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# VENTRICULAR FIBRILLATION IN A CONSCIOUS CANINE MODEL – ITS PREVENTION BY UM-272

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The antifibrillatory properties of UM-272 (dimethylpropranolol; Pranolium) were evaluated in a conscious canine model of sudden coronary death. The initial preparation of the animal model was carried out under surgical anesthesia and involved the intraluminal implantation of a Teflon-coated silver wire into the circumflex coronary artery so that 3 mm of the bared electrode was in contact with the endothelial surface. The left anterior descending coronary artery then was occluded for a period of 90 min and reperfused in the presence of a critical stenosis. Three days after myocardial infarction, they were randomized into two groups. One group (n = 10) served as controls and received saline. The second group (n = 10) received UM-272 in a dose of 5 mg/kg every 6 h. On day 4, a 150 $\mu$A current was applied to the intimal surface of the left circumflex coronary artery, resulting in transient or permanent alterations in circumflex coronary blood flow accompanied by electrocardiographic evidence of regional myocardial ischemia. The time to onset of ST-segment changes in the saline control group was 99 $\pm$ 34 min and was followed by the appearance of premature ventricular complexes (111 $\pm$ 34 min) and subsequent ventricular tachycardia (131 $\pm$ 37 min) which terminated in ventricular fibrillation in each of the 10 dogs. Animals treated with UM-272 likewise developed ST-segment changes (156 $\pm$ 28 min) and premature ventricular complexes (168 $\pm$ 29 min), but 4 of 10 animals failed to develop ventricular fibrillation ($P < 0.05$ vs. saline). These results demonstrate that UM-272, the dimethyl quaternary analog of propranolol, is effective in reducing the incidence of ventricular fibrillation in a conscious canine model in which the superimposition of a transient ischemic event upon an already jeopardized heart leads to the development of sudden death.

Model of sudden coronary death  Ventricular arrhythmias  Ventricular fibrillation  Pranolium

## 1. Introduction

Ventricular tachyarrhythmias leading to the development of ventricular fibrillation are a frequent complication of coronary artery disease in man and are a major cause of death in the industrialized world. The introduction of effective, well-tolerated antifibrillatory agents could add significantly to the salvage of human lives, for short of eradicating coronary atherosclerosis, pharmacologic therapy may provide the major means for treatment of patients at high risk for the development of sudden coronary death. The effective identification of useful interventions capable of reducing this mortality is dependent upon the development of animal models with cardiac electrophysiologic derangements comparable to those in patients at risk for developing ventricular fibrillation. In this report, we present a conscious canine model of sudden coronary death which reliably develops spontaneous ventricular fibrillation when acute myocardial ischemia is superimposed upon previous myocardial infarction and we examine the actions of the antiarrhythmic agent UM-272 (Pranolium; N,N-dimethyl-1-isopropylamino-3-(1-naphthyloxy)-propran-2-ol) in this model.

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## 2. Materials and methods

### *2.1. Surgical preparation*

Male mongrel dogs weighing between 14.8 and 22.1 kg were anesthetized with intravenous sodium pentobarbital, 30 mg/kg. An endotracheal tube was inserted and the animals ventilated with room air using a Harvard respirator. Using aseptic technique, a cannula was inserted into the left external jugular vein. A left thoracotomy was performed in the fourth intercostal space. The anterior surface of the heart was exposed and the heart suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free from surrounding tissue at the tip of the left atrial appendage. A blunt hypodermic needle (19 gauge) was placed parallel to the left anterior descending coronary artery and a suture placed around both the artery and the needle. The needle was then removed, leaving a critical stenosis. The artery was occluded using a snare formed from a loop of silastic tubing passed through a flared polyethylene tube. After 90 min of occlusion, reperfusion was allowed through the critical stenosis.

The left circumflex coronary artery was dissected free from surrounding myocardium approximately 1 cm from its origin and a bared 3 mm tip of a 30 gauge insulated silver wire inserted into the lumen of the artery. The wire was then secured to the surface of the heart using 3-0 suture. A bipolar plunge electrode (25 gauge insulated stainless steel, 4 mm in length, 3 mm apart) was placed into the interventricular septum adjacent to the right ventricular outflow tract, approximately 1 cm from the area of cyanosis. Silver disc electrodes were implanted subcutaneously to approximate locations for recording a lead II electrocardiogram. The chest was closed in layers and the animals allowed to recover from surgery.

### *2.2. Studies in the conscious dog*

On the third post-operative day, the animals were randomized to control and UM-272 treatment groups. The UM-272 treated animals received 5 mg/kg UM-272 every 6 h in 10 ml saline while control animals received 10 ml saline. On the fourth postoperative day, programmed electrical stimulation was performed immediately before administration of the fourth treatment dose at 24 h. All animals were studied while conscious and resting comfortably in a sling. One, two, or three premature ventricular stimuli (4 ms duration pulses, twice diastolic threshold) were introduced into the interventricular septum using a Grass model S-88 stimulator and SIU-5 stimulus isolation unit. Diastole was scanned at sequential 10 ms periods using a procedure described previously (Patterson and Lucceshi, 1981; Patterson et al., 1981). The procedure was performed until nonsustained ventricular tachycardia or sustained ventricular tachycardia was reproduced in triplicate. A greater number of premature stimuli or stimuli at shorter coupling intervals were not introduced in order to circumvent the production of ventricular fibrillation.

Immediately after completion of programmed stimulation, the next drug dose or saline was administered. An anodal current of 150 $\mu$A was applied to the intimal surface of the left circumflex coronary artery using a 9 V battery and variable potentiometer. A lead II electrocaridogram was transmitted by radiotelemetry and recorded on a Grass model 7 polygraph. Stimulation of the intimal surface of the left circumflex coronary artery was performed in the conscious ambulatory dog as all necessary equipment was contained in a $6 \times 12 \times 15$ cm package secured in the pouch of a nylon mesh vest.

After 24 h of intimal stimulation, or after ventricular fibrillation occurred, the hearts were excised and the left circumflex coronary artery examined for the presence of intimal damage and thrombus formation. The hearts were then sectioned into 5 mm thick slices from apex to base, parallel to the atrioventricular groove. The heart sections were stained for the presence of intracellular dehydrogenases using 0.5% triphenyltetrazolium hydrochloride in 0.01 M phosphate buffer, pH 7.4. Triphenyltetrazolium stains viable tissue brick-red allowing irreversibly damaged tissue to be distinguished by its pale appearance. Infarct size was determined gravimetrically and expressed as a percentage of total left ventricular mass.

### 2.3. Statistical evaluation

Data are expressed as mean ± the standard error of the mean. Differences in survival between control and UM-272 treatment groups were analyzed using Fisher's exact test. Student's t-test for unpaired data was used to determine significance for differences between means in control and UM-272 treatment groups.

## 3. Results

### 3.1. Programmed stimulation

The introduction of premature ventricular stimuli in control (saline-treated) animals on day 4 after myocardial infarction produced nonsustained ventricular tachycardia in 7 animals, sustained ventricular tachycardia in 2 animals, and failed to produce ventricular arrhythmias in 1 animal. Programmed stimulation failed to produce ventricular tachycardia in 9 of 10 animals treated for 24 h with UM-272 (5 mg/kg every 6 h). When analyzed using Fisher's exact test, this was a significant ($P < 0.001$) reduction in ventricular instability in the UM-272 treated group.

No difference in sinus heart rates was present between saline (122 ± 8 beats/min) and UM-272 (113 ± 9 beats/min) groups. Ventricular effective refractory periods were significantly elevated in the UM-272 group (200 ± 5 ms vs. 150 ± 3 ms, $P < 0.01$).

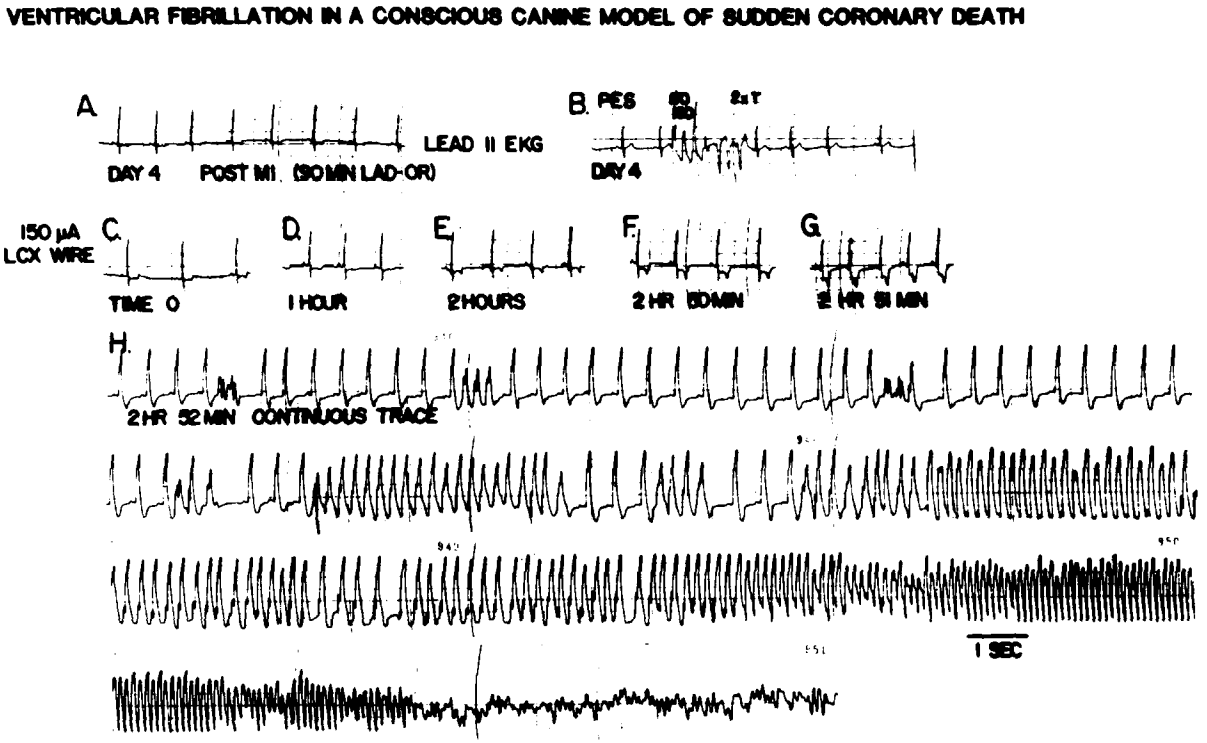


Fig. 1. Ventricular fibrillation in a conscious canine model of sudden coronary death. In panel A, the lead II electrocardiogram is shown during normal sinus rhythm on day 4 after myocardial infarction. Introduction of two premature ventricular stimuli at two times diastolic threshold (coupling intervals: 170 and 180 ms) produced non-sustained ventricular tachycardia (panel B). At time 0 (panel C), a current of 150 µA is applied to the intimal surface of the left circumflex coronary artery. No changes were noted at 1 h (panel D) and 2 h (panel E) with ST-segment changes observed at 2 h 50 min and 2 h 51 min (panels F and G). At 2 h 52 min, panel H, ventricular tachycardia appears and degenerates 30 s later to ventricular fibrillation.

*3.2. Initiation of current flow to the intimal surface of the left circumflex coronary artery*

In saline-treated animals, the application of 150 $\mu$A anodal current to the intimal surface of the left circumflex coronary artery produced ST-segment changes indicative of regional myocardial ischemia at 99 $\pm$ 34 min. These changes were followed by the development of premature ventricular beats (111 $\pm$ 34 min), ventricular tachycardia (131 $\pm$ 37 min), and ventricular fibrillation (173 $\pm$ 45 min) in all 10 animals. An example of the development of ST-segment changes, premature ventricular beats, and ventricular fibrillation in a saline-treated animal is shown in fig. 1. The development of ST-segment changes and the progression to ventricular fibrillation was dependent upon the production of intimal damage and the development of partial or complete occlusion of the left circumflex coronary artery by a platelet thrombus. The mean thrombus mass present within the left circumflex coronary artery was 8 $\pm$ 2 mg.

Treatment with UM-272 did not prevent the development of ST-segment changes (156 $\pm$ 28 min) or premature ventricular beats (168 $\pm$ 29 min). The onset of ST-segment changes and premature ventricular beats did not differ temporally from saline-treated animals ($P > 0.10$). However, the incidence of ventricular fibrillation was reduced in UM-272-treated animals with 4 of 10 animals surviving to 24 h ($P < 0.05$). An example is shown in fig. 2. Thrombus mass in UM-272-treated animals was 14 $\pm$ 4 mg.

*3.3. Myocardial infarct mass*

Irreversibly injured tissue comprising 19 $\pm$ 3% of total left ventricular mass was detected within the distribution of the left anterior descending coronary artery of saline-treated animals using the histochemical stain, triphenyltetrazolium chloride. No evidence of irreversible injury was detected within the distribution of the left circumflex coronary artery.

Irreversible injury comprising 19 $\pm$ 4% of left ventricular mass was observed in the left anterior descending coronary artery distribution of UM-272-treated animals with irreversible injury ob-

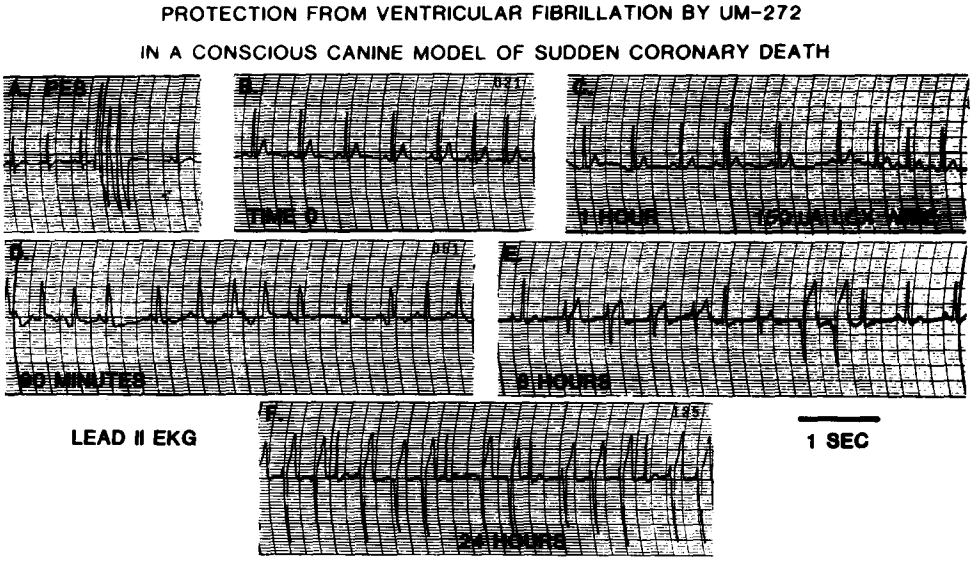


Fig. 2. Protection from ventricular fibrillation by UM-272 in a conscious canine model of sudden coronary death. In panel A, three premature ventricular stimuli at two times diastolic threshold failed to produce ventricular tachycardia in a UM-272-pretreated animal (5 mg/kg every 6 h for 4 doses, 6 h post-dose). In panel B, a current of 150 $\mu$A is applied to the intimal surface of the left circumflex coronary artery. No ST-segment changes are evident at 1 h (panel C). Ischemic changes are observed at 90 min (panel D) with premature ventricular beats at 6 h and 24 h (panels E and F).

served within the left circumflex distribution of 4 animals surviving to 24 h (17 ± 3% of left ventricular mass).

## 4. Discussion

Many of the animal models currently used to evaluate antiarrhythmic drugs are poorly suited for examining the potential of drugs to prevent sudden coronary death. Ventricular tachyarrhythmias present 24–72 h after ligation of a major coronary artery, the commonly used model of Harris (1950), poorly simulate the continued electrical instability observed in patients at risk for developing sudden coronary death. The ventricular arrhythmias slow and then disappear spontaneously over several days after infarction. Rapid ventricular tachyarrhythmias (> 250 beats/min) rarely develop and ventricular fibrillation occurs infrequently. These arrhythmias are believed to result from enhanced ventricular automaticity rather than localized myocardial reentry, the proposed mechanism in man (Lazzara et al., 1978; Ruskin et al., 1980; Josephson et al., 1980).

The majority of experimental work on lethal ventricular arrhythmias has been performed in animals subjected to acute occlusion and/or reperfusion of a major coronary artery. Either occlusion of a major coronary artery or rapid reinstitution of blood flow after a period of coronary artery occlusion provides a severe test of a drug's ability to prevent reentrant ventricular arrhythmia and fibrillation. Although these models may be applicable to a subset of patients at risk for development of sudden coronary death (i.e., coronary spasm), both models poorly simulate continued ventricular electrical instability associated with sudden coronary death and the presence of chronic ischemic heart disease with prior infarction/ischemia (Dreifus et al., 1981).

Recently, considerable interest has focused upon the electrophysiology of arrhythmias occurring 3–30 days after experimental myocardial infarction in the dog. During rapid ventricular pacing or after appropriately timed ventricular premature beats, slowly conducting electrical activity within ischemically injured ventricular myocardium spans diastole and ventricular arrhythmias occur. The diastolic interval between ectopic beats is accompanied by continuous electrical activity within ischemically injured ventricular myocardium, presumptive evidence of localized myocardial reentry (El-Sheriff et al., 1977; Karaguezian et al., 1979; Gibson and Lucchesi, 1980; Michelson et al., 1980; Garan et al., 1980). The ability of these arrhythmias to be reliably initiated and terminated with critically timed ventricular stimuli in a manner identical to that in patients resuscitated from a previous episode of sudden coronary death (Ruskin et al., 1980; Josephson et al., 1980) has led to the use of the canine model for the study of drug efficacy for prevention of reentrant ventricular arrhythmias and sudden coronary death. Reentrant ventricular arrhythmias occur only rarely unless provoked by programmed stimulation.

The conscious canine model described in this report overcomes many of the objections raised with other arrhythmia models. The model is characterized by previous irreversible ischemic injury and the ability of programmed stimulation to produce reentrant ventricular arrhythmias, Ventricular arrhythmias and ventricular fibrillation both develop spontaneously, a result of either partial or complete occlusion of the left circumflex coronary artery by a platelet thrombus. The evaluation of pharmacologic therapy also takes place in the conscious animal, eliminating the effect of anesthesia upon the autonomic nervous system and baseline electrophysiologic properties. When a similar lesion is produced in the left circumflex coronary artery of animals without previous ischemic injury, ventricular fibrillation occurs at a significant lower incidence (20%) (Patterson et al., 1982). Thus, ventricular fibrillation is provoked by a generally non-lethal ischemic event.

UM-272 is a dimethyl quaternary ammonium derivative of the β-adrenergic receptor antagonist, propranolol. The quaternary analog lacks β-adrenergic receptor antagonism, but retains many of the direct membrane actions of propranolol (Schuster et al., 1973). UM-272 is an effective antiarrhythmic agent, converting ouabain-induced ventricular tachycardias and 48 h two-stage coronary artery occlusion arrhythmias to normal sinus rhythm (Schuster et al., 1973). Similar ef-

ficacy has also been observed in patients with ventricular premature beats (Reele et al., 1978).

UM-272 increases ventricular fibrillation thresholds determined during normal sinus rhythm and during acute occlusion of a branch of the left anterior descending coronary artery (Kniffen et al., 1973). Ventricular fibrillation produced by acute occlusion and reperfusion of the left anterior descending coronary artery in the anesthetized dog is reduced in incidence by pretreatment with UM-272 (Schuster et al., 1973). Ventricular arrhythmias produced by programmed electrical stimulation 3–10 days after experimental canine myocardial infarction are suppressed by acute and chronic UM-272 administration (Gibson and Lucchesi, 1980; Patterson and Lucchesi, 1981). The latter action was confirmed in the present study as UM-272 significantly reduced the incidence of ventricular arrhythmias produced by programmed stimulation. Furthermore, the drug also significantly reduced the incidence of spontaneous ventricular fibrillation produced by acute myocardial ischemia in the presence of a previous myocardial infarction.

UM-272 does not appear to exert its protective effect via an antithrombotic action, since drug treated animals developed occlusive thrombi as a result of intimal injury to the circumflex coronary artery. The time to development of ST-segment changes did not differ between the two groups, suggesting that ischemic changes occurred at the same time after the initiation of the anodal stimulus to the vessel. Those animals in the UM-272-treated group which went on to survive for a period of 24 h, in each instance had sufficient time to develop a fully occlusive thrombus mass which resulted in extensive irreversible injury in the myocardial region subserved by the left circumflex coronary artery. The surviving animals thus had a significant degree of myocardial tissue subjected to ischemic injury and cell death in contrast to those animals which died suddenly as a result of ventricular fibrillation. The suddenness of the terminal event gave little time for development of a fully occlusive thrombus mass and/or signs of irreversible myocardial cell injury as determined by histochemical staining.

We cannot eliminate the actions of UM-272 upon the ischemic process as a mechanism for its protective effect. UM-272 has been shown to reduce myocardial oxygen consumption and the ultimate extent of irreversible injury produced by regional myocardial ischemia (Kniffen et al., 1975; Lucchesi et al., 1976; Ku and Lucchesi, 1978; Olson et al., 1976; Warltier et al., 1978). These actions could be beneficial in limiting the extent of ischemic injury and thereby prevent ventricular fibrillation.

Since the development of the conscious canine model of sudden coronary death in our laboratory, we have had the opportunity to examine a number of pharmacologic agents for their ability to protect against the development of ventricular fibrillation in response to an acute ischemic event superimposed upon a previously injured myocardium. Three agents, bretylium (Patterson et al., 1981; Holland et al., 1982), nadolol (Patterson and Lucchesi, 1982a), and amiodarone (Patterson and Lucchesi, 1982b) have provided beneficial effects similar to those observed with UM-272. On the other hand, bethanidine (unpublished observation) and diltiazem (Patterson, Eller and Lucchesi, to be published) were without effect and could not be distinguished from the vehicle treated control group. Thus, the animal model, as described, permits one to evaluate an agent using programmed electrical stimulation to determine if induced ventricular tachycardia can be prevented and to assess a given drug's potential to prevent ventricular fibrillation when a transient ischemic event is superimposed on a previously injured myocardium. On the basis of our experience with the model, we are of the impression that it can discriminate among the different agents so as to permit one to distinguish between a drug which is antiarrhythmic and one which is antifibrillatory. We have demonstrated that the quaternary ammonium compound, UM-272, possesses antifibrillatory properties, which would suggest that further exploration of this or related compounds is justified.

The use of animal models to determine efficacy of drug therapy for human disease is always tenuous. However, the canine model described in this report does provide a model which closely simulates pathophysiologic and electrophysiologic correlates in man.

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