Proarrhythmia of Azimilide and Other Class III Antiarrhythmic Agents in the Adrenergically Stimulated Rabbit (44478)

ROBERT R. BROOKS,[1] ANNE P. DREXLER, ANNE E. MAYNARD, HUSSEIN AL-KHALIDI, AND DAVID R. KOSTREVA
Procter & Gamble Pharmaceuticals, Cincinnati, Ohio 45253–8707

Abstract. **The ventricular proarrhythmic actions of five class III antiarrhythmic agents were compared in the Carlsson rabbit model. In adrenergically stimulated anesthetized rabbits, azimilide, clofilium, dofetilide, sematilide, and *d,l*-sotalol caused premature ventricular contractions and nonsustained and sustained ventricular tachyarrhythmias (NSVT and SVT) at pharmacologically equivalent intravenous doses that increased QTc intervals 20% (ED_{20}). There were no significant differences between agents in the percentage of rabbits with serious arrhyhthmias at the ED_{20} doses of 5.2, 0.033, 0.015, 0.66, and 2.8 mg/kg iv, respectively. Proarrhythmia was dose-dependent. Linear regression analysis of arrhythmia score versus log dose estimated the NSVT doses as 6.2, 0.055, 0.0089, 1.5, and 5.7, respectively. Analysis of arrhythmia states during a 10-min window after infusion when QTc prolongation was 20% showed that the compounds differed significantly in the proportion of time treated rabbits spent in SVT and combined NSVT and SVT. Rabbits treated with azimilide spent significantly less time in SVT and combined NSVT and SVT, followed in order of increasing time by *d,l*-sotalol, sematilide, clofilium, and dofetilide.** [P.S.E.B.M. 2000, Vol 223]

The main cardiac action of azimilide (NE-10064, (E)-1-[[[5-(4-chlorophenyl)-2-furanyl]methylene]-amino]-3-[4-(4-methyl-1-piperazinyl)butyl]-2,4-imidazolidinedione dihydrochloride) is prolongation of refractoriness, demonstrated in cardiac fibers of calf, dog, ferret, guinea pig, sheep, and human (1–3). This increased refractoriness has been ascribed to blockade of the fast (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier potassium current. These *in vitro* electrophysiologic properties could account for azimilide's antiarrhythmic efficacy in several rodent models and a dog model of ischemia-induced ventricular arrhythmia (4, 5). This agent suppressed fibrillation and increased survival in one dog model of sudden cardiac death (6). Clinical efficacy in treating supraventricular arrhythmias has also been reported (7, 8).

Proarrhythmia potential continues to limit the use of antiarrhythmic drugs (9). Class III agents that increase the QT interval can cause excessive prolongation of repolarization, an increased likelihood of early afterdepolarizations, and triggered arrhythmias (10) and may induce torsade de pointes, a potentially life-threatening ventricular arrhythmia (11). For nonclinical assessment of proarrhythmic actions, Carlsson has documented the sensitivity of the adrenergically stimulated rabbit to proarrhythmia induced by class III antiarrhythmic agents (12, 13).

This study compares azimilide and four other class III compounds in the Carlsson rabbit model, with attention to dose and rate of infusion. In addition to the incidence of ventricular arrhythmias, the length of time spent in a given arrhythmic state and the probability of being in malignant arrhythmic states were assessed (14). Preliminary findings have been reported (15).

Materials and Methods

Chemicals. Azimilide dihydrochloride, dofetilide, and sematilide were synthesized. *d,l*-Sotalol hydrochloride (Bristol-Myers Squibb, Wallingford, CT) was a gift, and clofilium tosylate (Research Biochemicals, Inc., Natick,

This study was conducted entirely within Procter & Gamble Pharmaceuticals without outside funding.
[1] To whom requests for reprints should be addressed at Drug Safety Assessment, Procter & Gamble Pharmaceuticals, 11810 E. Miami River Road, Ross, OH 45061. E-mail: brooks.rr@pg.com

Received May 3, 1999. [P.S.E.B.M. 2000, Vol 223]
Accepted September 1, 1999.

0037-9727/00/2232-0183$14.00/0

MA) was purchased. Solutions of these class III antiarrhythmic agents were prepared fresh daily in 0.9% (w/v) saline.

Husbandry. Purpose-bred male and female New Zealand White rabbits from Hazelton (Denver, PA) were fed Purina Rabbit Chow (Purina Mills, Richmond, IN) and water *ad libitum* and housed in a USDA-approved facility. Experiments were approved by the facility's Institutional Animal Care and Use Committee.

Surgical Preparation and Instrumentation. Fasted (3 hr) rabbits (2.4–4.5 kg) were anesthetized with thiopental sodium (47–120 mg/kg iv). Supplements were given as needed to maintain the surgical plane of anesthesia. Rabbits were intubated and mechanically ventilated with oxygen. Arterial blood gases and blood pH were measured (ABL505 blood gas analyzer, Radiometer, Copenhagen, Denmark). The rate and peak inspiratory pressure were adjusted to achieve respiratory $pCO_2 \approx 35$–40 mmHg and blood pH≈7.35–7.55. The femoral arteries and vein were isolated and cannulated for measurement of arterial blood pressure, withdrawal of arterial blood samples, and drug administration, respectively.

During experimentation, electrocardiogram (ECG, lead II and/or other leads), heart rate (HR), mean arterial blood pressure (BP), and body temperature were recorded continuously with Gould Electronics physiologic amplifiers (East Rutherford, NJ) and a computer data acquisition system (MacLabs, Milford, MA). Rabbits with a control BP of at least 50 mmHg and with stable BP and HR (≤5% change over 10 min) were used. At the end of the study, the rabbits were euthanized (e.g., 1 cc Socumb®, iv) without recovery.

ED_{20} Determination and Validation. At least three cumulative doses of antiarrhythmic agent were given to anesthetized (α-chloralose 90–105 mg/kg iv over 20 min) rabbits with 20 min between each 10-min infusion. QTc was derived from the QT interval on the ECG. Least-squares regression analysis of plots of log dose versus percentage QTc change yielded the dose that would prolong QTc 20% at 15–20 min after infusion (ED_{20}).

Proarrhythmia Studies. After α-chloralose anesthesia, rabbits were infused with the α_1 agonist methoxamine (15 μg/kg/min, 2-ml/kg/ hr). Starting 12–13 min into the methoxamine infusion, saline (vehicle control) or a class III drug was given over 10 min. Rabbits were monitored for 60 min after saline or drug infusion. For each animal, the most serious observed arrhythmia was identified.

In separate rabbits the effects of infusion rate were evaluated. The ED_{20} of azimilide was delivered at 0.52, 0.17, and 0.09 mg/kg/min as 10-, 30-, and 60-min infusions, respectively. The ED_{20}s of dofetilide and sotalol were also delivered over 60 min.

Data Collection and Analysis. One-minute means of HR and BP were calculated (MacLabs, Millford, MA). The last 5 one-min means before the first treatment (methoxamine, saline, or class III agent) were averaged and used as the baseline value. QT intervals were measured and corrected for HR changes with Bazett's formula, QTc = QT/$\sqrt{R\text{–}R}$ (16). The QT interval included the U-wave, when present. Five QTc values were averaged for each time point, and five values before the start of infusion of saline or class III agents were used as the baseline QTc value. Group mean and standard error of the percentage change from baseline were calculated for HR, BP, and QTc. QTc data from the log dose-response curve were used to calculate the ED_{20} by least-squares linear regression.

ECG records for the 8 saline-treated and 12 or 13 class III agent-treated rabbits were analyzed for arrhythmias. The most serious ventricular arrhythmia in each animal was identified, and arrhythmia scores were assigned as follows: no arrhythmia (normal ECG) = 0; premature ventricular contractions (PVCs), including single ectopic beats = 1; complexes (couplets, triplets, and bigeminy) = 2; nonsustained ventricular tachyarrhythmia (NSVT) (a run of 4–15 ectopic beats) = 3; sustained ventricular tachyarrhythmia (SVT) (a run of 16 or more ectopic beats, including polymorphic ventricular tachycardia and apparent *torsade de pointes*) = 4; and ventricular fibrillation (VF), usually followed by death = 5. The observation period for assignment of an arrhythmia score varied with the type of analysis (e.g., whole experiment vs. 5- or 10-min intervals). For the period of pharmacologic equivalence (12.5–22.5 min after the completion of the test infusion when QTc was increased ≈ 20% by the ED_{20}), a continuous arrhythmia classification was taken from the ECG data. The arrhythmia and its duration were recorded for the 10-min period.

Statistical Analysis. Possible intergroup differences in baseline BP, HR, QTc, and arrhythmia scores were assessed by one-way analysis of variance and Dunnett's test for comparison with the saline group or either the Student-Newman-Keuls method or Dunn's test based on ranks for pairwise, multiple comparisons among all compound-treated groups. Slopes of regression lines were compared by one-way analysis of variance. The data for most severe arrhythmia were analyzed with a proportional odds model. The continuous arrhythmia classification data were analyzed with the Markov chain model, a statistical procedure designed to determine the probability of existing in different arrhythmic states (14). Proportion of time spent in NSVT and SVT was compared between treatments by a *z*-test.

Results

Effects on QTc. Baseline QTc values ranged from 301 to 331 ms and did not differ significantly between groups. Based on the QT values at 15–20 min after a 10-min infusion of three or more intravenous doses, the dose (mg/kg) that increased QTc 20% was estimated as 0.033 for clofilium, 0.015 for dofetilide, 0.66 for sematilide, 2.8 for sotalol, and 5.2 for azimilide (Table I). In separate rabbits, infusion of the ED_{20} dose of clofilium, sematilide, and *d,l*-sotalol produced a gradual increase in QTc that reached 20% at the end of the 10-min infusion of the ED_{20}. Both azimilide and dofetilide caused an overshoot of QTc pro-

Table I. Potency of Class III Antiarrhythmics for QTc Prolongation and Proarrhythmia in Rabbits

Compound	ED_{20} (mg/kg iv)	NSVT dose (mg/kg iv)
Azimilide	5.2	6.18
Clofilium	0.033	0.055
Dofetilide	0.015	0.0089
Sematilide	0.66	1.47
d,l-Sotalol	2.8	5.70

Note. For ED_{20} (the dose that increased QTc 20%) determination, compounds were administered to three to six α-cloralose-anesthetized rabbits over 10 min with 20 min between doses. QT and RR intervals were measured before dosing and at 10 and 15 min after dosing. Both 10- and 15-min QTc values were used in a linear regression analysis to calculate the ED_{20} (dose causing 20% QTc increase at 15–20 min postinfusion). For determination of the dose that caused nonsustained ventricular tachyarrhythmia (NSVT), the ED_{20} dose, 1/3, and 3 times the ED_{20} doses of compounds were given to 5–13 α-chloralose-anesthetized, methoxamine-infused rabbits, and regression analysis of arrhythmia score on log dose was used.

longation that peaked at an increase of ≈ 35% at the end of the infusion period. The percentage QTc increase returned to ≈ 20% at 10 min post-dose for dofetilide and 15 min post-dose for azimilide (Fig. 1). Methoxamine alone did not affect QTc (data not shown). In the presence of methoxamine, the ED_{20} dose of clofilium increased QTc only 4% (Table II). Buchanan *et al.* also found that methoxamine reduced the QTc prolongation caused by clofilium in rabbits (13).

Hemodynamics. Methoxamine alone increased BP and decreased HR. After 30 min of methoxamine infusion, BP had increased 70% and HR had decreased 30% compared to baseline. Infusion of saline or the ED_{20} of the five class III agents slightly decreased BP (<10%) and HR (15%). (data not shown).

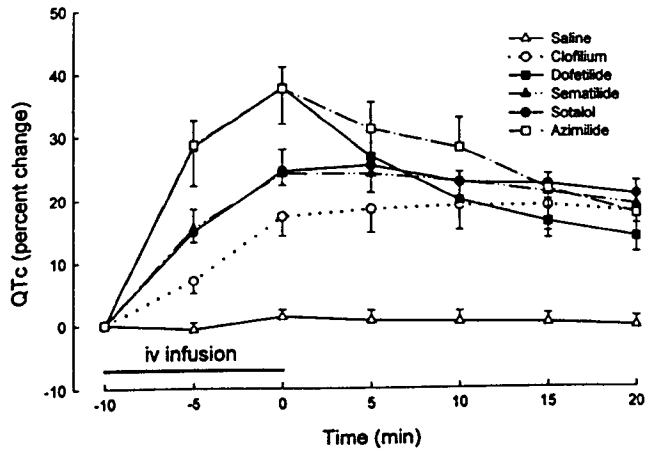

Figure 1. Time-dependent QTc changes during and after infusion of the iv ED_{20} (the dose that increased QTc 20% at 15–20 min after infusion) of class III antiarrhythmic agents in α-chloralose-anesthetized rabbits. Groups of five rabbits received 10-min infusions of saline (10 ml/kg), azimilide (5.2 mg/kg), clofilium (0.033 mg/kg), dofetilide (0.015 mg/kg), sematilide (0.66 mg/kg), or *d,l*-sotalol (2.8 mg/kg). QT values were corrected for heart rate and expressed as mean (SEM) percentage QTc change from the preinfusion baseline.

Proarrhythmia. Each class III agent was tested at three doses (administered as 10-min infusions): the ED_{20}, ≈ 1/3 the ED_{20}, and ≈ 3 times the ED_{20}. Arrhythmias were observed during and after administration of low, medium, and high doses of all tested class III agents (except for the low dose of sotalol) (Fig. 2). A dose-dependent increase in arrhythmia score, based on the whole 10-min infusion and 60-min post-infusion observation period, was observed for all agents (Fig. 3). The score-log dose relationship was linear, and the slopes of the least-squares regression lines were not significantly different between agents. Because the dose-response curves for each compound included the arrhythmia score of 3, corresponding to induction of NSVT, potency comparisons between the five agents were made based on an estimate of the dose that produced an arrhythmia score of 3. The NSVT doses were approximately twice the ED_{20} values for clofilium, sematilide, and sotalol and were similar to the ED_{20} values for dofetilide and azimilide (Table I).

When the worst arrhythmia was the basis of comparison (regardless of time or duration of the arrhythmia), no significant differences between treatments were noted (Table II). At 1/3 the ED_{20}, however, only dofetilide caused SVT and VF (one of five rabbits). At the ED_{20}, SVT occurred with all agents; dofetilide caused VF in 1 of 12 rabbits. At three times the ED_{20}, VF occurred with all agents at a 20%–60% incidence.

Of the 118 rabbits used to determine the dose response for proarrhythmia, 29 exhibited *torsade de pointes* (i.e., QRS complexes that twisted about the isoelectric axis). This arrhythmia occurred in 17% (4/23) of rabbits treated with clofilium or *d,l*-sotalol, 22% (5/23) of those treated with azimilide, 32% (7/22) of those treated with sematilide, and 41% (9/22) of those treated with dofetilide.

Arrhythmias appeared during the infusion of all agents. For the ED_{20} dose, the arrhythmia experience was similar for clofilium, sotalol, and sematilide (5 of 12 rabbits exhibited PVCs, complexes, NSVT, and SVT). For azimilide 9 of 13 exhibited the above arrhythmias. For dofetilide the arrhthmia experience was worse—11 of 12 rabbits exhibited the above arrhythmias and one exhibited VF. Both azimilide and dofetilide caused an overshoot of the QTc increase, which then fell back to the target level (20% at the ED_{20}). To better approximate a more clinically relevant situation in which an oral dose of a class III agent might avoid any overshoot of QTc prolongation, arrhythmias were considered only during a window of pharmacologic equivalence in which the ED_{20} dose actually increased QTc 20% (i.e., the 10-min period from 12.5–22.5 min after infusion). The worst arrhythmia was then determined in this period. Saline caused no arrhythmias. At the ED_{20}, all agents produced a similar incidence of SVT (15%–25%) (Table III). The incidence of the worst arrhythmias did not differ significantly between the tested compounds.

During the window of pharmacologic equivalence, arrhythmia duration was determined. Rabbits spent most of

Table II. Arrhythmias Caused by Class III Antiarrhythmic Agents in Rabbits

Treatment	Dose (mg/kg iv)	*n*	QTc (percent change)	Arrhythmia incidence (percent of rabbits)						Arrhythmia score
				None	PVCs	Complexes	NSVT	SVT	VF	
Saline	10 ml/kg	8	1 ± 2	100	0	0	0	0	0	0 ± 0
d,l-Sotalol	0.89	5	12 ± 5	100	0	0	0	0	0	0 ± 0
	2.8	12	21 ± 4[a]	33	17	25	0	25	0	1.7 ± 0.5[d]
	8.9	6	17 ± 3[a]	0	0	17	0	67	17	3.8 ± 0.4
Clofilium	0.01	6	5 ± 2	50	17	33	0	0	0	0.8 ± 0.4
	0.033	12	4 ± 1[b]	25	0	25	25	25	0	2.3 ± 0.4[d]
	0.1	5	28 ± 7[a]	0	20	0	0	40	40	3.8 ± 0.7[c]
Sematilide	0.21	5	7 ± 2	60	0	20	20	0	0	1.0 ± 0.6
	0.66	12	20 ± 4[a]	33	8	17	0	42	0	2.1 ± 0.5[d]
	2.1	5	21 ± 6	20	0	0	0	60	20	3.4 ± 0.9
Azimilide	1.6	5	9 ± 2	40	60	0	0	0	0	0.6 ± 0.2
	5.2	13	21 ± 3[a]	8	8	23	15	46	0	2.8 ± 0.4[c]
	16	5	24 ± 9[a]	0	0	0	0	40	60	4.6 ± 0.2[c]
Dofetilide	0.0047	5	20 ± 6[a]	20	20	20	20	0	20	2.2 ± 0.9[c]
	0.015	12	18 ± 4[a]	0	0	8	8	75	8	3.8 ± 0.2[c]
	0.047	5	15 ± 5	0	0	0	0	40	60	4.6 ± 0.2[c]

Note. α-Chloralose-anesthetized rabbits received a continuous infusion of 15μg/kg/min methoxamine, followed at 12–13 min by saline or the class III antiarrhythmic agent. The most severe arrhythmia was identified whenever it occurred during the 10-min infusion and for 60 min thereafter and used to calculate the arrhythmia score (0 for no arrhythmia (normal ECG); 1 for PVCs, including single ectopic beats; 2 for complexes (couplets, triplets, and bigeminy); 3 for NSVT (a run of 4–15 ectopic beats); 4 for SVT (a run of 16 ectopic beats or more, including polymorphic ventricular tachycardia and apparent *torsade de pointes*); and 5 for VF, usually followed by death). The mean (± SEM) QTc percentage change is from the preinfusion baseline.

[a] Significantly different from saline group ($p < 0.05$, Dunnett's test).

[b] Significantly different from other ED_{20}-dosed (middle dose) groups ($p < 0.05$, Student-Newman-Keuls test).

[c] Significantly different from the saline group ($p < 0.05$, Dunn rank comparison).

[d] Significantly different from dofetilide score at ED_{20} dose.

the time in normal sinus rhythm: clofilium 49%, azimilide 70%, sotalol 71%, dofetilide 77%, and sematilide 84%. The number of seconds spent in arrhythmic states varied between agents, particularly for the serious SVT state (Fig. 4). The percentage of time spent in SVT varied from 0.1% with azimilide to 11.8% with dofetilide, and each agent's time in SVT (and combined NSVT + SVT) was significantly different from all other agent's time (*z*-test) (Table III). A Markov chain model analysis of these window data allowed calculation of the probability of occurrence of any arrhythmic state. The probability of being in SVT varied from 0.001 with azimilide to 0.142 with dofetilide (Table III). The rank order of time in and probability of SVT and combined NSVT and SVT was the same: lowest with azimilide, followed by *d,l*-sotalol, sematilide, clofilium, and dofetilide.

Because azimilide and dofetilide caused a QTc increase greater than 20% during the infusion of the ED_{20}, the effects of slower rates of administration were investigated. When azimilide 5.2 mg/kg was infused over 10, 30, and 60 min (infusion rates of 0.52, 0.17, and 0.09 mg/kg/min, respectively), rabbits had an ≈ 20% increase in QTc during the 1-hr observation period after infusion (Fig. 5). The fast (10-min) infusion rate produced a peak in both the QTc percentage increase (35%) and the arrhythmia score (Fig. 5). For dofetilide and *d,l*-sotalol, infusion of the ED_{20} over 60 min failed to produce a 20% QTc increase (data not shown); maximum QTc increase was about 13%. No proarrhythmia analysis was performed for these agents delivered at this infusion rate.

Discussion

The five tested class III antiarrhythmic agents increased QTc in rabbits at intravenous doses similar to those reported in dogs and rabbits (5, 12, 13). Because different potency estimates may arise due to species, method of administration, and the time at which a QT measurement is taken after infusion, it is prudent to determine a pharmacologically equivalent dose in the system under study.

Proarrhythmia is a function of dose and delivery rate in this rabbit model (12). In this study, the five class III agents induced arrhythmias as a function of dose over the same range of approximately one-third to three times the ED_{20}. Although all agents caused VF at the highest dose, only dofetilide-treated rabbits experienced VF at the ED_{20} and at the lowest dose. Because clofilium at the ED_{20} dose did not increase QTc 20% in the presence of methoxamine, its proarrhythmic potency may be understated. Nevertheless, based on occurrence of the worst arrhythmia at any time during or for 60 min after infusion, the proarrhythmia induced by azimilide was not significantly different from that caused by the four other class III compounds.

Carlsson *et al.* (17) found that infusion rate can be a contributing factor in the proarrhythmic potential of almokalant. Slowing the infusion rate substantially reduced the incidence of *torsade de pointes* in this rabbit model. Slower administration may limit the dispersion of refractoriness, early after-depolarizations, and arrhythmia initiation. With azimilide both the overshoot of QTc prolongation and the

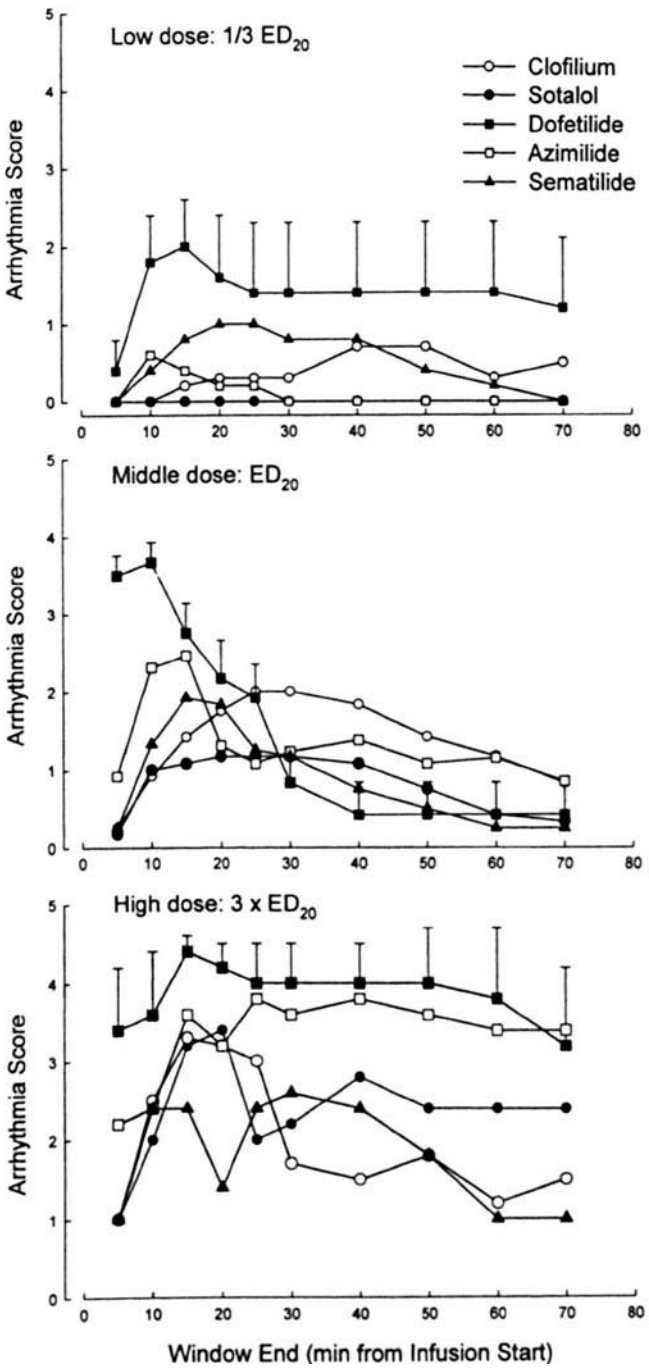

Figure 2. Time-dependent proarrhythmic effects in α-chloralose-anesthetized methoxamine-stimulated rabbits. Class III agents were infused at the dose that increased QTc 20% = ED_{20}, at ≈ 1/3, and 3 times the ED_{20} over 10 min starting 12–13 min into a methoxamine infusion. The worst arrhythmia seen during each 5- or 10-min window produced an arrhythmia score (none = 0; PVCs, including single ectopic beats = 1; complexes (couplets, triplets, and bigeminy) = 2; NSVT (4–15 ectopic beats) = 3; SVT (>15 ectopic beats, including polymorphic ventricular tachycardia and apparent *torsade de pointes*) = 4; VF, usually followed by death = (5). Group means (n = 5–13) are plotted with standard error bars for dofetilide.

peak in arrhythmia score seen at a high infusion rate of 0.52 mg/kg/min were avoided at the slower rate of 0.09 mg/kg/min.

By monitoring arrhythmic status over time and assessing the duration of arrhythmias, it is possible to evaluate proarrhythmic effects beyond a simple enumeration of the proportion of animals with a given arrhythmia. Comparison of seconds spent in SVT and combined NSVT + SVT (during the 10-min window in which all compounds increased QTc ≈ 20%) showed that azimilide-treated rabbits spent significantly less time in such states. Markov chain analysis is a useful statistical model to determine a sequence of events that involves continuous risk, the time to event, and differing severity of events occurring more than once in a random fashion (14). An analysis based on a finite-state, continuous-time model may assess the risk of transitioning from one arrhythmia condition (including baseline) to another. The approach assumes that a subject is always in one of a finite number of discrete cardiac rhythm states, called Markov states. All new occurrences of or increased frequency in an arrhythmic event (i.e., proarrhythmia) can be represented as transitions from one state to another. The Markov model is known to demonstrate both the probability of repetitive events and its time dependence, allowing for more accurate representation of the rhythmic state involving higher risks of occurrences of arrhythmias.

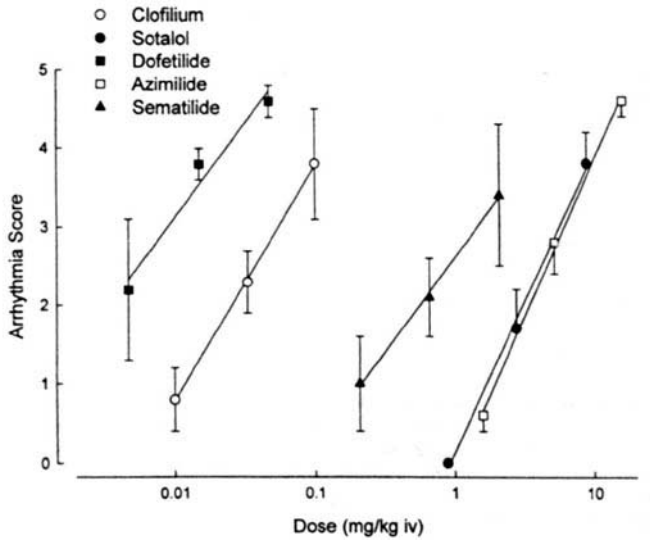

Figure 3. Dose-dependent proarrhythmic effects of class III antiarrhythmic agents in anesthetized methoxamine-stimulated rabbits. Compounds were infused over 10 min starting 12–13 min into the methoxamine infusion to 5–13 rabbits. Arrhythmia scores (mean ± SEM) were assigned based on the worst arrhythmia that occurred from the start of infusion until 60 min after: none = 0; PVCs, including single ectopic beats = 1; complexes (couplets, triplets, and bigeminy) = 2; NSVT (4–15 ectopic beats) = 3; SVT (16 or more ectopic beats, including polymorphic ventricular tachycardia and apparent *torsade de pointes*) = 4; VF, usually followed by death = 5. Regression lines are fitted to the mean score data.

In comparing treatments by the Markov analysis, it would be ideal if all animals had the same history of arrhythmic events prior to the Markov window. Preferably the rabbits should have experienced no prior arrhythmias, thus avoiding any effects of arrhythmia, such as calcium overload, that might influence susceptibility to arrhythmias during the analysis window. It was not possible to achieve this ideal for rabbits given the ED_{20} doses of the class III agents

Table III. Proarrhythmic Actions of Intravenous ED_{20} of Class III Antiarrhythmic Agents During the Period of Pharmacological Equivalence in Anesthetized Rabbits

	Saline	Azimilide	Clofilium	Dofetilide	Sematilide	*d,l*-Sotalol
Dose (mg/kg iv)	10 ml/kg	5.2	0.033	0.015	0.66	2.8
No arrhythmia (%)	100	38	33	42	58	42
PVCs (%)	0	15	0	17	0	17
Complexes (%)	0	23	25	25	17	25
NSVT (%)	0	8	17	0	8	0
SVT (%)	0	15	25	17	17	17
Time in NSVT + SVT (%)	0	1.0[a]	8.9[a]	11.8[a]	4.7[a]	3.8[a]
Time in SVT (%)	0	0.1[a]	5.0[a]	11.8[a]	3.9[a]	1.5[a]
Probability of NSVT + SVT	0	0.01	0.09	0.142	0.046	0.038
Probability of SVT	0	0.001	0.051	0.142	0.039	0.015

Note. Rabbits anesthetized with α-chloralose received a continuous infusion of 15 μg/kg/min methoxamine. Saline or the class III antiarrhythmic agent was administered at the ED_{20} (the dose that increased QTc 20%) 12–13 min after the start of methoxamine. The worst arrhythmia was identified during the pharmacologically equivalent 12.5–22.5-min postinfusion window when QTc was increased approximately 20%. PVC: premature ventricular contractions; NSVT: nonsustained ventricular tachyarrhythmia; SVT: sustained ventricular tachyarrhythmia, including *torsade de pointes.*

[a] Significantly different from all other treatment groups ($p < 0.01$, *z*-test).

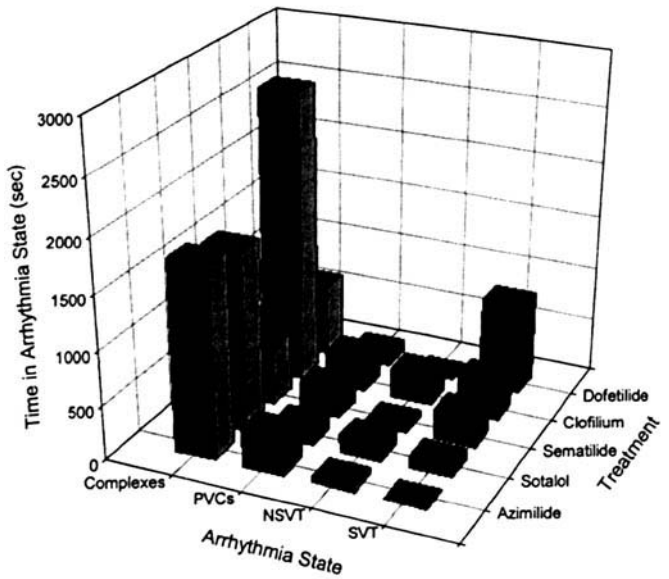

Figure 4. Arrhythmia durations during a 10-min window of pharmacological equivalent effect in rabbits treated with class III antiarrhythmic agents. ED_{20} doses of compounds were infused over 10 min to anesthetized rabbits receiving a continuous methoxamine infusion. Between 12.5 and 22.5 min after infusion, arrhythmias were timed. The number of seconds spent in four arrhythmic states for 12 rabbits (normalized from 13 for azimilide) is displayed in order of increased duration of SVT.

since each agent caused arrhythmias during intravenous administration (Fig. 2, center panel). Nevertheless, between-treatment comparisons seemed reasonable since all the compounds caused early arrhythmias. By this analysis rabbits treated with dofetilide were 10 times more likely to be in combined NSVT and SVT than those treated with azimilide. The ranking by these criteria of azimilide first among the five class III agents, even though azimilide caused more arrhythmias than clofilium, sematilide, and sotalol in the infusion period, suggests that the arrhythmias experienced before the Markov window neither suppressed nor augmented the arrhythmias seen during the Markov window.

The basis for apparent differences between azimilide and dofetilide may be related to different ion-channel effects. Whereas dofetilide blocks I_{Kr}, azimilide blocks both I_{Kr} and I_{Ks} and, at higher concentrations, I_{CaL}. Combined inhibition of potassium and calcium currents has been invoked to explain a lesser proarrhythmic activity of BR-32872 as compared with clofilium, dofetilide, E-4031, and RP-58866 (18). Therefore, if multiple-channel blockade results in less proarrhythmia, the rabbit may have limited value in assessing the relative proarrhythmia of an I_{Ks}-blocking class III drug because the contribution of I_{Ks} to repolarization in this species is less than that in other species (19).

The current study shows that azimilide, like other class III antiarrhythmic agents, can cause serious ventricular arrhythmias in the adrenergically stimulated rabbit model. By conventional analysis of proarrhythmia data, azimilide appears similar to other class III agents, including dofetilide and sotalol, in causing dose-dependent proarrhythmia. However, analysis of time spent in and probability of severe arrhythmic states with pharmacologically equivalent doses suggests that azimilide has a lesser proarrhythmic action than clofilium, dofetilide, sematilide, and *d,l*-sotalol. Whether this difference will be manifested in a clinical setting will require much greater clinical experience with azimilide. Since this model involves animals sensitized to the proarrhythmic effects of class III agents, arrhythmia incidence is greater than that seen in the clinic. For example, Soyka *et al.* (20) reported a proarrhythmia incidence of 4.3% in 1288 patients treated with therapeutic doses (2.1–8.5 mg/kg) of sotalol, whereas the 2.8 mg/kg caused complexes or SVT in 50% of rabbits. Additional clinical experience with newer class III agents will be needed to determine if the same relationship between high rabbit proarrhythmia and low clinical proarrhythmia holds for different agents.

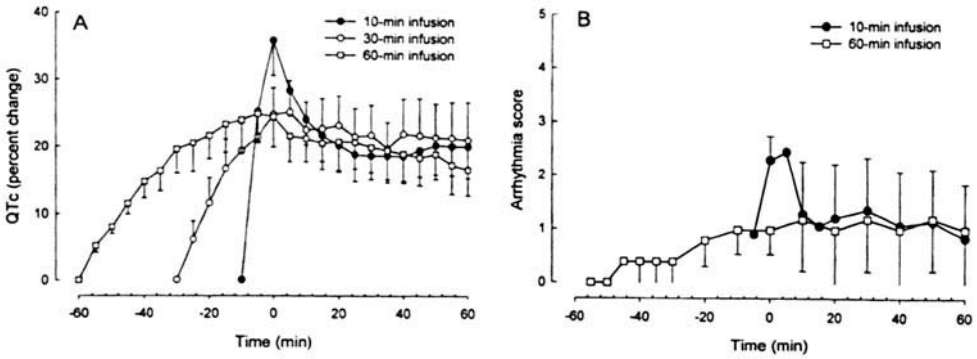

Figure 5. Effect of rate of infusion of azimilide 5.2 mg/kg on QTc and proarrhythmia in anesthetized rabbits. (A) Azimilide was infused over 10, 30, and 60 min to separate groups of five or six rabbits with mean baseline QTc values of 267, 320, and 300 ms, respectively. Data are expressed as mean (SEM) percentage QTc change from baseline. (B) Azimilide was infused over 10 and 60 min to groups of 13 and 6 rabbits, respectively. Arrhythmia scores are expressed as mean (SEM) and were assigned based on the worst arrhythmia that occurred in the preceding 5-min interval: none = 0; premature ventricular contractions, including single ectopic beats = 1; complexes (couplets, triplets, and bigeminy) = 2; nonsustained ventricular tachyarrhythmia (a run of 4–15 ectopic beats) = 3; sustained ventricular tachyarrhythmia (a run of 16 or more ectopic beats, including polymorphic ventricular tachycardia and apparent *torsade de pointes*) = 4; ventricular fibrillation, usually followed by death = 5.

The authors thank Dr. Richard Jorgensen and Nathan Johnson for technical assistance, Dr. Jose Brum for discussions, and Priscilla Davis for editorial assistance.

1. Mcintosh MA, Tanira M, Pacini D, Kane KA. Comparison of the cardiac electrophysiologic effects of NE-10064 with sotalol and E-4031 and their modification by simulated ischaemia. J Cardiovasc Pharmacol **23**:653–657, 1994.
2. Fermini B, Jurkiewicz NK, Jow B, Guinosso PJ Jr., Baskin EP, Lynch JJ Jr., Salata JJ. Use-dependent effects of the class III antiarrhythmic agent NE-10064 (azimilide) on cardiac repolarization: Block of delayed rectifier potassium and L-type calcium currents. J Cardiovasc Pharmacol **26**:259–271, 1995.
3. Salata JJ, Brooks RR. Pharmacology of azimilide dihydrochloride (NE-10064). Cardiovasc Drug Rev **15**:137–156, 1997.
4. Brooks RR, Carpenter JF, Miller KE, Maynard AE. Efficacy of the class III antiarrhythmic agent azimilide in rodent models of ventricular arrhythmia. Proc Soc Exp Biol Med **212**:84–93, 1996.
5. Drexler AP, Micklas JM, Brooks RR. Suppression of inducible ventricular arrhythmias by intravenous azimilide in dogs with previous myocardial infarction. J Cardiovasc Pharmacol **28**:848–855, 1996.
6. Black SC, Butterfield JL, Lucchesi BR. Protection against programmed electrical stimulation-induced ventricular tachyarrhythmia and sudden cardiac death by NE-10064, a class III antiarrhythmic drug. J Cardiovasc Pharmacol **22**:810–818, 1993.
7. Pritchett E, Page R, Connolly S, Marcello S, Schnell D, Wilkinson W, ASAP Investigators. Azimilide treatment of atrial fibrillation. (abstract) Circulation **17**:I-633, 1998.
8. Page RL, Connolly SJ, Marcello SR, Schnell DJ, Wilkinson WE, Pritchett ELC, ASAP Investigators. Dose response for azimilide treatment of paroxysmal supraventricular tachycardia. (abstract) Pacing Clin Electrophysiol **44**:770, 1999.
9. Stanton MS, Prystowsky EN, Fineberg NS, Miles WM, Zipes DP, Heger JJ. Arrhythmogenic effects of antiarrhythmic drugs: A study of 506 patients treated for ventricular tachycardia or fibrillation. J Am Coll Cardiol **14**:209–215, 1989.
10. Roden DM. Early after-depolarizations and *torsade de pointes*: Implications for the control of cardiac arrhythmias by prolonging repolarization. Eur Heart J **14**:56–61, 1993.
11. Yang T, Roden DM. Extracellular potassium modulation of drug block of I_{Kr}: Implications for *torsade de pointes* and reverse use-dependence. Circulation **93**:407–411, 1996.
12. Carlsson L, Almgren O, Duker G. QTU-prolongation and *torsades de pointes* induced by putative class III antiarrhythmic agents in the rabbit: Etiology and interventions. J Cardiovasc Pharmacol **16**:276–285, 1990.
13. Buchanan LV, Kabell G, Brunden MN, Gibson JK. Comparative assessment of ibutilide, *d*-sotalol, clofilium, E-4031, and UK-68,798 in a rabbit model of proarrhythmia. J Cardiovasc Pharmacol **22**:540–549, 1993.
14. Al-Khalidi H, Schnell DJ. Application of a continuous-time Markov chain to a preclinical study. Drug Inf J **31**:607–613, 1997.
15. Al-Khalidi H, Drexler AP, Kostreva DR, Brum JM. Proarrhythmic effects of the class III antiarrhythmic drugs using continuous-time Markov chain model. (abstract) Pacing Clin Electrophysiol **20**:1233, 1997.
16. Bazett HC. An analysis of the time-relations of electrocardiograms. Heart J **7**:353–370, 1920.
17. Carlsson L, Abrahamsson C, Andersson B, Duker G, Schiller-Linhardt G. Proarrhythmic effects of the class III agent almokalant: Importance of infusion rate, QT dispersion, and early after-depolarisations. Cardiovasc Res **27**:2186–2193, 1993.
18. Bril A, Gout B, Bonhomme M, Landais L, Faivre JF, Linee P, Poyser RH, Ruffolo RR. Combined potassium and calcium channel blocking activities as a basis for antiarrhythmic efficacy with low proarrhythmic risk: Experimental profile of BRL-32872. J Pharmacol Exp Ther **276**:637–646, 1996.
19. Salata JJ, Jurkiewicz NK, Jow B, Folander K, Guinosso PJ Jr., Raynor B, Swanson R, Fermini B. I_K of rabbit ventricle is composed of two currents: Evidence for I_{Ks}. Am J Physiol **271**:H2477–H2489, 1996.
20. Soyka LF, Wirtz C, Spangenberg RB. Clinical safety profile of sotalol in patients with arrhythmias. Am J Cardiol **65**:74A–81A, 1990.