elevated intracellular calcium consequent to inhibition of Na,K-ATPase, elevated intracellular sodium, and augmented Na-Ca exchange (23). Class I agents, perhaps because of their membrane-stabilizing actions, are effective against ouabain-induced arrhythmias in the guinea pig (11). Some, but not all, β-adrenergic blocking agents also inhibit these arrhythmias, a property that may also involve membrane stabilization (43, 44). Azimilide lacks class I electrophysiological effects, precluding that as the mechanism by which it suppresses ouabain-induced arrhythmias. Although azimilide may have some β-adrenergic antagonist properties (32), they are too weak to account for its activity in this setting. Furthermore, the profiles of azimilide and *dl*-sotalol, whose β-adrenergic blocking potency is significant, differ.

Azimilide demonstrates significant antiarrhythmic activity in several rodent arrhythmia models. Its profile, including limited efficacy in the mouse chloroform model, ability to suppress VES and VT partially in the CALR rat model, and protection in the guinea pig ouabain models, differs from that of other reference class III agents. These differences may be the result of different ion channel blocking properties (I_{Kr}, I_{Ks}, I_{K1}, and I_{CaL} inhibition) as well as other pharmacological actions (at α- and β-adrenergic receptors and at muscarinic receptors).

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