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**Short communication**

# THE EFFECT OF DILTIAZEM ON CORONARY THROMBOSIS IN THE CONSCIOUS CANINE [1]

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The effect of diltiazem vs. saline was studied in a conscious canine model of coronary thrombosis. Diltiazem given as a 0.75 mg/kg loading dose intravenously followed by 0.4 mg/kg intravenously every 4 h for 24 h had no significant effect on thrombus wet weight, left ventricular infarct size, frequency of ventricular arrhythmias or ex vivo platelet aggregation. The search for antithrombotic agents using in vitro or ex vivo platelet aggregation studies should include concomitant in vivo thrombosis studies using therapeutic dosages of the drug in question.

Diltiazem  Calcium antagonist  Thrombosis  Platelet aggregation  Myocardial infarction

## 1. Introduction

Recent work in our laboratory indicates that diltiazem protects against both regional and global myocardial ischemia by inhibition of calcium ingress into ischemic myocardium (Bush et al., 1981). We have extended these studies in a conscious canine model of coronary thrombosis. While the effects of calcium channel blockers on non-cardiac and non-smooth muscle cells have been studied less extensively than in the cardiac cells, it is appealing to consider the potential effect of these drugs on the platelet which is centrally involved in arterial thrombosis. A variety of direct and indirect evidence suggests that calcium plays an important regulatory role in platelet aggregation and release reactions (Detwiler et al., 1978). Thus, calcium channel blockers might be expected to inhibit platelet aggregation in response to vascular injury.

We hypothesized that diltiazem might have antithrombotic effects in view of the drug's potential for antiplatelet activity in addition to its known effect in relieving coronary artery spasm which may also contribute to the thrombotic process.

Our model of coronary thrombosis (Romson et al., 1980) is attractive because the animals are conscious during thrombus induction and thus the intact neural, metabolic and other homeostatic processes more closely resemble the clinical state. In addition, pharmacologic doses of drugs easily tolerated by the open-chest anesthetized animal are often poorly tolerated by the conscious animal; thus the dosage of a drug given to the conscious animal will more closely resemble the clinical situation.

## 2. Materials and methods

### 2.1. Surgical procedure

Male mongrel dogs, 12–15 kg, were anesthetized with intravenous sodium pentobarbital (30

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mg/kg) and artificially respirated. Cannulae were placed in the left common carotid artery and jugular vein and exteriorized through the nape of the neck. The heart was suspended in a pericardial cradle and the proximal left circumflex coronary artery was isolated. A 28 gauge Teflon-coated silver wire with 5 mm of the tip of a 25 gauge hypodermic needle secured on the wire's leading end was inserted into the circumflex artery and affixed to the heart with suture. The pericardium and chest were closed in layers. Grass disc electrodes were tunneled subcutaneously and exteriorized along with the intracoronary wire at the neck. The dogs were given a single intramuscular injection of ampicillin (3 mg/kg). A dilute heparin solution was placed in the cannulae to maintain patency.

*2.2. Electrical stimulation of the left circumflex artery and drug treatment*

On the morning following surgery, the animals heart rate, blood pressure and electrocardiogram were monitored. Only animals free of electrocardiographic evidence of myocardial injury or significant arrhythmias were studied further.

Venous blood was drawn from a foreleg for platelet studies (vide infra). Diltiazem 0.75 mg/kg or saline was administered as an intravenous loading dose over 10 min with continuous monitoring of the blood pressure and electrocardiogram. Venous blood for platelet studies was drawn 60 min after this loading dose. Subsequently, diltiazem 0.4 mg/kg or saline was given intravenously every 4 h for 5 consecutive doses. Two additional animals were given a loading dose of 1.5 mg/kg of diltiazem followed by 0.8 mg/kg every 4 h. An individual not directly involved with the experiment made up and administered the solutions according to a random number table.

One hour after the loading dose of drug and phlebotomy for platelet studies, anodal current from a 9 V battery was delivered to the intimal surface of the circumflex coronary artery via the Teflon-coated wire. The current output of 50 $\mu$A was adjusted through a 250000 $\Omega$ potentiometer placed in series. The Grass electrodes were connected to a telemetry transmitter. Electrocardiographic data were received via telemetry and in turn were recorded by a tape recorder programmed to record 28 sec of tracing every 15 min. The tape was played back later through a Grass polygraph for analysis or arrhythmias.

*2.3. Sacrifice*

The 50 $\mu$A current to the circumflex artery was maintained for 24 h, then stopped. Venous blood was again drawn for platelet studies. Ventricular fibrillation was induced by DC current and the heart was removed. The circumflex coronary artery in the area of the wire insertion was dissected free and the artery was opened lengthwise. The wet thrombus was scraped free from the intima and weighed. The heart was then 'breadloafed' from apex to base into 1.0 cm thick slices and incubated in 2,3,5-triphenyltetrazolium chloride for 30 min. This dye stains normal tissue 'brick-red' by virtue of its reaction with myocardial dehydrogenases, while unstained dead tissue appears white. The non-stained areas were removed and weighed.

*2.4. Ex vivo platelet aggregation*

Platelet aggregation studies were performed using previously described spectrophotometric methods (Mills and Roberts, 1967) utilizing a Bio-Data platelet aggregometer. Platelet rich plasma was prepared by collecting venous blood in 1.0 ml of 3.8% sodium citrate to a total volume of 10 ml. This was centrifuged at $310 \times g$ for 3 min to obtain the platelet rich plasma fraction and then at $2200 \times g$ for 10 min to obtain the platelet poor plasma fraction. Platelet rich plasma was diluted with platelet poor plasma to a platelet count of 200000/mm$^3$ before use in the aggregation assays. All platelet samples were assayed within 3 h of the time of phlebotomy. Aggregation was initiated under 3 different conditions using a 50 $\mu$l aliquot of aggregating agent added to 450 $\mu$l of diluted platelet rich plasma. Aggregating conditions include: collagen (1:80 dilution of Ethicon Collagen Dispersion-TD150); 5.0 $\mu$g ADP (Sigma); and arachidonic acid (Sigma), 0.65 mM with an additional 10 $\mu$l of L-epinephrine (Sigma), 0.55 $\mu$M in saline at pH 3.

*2.5. Statistical methods*

All data are expressed as the mean ± standard deviation. Student's t-test for unpaired observations and the Wilcoxon rank sum test were used to determine statistical significance. A P value of $< 0.05$, treated vs. controls, was considered a significant difference.

## 3. Results

The mean weight of the saline treated animals ($n = 5$) was $13 \pm 1$ kg whereas the mean weight of the diltiazem treated animals ($n = 5$) was $14 \pm 1$ kg ($P > 0.02$).

The administration of the loading doses of diltiazem or saline revealed no significant differences in heart rate or mean arterial pressure in the pre-drug period as compared to the immediate post-drug period. A between group comparison revealed higher pre- and post-drug heart rates for the saline group (pre, $140 \pm 19$; post, $140 \pm 18$) as compared to the diltiazem group (pre, $120 \pm 7$; post, $121 \pm 7$) ($P < 0.05$). This difference is unexplained. There were no differences between groups in mean arterial pressure.

There was no difference between the diltiazem group vs. saline group with respect to thrombus wet weight, left ventricular infarct size, infarct as a percent of left ventricle or infarct as a percent of total heart weight (table 1). A trend for the control group to be lower for some of these measures was evident. Of interest, two diltiazem treated animals had no evidence of infarction despite the presence of thrombi. Two animals receiving higher doses of diltiazem died before the end of the electrical stimulation period. Both animals developed intermittent marked atrioventricular block and the terminal arrhythmias were sinus bradycardia followed by asystole in one animal and ventricular fibrillation in the other. Coronary thrombi weighed 19 mg and 22 mg—approximately the same weights as animals treated with diltiazem at a lower dose.

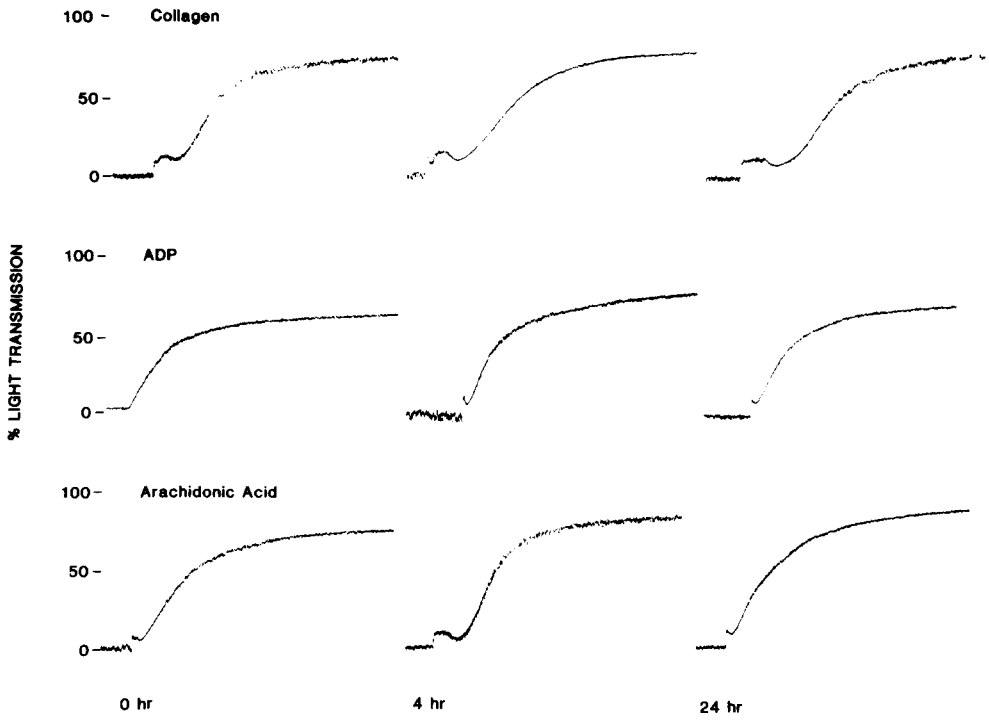


Fig. 1. Effect of diltiazem on ex vivo platelet aggregation.

TABLE 1

Effects of diltiazem vs. saline on coronary thrombosis and left ventricular infarction after circumflex stimulation.

| | Saline (n=4) | Diltiazem (n=5) |
|---|---|---|
| Thrombus Wet Weight (mg) | 13±7 [a] | 19±14 |
| Infarct (gm) | 13±5 | 15±17 |
| Left Ventricle (gm) | 49±7 | 69±18 |
| Total Heart (gm) | 79±9 | 98±25 |
| Infarct/Left Ventricle (%) | 26±8 | 23±23 |
| Infarct/Total Heart (%) | 16±7 | 17±17 |

[a] $\bar{X}\pm$S.D.

Examination of the frequency of premature ventricular contractions occurring during the 24 h period of stimulation revealed no differences between the treated and untreated groups.

Platelet aggregation studies revealed no differences in the diltiazem group or saline group when the one-h and 24-h post-baseline aggregation curves were compared to the baseline aggregation curve. A representative diltiazem platelet aggregation study is displayed in fig. 1.

## 4. Discussion

Diltiazem has no apparent antithrombotic effect in this model of coronary thrombosis. This result may not be surprising in view of the lack of effect of diltiazem on platelet aggregation. It has been suggested that platelet aggregation responses may not be predictive of in vivo antithrombotic effects. While this is especially true in the case of drugs which demonstrate clear-cut in vitro inhibition of platelet aggregation without concomitant in vivo antithrombotic effects, the opposite situation is rarely observed since most drugs which have no in vitro platelet inhibiting properties are not studied further with respect to thrombosis.

An interesting finding in two diltiazem-treated animals was the presence of thrombi without infarction. In both cases the thrombi were of sufficient size to be occlusive. This suggests that diltiazem may have been limiting the ischemic injury as has been shown in this laboratory using models of global and regional ischemia (Bush et al., 1981).

Little is known about the effect of diltiazem in suppression in ischemic ventricular arrhythmias. While no antiarrhythmic effect was observed in the current study, it should be noted that this model prohibits conclusions because of the experimental design. Appropriate study of ventricular fibrillation thresholds, response to programmed electrical stimulation and related studies would better answer the question of antiarrhythmic potential.

Diltiazem's inability to inhibit platelet aggregation is most likely a reflection of the dosage of drug used since in vitro platelet aggregation studies of calcium channel blockers indicate an absence of antiaggregatory effect at therapeutic concentrations ($10^{-7}$ M) whereas higher in vitro concentrations ($10^{-5}$ and $10^{-6}$ M) inhibit platelet aggregation (Margolis et al., 1980).

Caution is advised in extrapolating antithrombotic potential from in vitro platelet aggregation studies without the use of concomitant in vivo thrombosis studies using therapeutic concentrations of the drug in question.

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