

ANTIARRHYTHMIC AND ELECTROPHYSIOLOGIC ACTIONS OF CLOFILIUM IN EXPERIMENTAL CANINE MODELS¹

GREGORY A. KOPIA^{2,3}, BRIAN T. ELLER, EUGENE PATTERSON, MICHAEL J. SHEA² and BENEDICT R. LUCCHESI⁴

Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan 48109, U.S.A.

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Clofilium was studied in three experimental models. In non-ischemic and chronically infarcted canine hearts, clofilium (0.5–2 mg/kg) produced a dose-dependent increase in electrical ventricular fibrillation threshold (VFT), but prolonged the effective refractory period (ERP) of normal myocardium in only the non-ischemic heart. When chronically infarcted hearts were subjected to programmed electrical stimulation, 1 mg/kg of clofilium inhibited the re-induction of either ventricular tachycardia or ventricular fibrillation in 5 of 6 animals and slowed the rate of the induced tachycardia in the sixth. Clofilium, however, failed to alter ventricular refractory periods of normal myocardium at either twice diastolic threshold current (176 ± 5 ms control vs. 187 ± 9 ms post-clofilium, $P > 0.05$) or at 10 mA (134 ± 6 ms control vs. 137 ± 13 ms post-clofilium, $P > 0.05$). In addition, chronic administration of clofilium (2 mg/kg, i.v., followed by 1 mg/kg every 12 h) was ineffective in decreasing mortality in a canine model of sudden coronary death. Of 10 saline-treated conscious animals subjected to an electrically-induced intimal lesion of the left circumflex coronary artery in the presence of a previous ischemic insult, all 10 died suddenly of ventricular fibrillation within 173 ± 45 min after current application. Under similar conditions, 7 clofilium-treated animals died suddenly within 249 ± 88 min ($P > 0.05$) after current application while 3 animals survived ($P > 0.10$). Clofilium did, however, elevate the effective refractory period in these animals (150 ± 3 ms saline-treated vs. 195 ± 7 ms clofilium-treated). It is concluded from our data that there is little relationship between clofilium's electrophysiologic actions in normal myocardium and antiarrhythmic effects. Furthermore, simple prolongation of refractoriness in normal non-ischemic myocardium may be insufficient for the prevention of ventricular fibrillation which develops in response to a transient ischemic event superimposed on a chronically injured myocardium.

Clofilium Ventricular fibrillation Ventricular tachycardia Sudden coronary death

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³ Current address: Smith, Kline and French Laboratories, 1500 Spring Garden Street, Philadelphia, PA 19101, U.S.A.

⁴ To whom all correspondence should be addressed: Department of Pharmacology, M6322 Medical Science Building I, The University of Michigan Medical School, Ann Arbor, Michigan 48109, U.S.A.

1. Introduction

There has been recent interest in the development and use of pharmacologic agents for the prevention of sudden coronary death. The β -adrenergic receptor antagonists timolol (Norwegian Multicenter Study Group, 1981), metoprolol (Hjalmarson et al., 1981), and propranolol (Beta-Blocker Heart Attack Study Group, 1981) have been reported to reduce the risk of sudden coronary death after myocardial infarction. The quaternary ammonium adrenergic blocking agent bretylium is

effective in preventing ventricular fibrillation in both experimental animals (Bacaner, 1968; Bacaner and Schrienmachers, 1968; Kniffen et al., 1975; Cervoni et al., 1971) and man (Holder et al., 1977; Sanna and Arcidiacono, 1973). Another quaternary ammonium agent, pranolium, has been reported to reduce the incidence of ventricular fibrillation in experimental animals (Eller et al., 1983). Clofilium, a third drug of the quaternary ammonium type with electrophysiologic and potential antifibrillatory actions, has been investigated recently (Steinberg and Molloy, 1979; Steinberg et al., 1981). Additional electrophysiologic studies using this drug are appropriate to correlate clofilium's reported *in vitro* actions and its potential *in vivo* antiarrhythmic and antifibrillatory effects.

In this report we examine the effect of clofilium on: (1) the ventricular fibrillation threshold in an anesthetized, previously infarcted canine preparation; (2) programmed electrical stimulation in anesthetized, previously infarcted dogs; and (3) ventricular fibrillation in a conscious canine model of sudden coronary death.

2. Materials and methods

2.1. Production of myocardial infarction

Male mongrel dogs were anesthetized with intravenous (*i.v.*) pentobarbital (30 mg/kg), intubated, and ventilated with room air. The heart was suspended in a pericardial cradle after exposure by means of a left lateral thoracotomy at the fifth intercostal space. The left anterior descending coronary artery (LAD) was separated from the surrounding tissue at or just below the level of the tip of the left atrial appendage. A critical stenosis was produced by tying a ligature around both the vessel and a 19 g needle and then withdrawing the latter. The vessel was then occluded using a snare formed from a loop of silastic tubing passed through a flared polyethylene tube. After 90 min of occlusion, the snare was released allowing reperfusion of the vessel through the critical stenosis. The pericardium was then loosely tied, the chest incision closed, and the dogs allowed to recover. Appropriate antibiotic therapy was administered during the recovery phase.

2.2. Determination of the ventricular fibrillation threshold (VFT)

Seven normal and seven previously infarcted (4-7 days) male mongrel dogs weighing between 12 and 14 kg were anesthetized with sodium pentobarbital (30 mg/kg *i.v.*), intubated, and ventilated with room air at a rate and tidal volume which maintained normal blood gases and pH. The heart was exposed via a left thoracotomy at the fourth or fifth intercostal space and suspended in a pericardial cradle. An acrylic plaque containing 2 silver-silver chloride electrodes (1 mm diameter, 3 mm apart) were sewn to the surface of the heart in the interventricular septum adjacent to the right ventricular outflow tract for the determination of VFT. A second acrylic plaque electrode was sewn to the heart in an area of tissue perfused by the left circumflex coronary artery. This electrode was used for the determination of the excitation threshold and the Q-EG interval (time in ms from the beginning of the Q-wave to the peak of the ventricular electrogram, *i.e.*, conduction time or CT). A bipolar plunge electrode (insulated stainless steel 25 g wire, 4 mm long, 2 mm apart) was placed high in the left ventricle for determination of the effective refractory period (ERP). For all VFT studies, the ERP was measured at twice diastolic threshold current. In addition, a small bipolar plaque electrode was attached to the left atrial appendage for left atrial pacing. All electrophysiologic measurements were made in normal, non-infarcted tissue regardless of whether or not the animal had previously undergone myocardial infarction. For most experiments, the heart was paced at a constant rate during the measurement of the electrophysiologic parameters.

VFT was measured by delivering electrical current to the heart through the VFT electrode using a Grass S88 stimulator coupled to a Grass CCU-1A constant current unit and an SIU-5 stimulus isolation unit. A Tektronix model 565 oscilloscope with a type 3A8 operational amplifier was used to trigger the ventricular stimulus from the peak of the R-wave of the Lead II ECG. The triggering stimulus was delayed 50 ms after R activation so that the impulse train scanned the T-wave. Current was delivered every 6-8 beats using a 200 ms

train of 4 ms pulses at a frequency of 60 Hz. Each current level was tested 3 times before moving on to the next highest current. An increasing amount of current was given until the animal fibrillated, at which time defibrillation was accomplished within 15-20 s using a Physio-control series 70 DC defibrillator.

Blood pressure via a cannulated carotid artery and the Lead II ECG were monitored continuously on a Grass model 7 polygraph. Drug injections were made through a cannulated external jugular vein.

For the determination of the Q-EG interval, both the Lead II ECG and the ventricular electrogram were displayed at high sweep speed (10 ms/division) on a Tektronix 5111 storage oscilloscope and the time between waveforms measured.

After two control determinations (separated by 1 h) of all parameters each dog received sequential and cumulative doses of 0.5, 1.0 and 2.0 mg/kg of clofilium i.v., each dose being separated by 1 h. One h after drug administration the electrophysiological parameters were measured again prior to administration of the next higher dose. After the last VFT determination, animals were not defibrillated and the experiment was concluded.

2.3. Programmed electrical stimulation

Seven dogs weighing between 12 and 17 kg were infarcted as described above and allowed to recover for 4 to 7 days. On the day of the experiment, each dog was reanesthetized with 30 mg/kg of sodium pentobarbital, intubated, and mechanically ventilated with room air. Cannulae were placed in the left common carotid artery for measurement of blood pressure and in the left external jugular vein for injection of drugs. Blood pressure, Lead II ECG, and heart rate derived from the ECG were monitored continuously throughout the experiment on a Grass model 7 polygraph and the ECG was stored on magnetic tape (Lockheed Store 4D) for later analysis. The chest was opened at the fourth or fifth intercostal space, the heart exposed and suspended in a pericardial cradle. Programmed electrical stimulation was performed in a manner similar to that of Michelson et al. (1980). Briefly, a single plunge wire electrode (0.1 mm

diameter silver wire, Teflon coated except at the tip) was inserted into the left ventricle in the area of the right ventricular outflow tract at, or slightly below, the level of the previous LAD occlusion in normal tissue at the border of the region of infarction. The electrode was connected to the cathode of a Grass constant current unit (CCU-1A) which was in turn connected to the S₂ output of a Grass S88 stimulator through a stimulus isolation unit (Grass SIU-5). The anode of the constant current unit was connected to the rib retractors which provided a contact surface area of 23 cm². A Tektronix model 565 oscilloscope with type 3A8 operational amplifier was used to trigger the ventricular stimulus from the R-wave of the Lead II ECG. Using this system, 1, 2 or 3 premature stimuli (4 ms) could be inserted at timed intervals after the R wave.

Each dog was scanned initially with 3 premature beats at twice diastolic threshold current with R-S₂ intervals ranging from 350 ms to the refractory period and S₂-S₃: S₃-S₄ intervals ranging from 200 to 125 ms. If sustained ventricular tachycardia (VT) or ventricular fibrillation could not be induced, the cathodal electrode was moved down the LAD distribution toward the apex of the heart or laterally across the surface of the left ventricle at the level of the LAD occlusion. Most dogs (4) required testing of only 1 site but no more than 6 sites were required in any dog (1 dog). Once an arrhythmia could be induced in a dog, the strength interval relationship for that site was determined. The current was set at diastolic threshold and the interval was reduced from 300 ms in 10 ms increments until the stimulus failed to result in a propagated beat. Then the current was increased until a propagated response could once again be obtained, after which the interval was decreased in 5 ms increments. Current was increased in 0.01 mA increments from threshold to 0.1 mA, in 0.05 mA increments from 0.1 to 1 mA, and in 0.5 mA increments from 1 to 10 mA. Data are presented as the refractory periods at both twice diastolic threshold current and at 10 mA. In both cases the value represents the longest interval at that particular current that failed to result in a propagated response.

After determining the strength interval relation-

ship, the stimulus current was reset to twice diastolic threshold and the precise parameters for inducing each animal determined. Once the arrhythmia could be induced reproducibly (at least 3 times), the animal was given 1 ml of 0.9% NaCl (saline) for each kg of body weight and allowed to stabilize for 45 min to 1 h. The ability to induce the arrhythmia was then re-tested, after which each animal was given clofilium, 1 mg/kg i.v. (in saline, pH 7.3-7.4), and again allowed to stabilize for 45 min to 1 h. The strength interval relationship was then redetermined and the animals retested for inducibility of the arrhythmia. Some animals were tested further at current intensities greater than twice diastolic threshold and with subsequent doses of clofilium.

At the termination of the experiment, each animal was sacrificed by excision of the heart. Cannulae were then inserted in the left anterior descending coronary artery at the site of the previous occlusion and into the root of the aorta. The hearts were then stained by perfusing, at equal pressure (100 mmHg), Evans blue (0.5% w/v) through the aortic root and triphenyltetrazolium (1% w/v) in 0.01 M potassium phosphate buffer into the LAD. After rinsing with tap water, the hearts were sliced in bread-loaf fashion from apex to base and the amount of infarcted tissue (unstained), the tissue at risk of infarction (stained brick red), and the remaining left ventricular mass (stained blue), were determined gravimetrically.

2.4. Canine model of sudden coronary death

The antifibrillatory action of clofilium was studied in a new, recently described, canine model of sudden coronary death (Patterson et al., 1982). Briefly, myocardial infarction was produced as described above in 20 dogs weighing between 18 and 22 kg. During the surgical procedure, the left circumflex coronary artery was exposed approximately 1 cm from its origin and the 3 mm tip of a 25 g needle which had previously been attached to a length of 30 g silver wire, was inserted into the lumen of the artery. The wire was then secured to the epicardial surface of the heart with 3-0 suture. A bipolar plunge electrode (25 g insulated stainless steel, 4 mm long and 3 mm apart) was inserted

into the interventricular septum in the area of the right ventricular outflow tract, approximately 1 cm above the level of the LAD occlusion in an area bordering the region of infarct. Silver disc electrodes were implanted subcutaneously (s.c.) to approximate locations for recording the Lead II ECG. A cannula was inserted into the left jugular vein. The chest incision was closed and the animals allowed to recover. The ECG of each dog was monitored daily and when sinus rhythm resumed (usually 48-72 h after surgery) animals were randomized to either control or clofilium treatment groups. Control animals received 10 ml saline and clofilium-treated animals received an initial i.v. dose of 2 mg/kg followed by additional doses of 1 mg/kg every 12 h. Programmed electrical stimulation was performed after 24 h of treatment, just prior to the next scheduled dose. Animals were studied while conscious and resting comfortably in a sling. One, two or three premature ventricular stimuli (4 ms pulses at twice diastolic threshold voltage) were introduced via the electrode in the interventricular septum in a manner similar to that described above. The procedure was performed until non-sustained ventricular tachycardia (self-terminating ventricular tachycardia lasting longer than 3 beats) or sustained ventricular tachycardia was produced in triplicate. A rigorous attempt at production of inducible arrhythmias was avoided in order to circumvent the induction of ventricular fibrillation.

After completion of programmed electrical stimulation, the scheduled dose of clofilium (1 mg/kg) or saline (10 ml) was administered. An anodal current of 150 μ A was then applied to the intimal surface of the left circumflex coronary artery by connecting the previously implanted wire to a 9 V battery and a variable potentiometer. The Lead II ECG was recorded via radiotelemetry on a Grass model 7 polygraph. Throughout the duration of intimal stimulation, the animals were ambulatory as all the necessary equipment was secured in the pouch of a nylon mesh vest.

At the time of ventricular fibrillation or the 24 h end point, animals were anesthetized and their hearts were excised. The left circumflex coronary artery was examined for the presence of intimal damage and thrombus formation. Hearts were then

sectioned in bread-loaf fashion from apex to base and incubated at 37°C in 0.5% triphenyltetrazolium in 0.01 M phosphate buffer, pH 7.4. Infarct size was determined gravimetrically and expressed as a percentage of the total left ventricular mass.

The Unit for Laboratory and Animal Medicine of the University of Michigan observed and approved the experimental protocols. Experiments were performed according to the 'Guiding Principles in the Care of Laboratory Animals' as outlined by the American Physiological Society.

2.5. Data analysis

Data are expressed as the means \pm S.E.M. Differences between doses of clofilium in the VFT study were assessed using 2-way ANOVA and Scheffe's test (Dixon and Massey, 1969). In the programmed electrical stimulation study, differences in electrophysiological parameters after clofilium administration were analyzed using the paired t-test. Fisher's exact test was used to determine whether there was a difference in survival between clofilium and saline-treated animals in the sudden death experiments and Student's unpaired t-test was used to determine significant differences between means of saline-treated and clofilium-treated groups of animals. For all tests, the null hypothesis was rejected only if the probability of the calculated statistic was less than 0.05.

3. Results

3.1. Effect of clofilium on electrical ventricular fibrillation threshold

Figures 1 and 2 summarize the effect of 0.5, 1 and 2 mg/kg doses of clofilium on both VFT and the effective refractory period in normal and chronically infarcted canine hearts. In normal, non-infarcted hearts, clofilium produced a maximum and sustained elevation in effective refractory period with the smallest dose (0.5 mg/kg), but failed to increase VFT significantly until after administration of the highest dose (2.0 mg/kg, fig. 1). Conversely, in the chronically infarcted heart, clofilium failed to significantly increase the effective

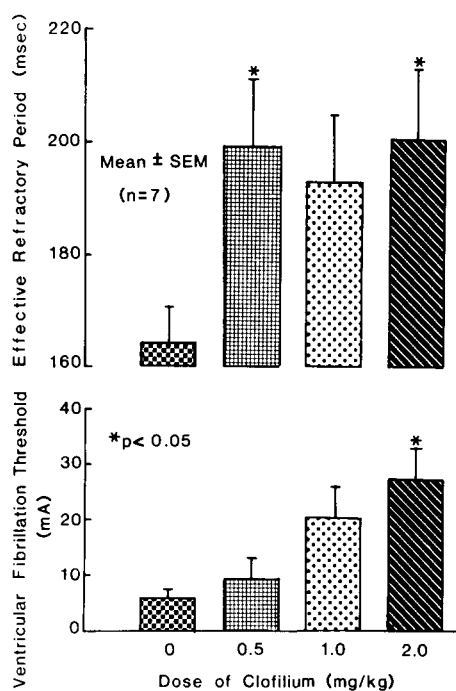


Fig. 1. Antifibrillatory action of clofilium (0, 0.5, 1.0, and 2.0 mg/kg) in the normal non-ischemic canine heart. The upper panel depicts the changes in the effective refractory period (in ms) at twice threshold current after a control determination and after the three doses of clofilium. The bottom panel shows the elevation in electrical VFT produced by clofilium. * Signifies significant difference from control.

refractory period at any dose level while producing a graded increase in VFT which achieved statistical significance only at the 2 mg/kg dose (fig. 2). Control VFT's for both groups were not different (6.4 ± 1.7 mA non-infarcted VFT vs. 9.8 ± 3.2 mA infarcted VFT) nor were the maximum VFT's after the 2 mg/kg dose of clofilium (28.4 ± 6.4 mA non-infarcted VFT vs. $26.2 \pm$ mA infarcted VFT). Figure 3 is an example of a ventricular fibrillation threshold experiment conducted in a dog subject to previous myocardial infarction.

Table 1 summarizes the remaining electrophysiological and cardiovascular actions of clofilium that were measured in this study. With the exception of a modest decrease in heart rate in the non-infarcted animals, clofilium failed to produce any alterations in mean blood pressure, excitation threshold or conduction time.

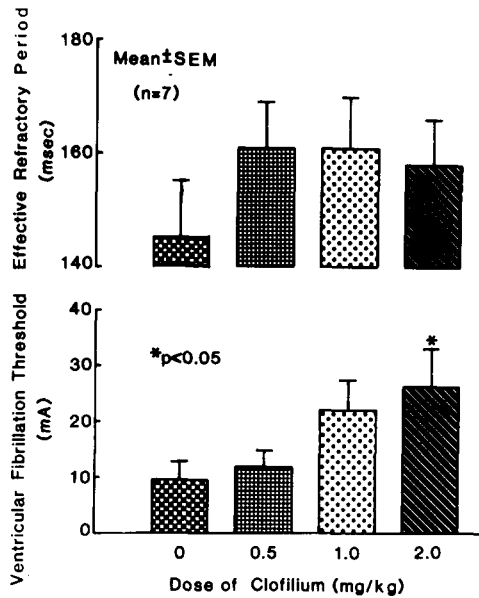


Fig. 2. Antifibrillatory action of clofilium in the chronically infarcted canine heart. Presentation of data similar to that in fig. 1.

3.2. Effect of clofilium in dogs subject to programmed electrical stimulation

Tables 2 and 3 summarize the effect of clofilium in a canine model of programmed electrical stimulation. Of the 7 dogs originally studied, 1 animal fibrillated during determination of the strength interval relationship after clofilium administration

and could not be revived despite vigorous direct heart massage and repeated cardioversion attempts. This animal is not included in the data of tables 2 and 3. Data from the remaining 6 animals, however, show that administration of 1 mg/kg of clofilium was effective in either preventing the induction of ventricular tachycardia by programmed electrical stimulation (5 dogs) or slowing the cycle length of the tachycardia (1 dog, table 2). This dose of clofilium, however, failed to alter excitation threshold or the effective refractory period at either twice diastolic threshold current or at 10 mA (table 3). Only 1 animal showed a substantial increase in the effective refractory period (table 3, dog 1) while 1 animal showed a small decrease in the effective refractory period at twice threshold current and a larger decrease at 10 mA (table 3, animal 6). The remaining animals showed only minor alterations in refractoriness.

After blocking the induced arrhythmia with 1 mg/kg of clofilium, 2 dogs (nos. 2 and 4) were tested further at 5 times diastolic threshold current and were found to be non-inducible even at the increased stimulation current. Two other animals (nos. 1 and 5) were tested at 5 times diastolic threshold current. In animal number 5, 3 premature beats at 5 times diastolic threshold current and with coupling intervals of 170, 125 and 125 ms produced a ventricular tachycardia with a cycle length of 138 ms which rapidly degenerated into

TABLE 1

Electrophysiologic and cardiovascular actions of clofilium in normal and chronically infarcted dogs.

		Dose of clofilium (mg/kg)			
		0	0.5	1	2
Mean blood pressure (mmHg)	N	92 ± 5 ^a	82 ± 6	82 ± 7	76 ± 12
	I	87 ± 6	86 ± 4	80 ± 3	83 ± 3
Heart rate (BPM)	N	158 ± 9	133 ± 14 ^b	121 ± 14 ^b	112 ± 17 ^b
	I	172 ± 8	165 ± 5	154 ± 7 ²	165 ± 5
Excitation threshold (mA)	N	0.097 ± 0.019	0.254 ± 0.129	0.151 ± 0.039	0.302 ± 0.176
	I	0.6 ± 0.1	2.1 ± 1.1	2.0 ± 1.0	1.5 ± 0.4
Conduction time (ms)	N	20 ± 2	19 ± 2	21 ± 2	20 ± 2
	I	31 ± 1	31 ± 1	30 ± 1	31 ± 1

^a Mean ± S.E.M., n = 7 for both normal (N) and chronically infarcted (I) groups. ^b Significantly less than without clofilium; P < 0.05 by 2-way ANOVA and Scheffe's test.

VENTRICULAR FIBRILLATION THRESHOLD AFTER CLOFILIUM

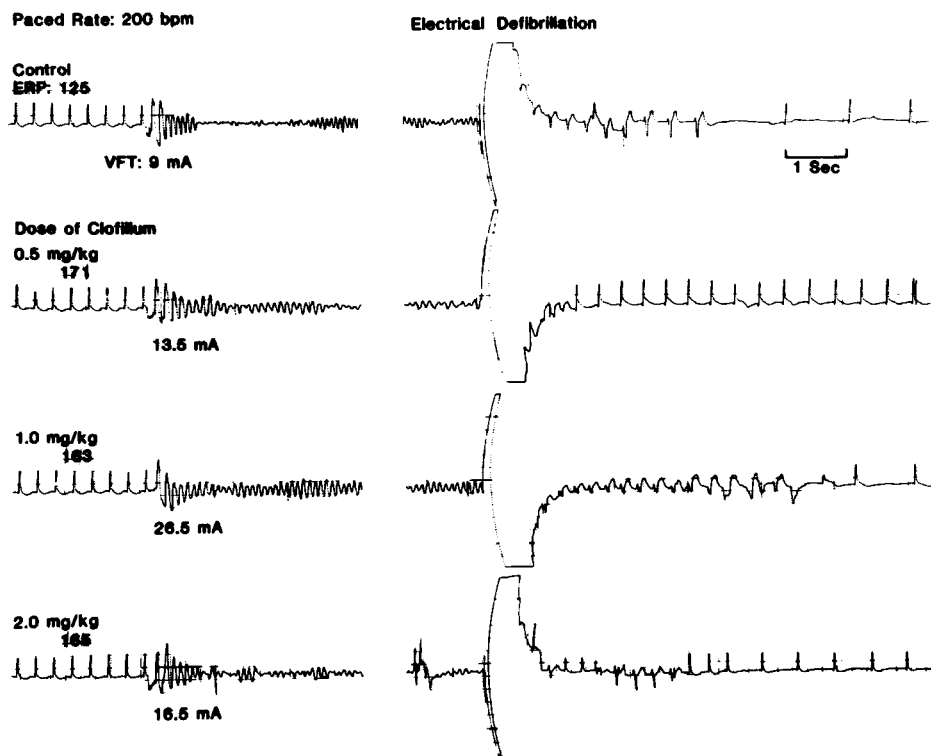


Fig. 3. An example of the antifibrillatory action of clofilium in a chronically infarcted canine heart. In this animal, 1.0 mg/kg of clofilium produced the largest increase in VFT to a value of 26.5 mA while the 0.5 mg/kg dose produced the largest increase in the ERP.

ventricular fibrillation. Further administration of 2 mg/kg of clofilium to this dog failed to prevent induction of the ventricular tachycardia, but increased the cycle length to 153 ms and prevented the degeneration of the ventricular tachycardia to ventricular fibrillation. The remaining animal (no. 1) responded to programmed electrical stimulation at 5 times threshold current and with 3 ventricular stimuli (230, 155, 155 ms) leading to ventricular tachycardia which degenerated into ventricular fibrillation. This dog received no further clofilium. However, prior to clofilium, and at twice threshold current, this animal required only 2 premature stimuli at coupling intervals of 180 and 165 ms. After 1 mg/kg of clofilium even 3 premature stimuli at twice threshold current and at intervals of 230, 140 and 140 ms failed to induce a ventricular tachycardia so that clofilium afforded this animal a considerable degree of protection against

the production of the tachycardia.

Staining of the hearts from all 7 dogs revealed mottled, non-confluent subendocardial infarcts that occasionally extended transmurally. Average

TABLE 2

Antiarrhythmic actions of clofilium in dogs subject to programmed electrical stimulation.

Dog	Pre-clofilium		Clofilium (1 mg/kg)	
	Arrhythmia	Cycle length (ms)	Arrhythmia	Cycle length (ms)
1	V-Tach ^a	200	Non-inducible	—
2	V-Tach Fib ^a	125	Non-inducible	—
3	V-Tach	168	Non-inducible	—
4	V-Tach	158	Non-inducible	—
5	V-Tach Fib	109	Non-inducible	—
6	V-Tach	183	V-Tach	203

^a V-Tach, sustained ventricular tachycardia; Fib, fibrillation.

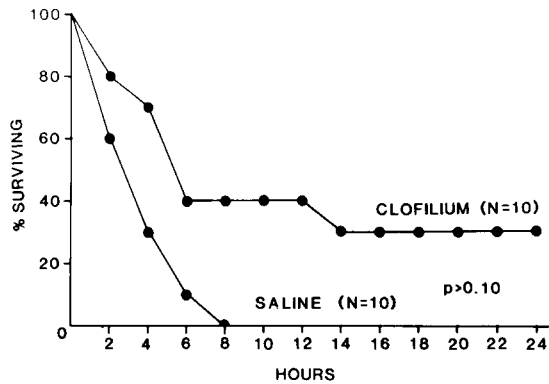


Fig. 4. Effect of clofilium on survival in a canine model of sudden coronary death. Clofilium failed to exert any significant protection against sudden death in this animal model when administered as a 2 mg/kg loading dose followed by 1 mg/kg every 12 h for 24 h. Data were analyzed using Fisher's exact test.

infarct size in these animals was $17.4 \pm 1.9\%$ of the total left ventricle. The single animal that fibrillated and could not be revived had the largest infarct of any animal: 25.8% of the left ventricle.

3.3. Effect of clofilium in a canine model of sudden coronary death

In conscious ambulatory dogs, subjected to acute ischemia at a site remote from a previous infarction, clofilium was ineffective in preventing sudden death due to ventricular fibrillation. Figure 4 depicts the mortality curves and table 4 summarizes the electrophysiologic changes, infarct size,

and thrombus mass data obtained from saline-treated control animals and animals treated chronically with clofilium. Of 10 dogs given 2 mg/kg clofilium followed by 1 mg/kg for 12 h, 7 dogs succumbed to ventricular fibrillation within an average of 249 ± 88 min after application of 150 μ A to the intimal surface of the left circumflex coronary artery. Each of the 10 saline-treated animals fibrillated (173 ± 45 min-post 150 μ A) and the difference between these 2 groups of animals is not significant ($P > 0.10$, fig. 3).

Clofilium did produce a significant prolongation of the effective refractory period (195 ± 7 ms in clofilium-treated vs. 150 ± 3 ms in saline controls, $P < 0.05$). However, this was not associated with a decrease in mortality in the clofilium-treated dogs suggesting that increasing refractoriness alone was not sufficient to prevent ventricular fibrillation in these animals.

Of the remaining parameters measured, clofilium-treated animals appeared slightly less susceptible to the production of sustained and non-sustained ventricular tachycardia, but this was not statistically significant. There were no significant differences between the thrombus mass, infarct size, or the time to electrocardiographic changes noted in table 4 in clofilium-treated and saline-treated dogs. These data suggest that clofilium exerted its principal electrophysiologic effect of prolongation of the effective refractory period, but failed to exert a significant protective antifibrillatory effect.

TABLE 3

Electrophysiological actions of clofilium in dogs subject to programmed electrical stimulation.

Dog	Excitation threshold (mA)		Effective refractory period (ms; twice threshold mA)		Effective refractory period (ms; 10 mA)	
	Pre-clofilium	Post-clofilium	Pre-clofilium	Post-clofilium	Pre-clofilium	Post-clofilium
1	0.22	0.06	171	228	125	195
2	0.05	0.07	176	175	125	115
3	0.06	0.05	169	175	135	130
4	0.06	0.08	181	185	150	145
5	0.06	0.13	160	167	115	115
6	0.11	0.11	198	190	155	120
Mean						
\pm S.E.M.	0.09 ± 0.03	0.08 ± 0.01	176 ± 5	187 ± 9	134 ± 6	137 ± 13

TABLE 4

Action of clofilium in a canine model of sudden coronary death^a.

Parameter		Saline	Clofilium
Effective refractory period (ms)		150 ± 3	195 ± 7 ^b
Programmed electrical stimulation	NI	10%	40%
	NSVT	70%	60%
	SVT	20%	-
Time to (min)	ST change	99 ± 34	184 ± 30
	PVB	111 ± 34	216 ± 41 (n = 8)
	VT	131 ± 37	213 ± 49 (n = 7)
	VF	173 ± 45	249 ± 88 (n = 7)
Infarct size (% Left ventricle)	LAD	19 ± 3	14 ± 3
	LCX	33 (n = 1)	16 ± 4 (n = 3)
LCX thrombus mass (mg)		7.5 ± 2.1	19.1 ± 5.9
Ventricular fibrillation		10/10	7/10

^a NI, non-inducible; NSVT, non-sustained ventricular tachycardia; SVT, sustained ventricular tachycardia; PVB, premature ventricular beats; VT, ventricular tachycardia; VF, ventricular fibrillation; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery. ^b P < 0.05 by non-paired t-test; n = 10 except where otherwise noted.

One surprising and unexpected finding in this study was that clofilium appeared to occasionally produce ventricular arrhythmias. While these were not quantitated, 2 of the 7 anesthetized dogs subjected to programmed electrical stimulation and 6 of the 10 conscious dogs used in the sudden death model responded to clofilium administration with ventricular arrhythmias, characterized by both single and multiple multifocal premature complexes and occasionally short runs of spontaneous non-sustained ventricular tachycardia. These arrhythmias occurred in two dogs anesthetized acutely after administration of clofilium, and also were present in the conscious animals 12 h after clofilium administration.

4. Discussion

Mortality as a result of sudden coronary death represents a major cause of loss of life in the United States today. Estimates are that as many as 199 000 to 386 000 individuals (Doyle, 1979) may die suddenly each year. Because most of these deaths result from ventricular fibrillation secondary to ischemic heart disease (Peter et al., 1980), much experimental effort has been aimed at finding effective pharmacologic agents for the prevention of ventricular fibrillation. Clinical trials with the β -adrenergic blocking agents timolol, metoprolol and propranolol have demonstrated that these drugs are capable of reducing mortality due to sudden coronary death in individuals with a previous myocardial infarction. The precise mechanism by which this is accomplished, however, is not yet certain, although β -adrenergic blockade most likely plays an important role.

Previous investigators have found that the adrenergic neuronal blocking agent, bretylium, was effective in elevating the electrical ventricular fibrillation threshold in animals (Bacaner, 1968; Bacaner and Schrienemachers, 1968; Kniffen et al., 1975; Cervoni et al., 1971) and later work demonstrated bretylium to be efficacious as an antifibrillatory agent in man (Holder et al., 1977; Sanna and Arcidiacono, 1973). The mechanism of bretylium's antifibrillatory action, however, was believed not to be due to its adrenergic neuronal blocking properties since: (1) another adrenergic neuron blocking drug, guanethidine, was ineffective in elevating ventricular fibrillation threshold (Bacaner, 1968; Cervoni et al., 1971); (2) in vitro electrophysiologic studies demonstrated that bretylium exerted its primary electrophysiologic property (prolongation of action potential duration and tissue refractoriness) despite prior sympathetic blockade with either reserpine pretreatment (Wit et al., 1970) or immunosympathectomy (Namm et al., 1975); and (3) the in vivo electrophysiologic and antifibrillatory actions of bretylium were intact despite prior reserpine administration (Cervoni et al., 1971) or surgical denervation (Waxman and Wallace, 1972). The above observations suggest that prolongation of the ventricular action potential duration (and ventric-

ular refractoriness) is bretylium's principal mechanism of action. Drugs which similarly prolong ventricular refractoriness may be effective in preventing ventricular fibrillation or reentrant arrhythmias (Steinberg and Molloy, 1979; Moe, 1975). Others have suggested that ventricular fibrillation is fundamentally a reentrant-type arrhythmia (Moe, 1975) where prolongation of the reentrant pathway by increasing refractoriness represents a reasonable approach to the prevention of ventricular fibrillation.

Recently, the new quaternary ammonium compound, clofilium, has been found to prolong action potential duration and myocardial refractoriness (Steinberg and Molloy, 1979; Steinberg et al., 1981). Clofilium produces a rate-dependent increase in action potential duration, increases the effective refractory period in proportion to the action potential duration, and appears to produce a greater increase in action potential duration in normal vs. acutely infarcted myocardium, thus decreasing the disparity in refractoriness of normal and infarcted tissue (Steinberg et al., 1981). Clofilium, however, has no effect on conduction, V_{max} , and unlike bretylium, has no adrenergic neuronal blocking properties (Steinberg and Molloy, 1979). In addition to the above *in vitro* effects, clofilium has been shown to elevate electrical ventricular fibrillation threshold in dogs (Steinberg and Molloy, 1979) and prevents ventricular tachycardia and ventricular fibrillation in dogs subjected to programmed electrical stimulation (Michelson et al., 1981). These observations are in agreement with data presented in this report. Clofilium was fully effective, although at somewhat higher doses than those used elsewhere (Steinberg and Molloy, 1979; Platia and Reid, 1980; Greene et al., 1981; Michelson et al., 1981), in elevating electrical ventricular fibrillation threshold in both normal and previously infarcted canine myocardium and in preventing induced ventricular tachycardia and fibrillation in dogs undergoing programmed electrical stimulation. Additionally, clofilium was effective in slowing the rate of the tachycardia in inducible animals.

Clofilium was not, however, effective in a recently described model of sudden coronary death (Patterson et al., 1982). This model overcomes

some of the objections raised with other animal models of ventricular arrhythmias and fibrillation. It is characterized by the presence of an area of previous myocardial infarction and the ability of programmed electrical stimulation to elicit reentrant ventricular arrhythmias. Drugs are evaluated in the conscious animal, thus eliminating the confounding effects of anesthesia upon both the autonomic nervous system and the myocardial electrophysiologic properties. In addition, ventricular dysrhythmias develop spontaneously as a result of either a partial or complete occlusion of the left circumflex coronary artery by a platelet thrombus (Patterson et al., 1982; Romson et al., 1980), resulting in mortality rates up to 100%. When a similar thromboembolic lesion is produced in animals without previous infarction, the mortality is 20% (Patterson et al., 1982). Therefore, in the model employed in the present report, ventricular fibrillation, is produced by a generally non-lethal ischemic event. While clofilium was effective in increasing the duration of the effective refractory period over that of control animals, no significant decline in mortality was observed in this model of sudden death, although the trend for clofilium-treated animals was favorable. In such studies with small numbers of animals there is always the risk that the failure of clofilium treatment was due to a type II statistical error *viz.*, clofilium really is beneficial in such a model but the use of too few animals failed to show a statistical benefit. While this is certainly a consideration it is important to point out that other drugs such as bretylium (Holland et al., 1983) nadolol (Patterson and Lucchesi, 1983) amiodarone (Patterson et al., 1983) pranolium (Eller et al., 1983) and sotalol (Lynch et al., 1985) have all been studied in an identical model with the same numbers of animals in each treatment group and have shown significant benefits when compared to appropriate vehicle control animals. Thus, a relative comparison would suggest that clofilium is less effective in the canine model of sudden death than these other agents, yet there is clearly a favorable trend. These pharmacologic agents, however, have multiple actions. As previously mentioned, in addition to increasing myocardial cellular refractoriness, bretylium is also an adrenergic neuronal blocking agent. Con-

versely, nadolol has little effect on conduction and refractoriness in myocardial tissue (Patterson and Lucchesi, 1982), but is a β -adrenergic blocking drug (Lee et al., 1975). Pranolium (UM-272) not only increases cellular refractoriness, but also decreases myocardial conduction both in vitro (Rosen et al., 1975) and in vivo (Gibson et al., 1978; Patterson and Lucchesi, 1981). Thus, the observation that clofilium failed to exert a significant antifibrillatory effect in the conscious dog suggests that, at least in this model, the milieu of factors which predisposes the heart to spontaneous ventricular fibrillation may require greater appeasement than simple prolongation of action potential duration and refractoriness. The underlying electrophysiological substrate during myocardial ischemia which ultimately provokes ventricular fibrillation is most likely the sum total of many individual factors working in concert. Even though clofilium is effective in prolonging the action potential duration in normal and ischemic myocardium in vitro, and is able to elevate electrical ventricular fibrillation threshold in vivo, during the dynamic process which ultimately leads to sudden death, one or a multiple of these factors may overcome any beneficial effect on action potential duration which has been produced by clofilium. So, while it is difficult to draw any firm conclusion based on the small number of animals used in the sudden death study, it is clear that further effort should be made to determine the precise benefit, if any, of prolongation of refractoriness in in vivo animal models of sudden coronary death.

In this regard, data from our study must question the benefit of prolonged refractoriness by clofilium as it regards clofilium's antifibrillatory effect. We saw no clear relationship between these two effects. In the VFT study, in the non-ischemic canine heart, clofilium clearly elevated myocardial ERP but at a dose which had no effect on VFT. Conversely, in chronically infarcted animals, clofilium produced no significant effect on ERP of normal myocardium but elevated the VFT to the same level as in the non-ischemic canine heart. This may be explained partially by the fact that the average paced heart rate in the non-ischemic group was somewhat lower than the paced rate in

the infarcted group (163 ± 8 vs. 190 ± 6 beats/min, respectively; $P < 0.05$). However, in those animals subjected to programmed electrical stimulation, no change in ERP occurred after clofilium and the paced heart rate was 161 ± 9 beats/min, a value similar to the paced rate in the non-infarcted VFT group where a significant increase in ERP occurred after clofilium. As previously mentioned, while the ERP was prolonged in the animals studied under conditions which provoked sudden death, certainly this did not provide any protection to this group of animals. While others have reported a relationship between clofilium's antiarrhythmic and electrophysiologic action (Platia and Reid, 1980; Greene et al., 1981; Michelson et al., 1981), our data can not confirm this relationship.

It should be pointed out that in no animal can clofilium administration be causally related to either ventricular fibrillation or sudden death. However, any further evaluation of this agent should be tempered with the knowledge that ventricular arrhythmias may be an undesirable side effect of therapy with clofilium.

It should also be pointed out that limitation of the present study relates to the sampling sites chosen for electrophysiologic investigation. In general, sampling sites were in normal regions or regions bordering ischemic areas, but no direct measurements were made from ischemic areas. To the extent that ischemia generates electrophysiologic disturbances in non-ischemic tissues our observations are valid, especially since each dog was its own control. However, further studies in ischemic tissues are warranted especially since the apparent lack of correlation between clofilium's electrophysiologic and antiarrhythmic actions might be further clarified if ischemic tissue were studied.

In summary, clofilium is a new quaternary ammonium antiarrhythmic agent which is capable of elevating electrical ventricular fibrillation threshold in both normal and chronically infarcted dogs and prevents the induction of reentrant ventricular arrhythmias in dogs undergoing programmed electrical stimulation. It does not, however, significantly reduce mortality in a canine model of sudden coronary death at the dose studied. Although

clofilium showed antiarrhythmic activity in the models studied, a significant protective effect was not observed in the sudden death model. This may indicate that a subtherapeutic drug level was achieved and a small increment in dose or change in schedule might show greater efficacy. Also, anesthetic effects may also account for this discrepancy. There is, however, a suggestion that a change in the ERP of normal myocardium induced by clofilium is not the sole mechanism of action of this drug.

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