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Pentoxifylline does not reduce infarct size in a canine model of acute myocardial infarction

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1 The effect of the haemorrheological agent pentoxifylline was investigated in a canine model of acute myocardial infarction, induced by occlusion of the left anterior descending coronary for 6 h. Thirty minutes post-occlusion the dogs were randomized to receive either distilled water or pentoxifylline (0.3 mg kg^{-1} min^{-1} for 1 h followed by 0.15 mg kg^{-1} min^{-1} for 4.5 h) intravenously.

2 At 6 h post-occlusion the *in vivo* area at risk was determined with monastral blue dye and the area of necrosis was determined with triphenyltetrazolium chloride. The area at risk was 16.5 ± 1.3% in the control group ($n = 10$) and 17.2 ± 1.8% in the pentoxifylline treated group ($n = 10$; NS). The area of necrosis was 12.3 ± 1.9% in the control group and 11.9 ± 2.2% in the pentoxifylline treated group (NS). The area of necrosis expressed as a percentage of the area at risk was 69.3 ± 7.7% in the control group and 63.6 ± 7.4% in the pentoxifylline treated group (NS).

3 Pentoxifylline had no significant effects on heart rate, systolic or diastolic blood pressure. Regional myocardial blood flow, measured by the radioactive microsphere technique, was not significantly different between the groups.

4 Thus, pentoxifylline does not reduce infarct size in this model of acute myocardial infarction and does not enhance coronary collateral blood flow.

Introduction

Pentoxifylline is a haemorrheological agent used clinically for the treatment of intermittent claudication. The mode of action of pentoxifylline is thought to be via increased flexibility of erythrocytes thereby reducing blood viscosity and increasing blood flow through zones of atherosclerotic narrowing of peripheral arteries (Dettelbach & Aviado, 1985).

The analogous situation in the heart would be the severe stenosis of a major coronary artery leading to acute myocardial ischaemia and infarction. Because of the beneficial effect of pentoxifylline in peripheral arteries, we reasoned that it might have beneficial effects during myocardial ischaemia; increased erythrocyte flexibility theoretically could enhance coronary collateral blood flow and reduce ischaemic damage. The aim of this study was to investigate the effect of pentoxifylline in a canine model of acute myocardial infarction.

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Methods

Surgical procedures

Mongrel dogs, of either sex weighing between 13.0 and 32.8 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, intravenously), intubated and artificially ventilated with room air using a Harvard respirator. Cannulae were positioned in the carotid artery for monitoring arterial blood pressure and for withdrawal of reference blood samples to determine regional myocardial blood flow, and in the jugular vein for the administration of fluids and drugs. A thoracotomy was performed at the fifth left intercostal space, a pericardial cradle was temporarily constructed and the left anterior descending coronary artery was isolated proximal to the first major diagonal branch. A third cannula was placed in the left atrium

for injection of radioactive microspheres to determine regional myocardial blood flow.

Experimental protocol

After obtaining baseline measurements of heart rate and arterial blood pressure on a Gould multi-channel pen recorder, a bolus of lidocaine (1.5 mg kg^{-1}) was injected intravenously and the left anterior descending coronary artery was occluded for 6 h with a Schwartz vascular clamp. Thirty minutes post-occlusion the dogs were randomized to receive either pentoxifylline dissolved in distilled water (0.3 mg kg^{-1} min^{-1} for 1 h followed by 0.15 mg kg^{-1} min^{-1} for 4.5 h; volume of 27.2 ml h^{-1}) or a control infusion of distilled water (27.2 ml h^{-1}) intravenously. This dose of pentoxifylline has been shown previously to reduce ischaemically induced ST segment elevation without affecting heart rate or blood pressure (Gallenkemper *et al.*, 1984). The volume of the infusion did not change the haematocrit. At 6 h post-occlusion, the *in vivo* area at risk was determined by intra-atrial injection of monastral blue (0.25 ml kg^{-1}). The dogs were killed with an overdose of sodium pentobarbitone and potassium chloride, and the hearts were excised. The atria and right ventricle were removed and the left ventricle sectioned transversely from apex to base into 4–5 mm sections. The area of tissue unstained by the monastral blue, representing the area at risk was traced onto acetate sheets as previously described (Lo *et al.*, 1985). The sections were then incubated in triphenyltetrazolium chloride at 35°C for 10 min and the area unstained by the triphenyltetrazolium chloride, representing the area of necrosis, was traced onto acetate sheets. The size of the area at risk and the area of necrosis were determined by planimetry. Validation of the triphenyltetrazolium chloride staining method to quantify the area of necrosis has been reported previously (Fishbein *et al.*, 1981). Since some studies have shown that risk areas must achieve a certain size before any necrosis occurs (Jugdutt *et al.*, 1979) dogs having an area of risk of less than 10% of the left ventricle were eliminated. The area at risk and area of necrosis were expressed as a percentage of the left ventricle.

Regional myocardial blood flow

Regional myocardial blood flow was determined 30 min post-occlusion (before randomization), 90 min post-occlusion and 6 h post-occlusion by the left atrial injection of 2.0×10^6 radioactive (^{141}cerium, ^{103}ruthenium, ^{95}niobium) microspheres (11 ± 1 μm diameter) while a reference arterial blood sample was withdrawn from the carotid artery at 15.3 ml min^{-1} by means of a Harvard withdrawal pump. At the end of the study, samples of myocardium weighing approximately 1 g were cut from the epicardial, midmyocardial and endocardial regions in the centres of the necrotic and non-necrotic area for determination of regional myocardial blood flow. Regional myocardial blood flow (RMBF) in the tissue sample (ml min^{-1} g^{-1}) was calculated by the formula: $RMBF = Cs \times (Cb/Cr)$, where Cs = counts in the myocardial tissue sample corrected per g; Cb = rate of withdrawal of the reference blood sample and Cr = total counts in the reference blood sample (Domenech *et al.*, 1969).

Statistics

Comparison of the incidence of areas at risk of $< 10\%$ in each group was made by use use of the Chi^2 test. Comparisons of the area at risk and area of necrosis between the groups were made by use of Student's *t* tests. Comparisons of heart rate, systolic and diastolic arterial blood pressure between the groups were performed by analysis of variance (Wallenstein *et al.*, 1980). A probability level of less than 0.05 was considered statistically significant. All values are expressed as mean ± s.e.mean. NS indicates that differences were not statistically significant.

Results

A total of 63 dogs were entered into the study, of which 32 died of ventricular fibrillation within 30 min of coronary occlusion, prior to randomization. Of the dogs randomized 11 were eliminated (3 in the control group compared with 8 in the pentoxifylline group, NS) since they showed an area at risk of less than 10%. Thus, the results of 10 dogs in each group are summarized herein.

Area at risk and area of necrosis

The area at risk was $16.5 \pm 1.3\%$ in the control group and $17.2 \pm 1.8\%$ in the treated group (NS). The area of necrosis was $12.3 \pm 1.9\%$ in the control group and $11.9 \pm 2.2\%$ in the treated group (NS). The area of necrosis expressed as a percentage of the area at risk was $69.3 \pm 7.7\%$ in the control group and $63.6 \pm 7.4\%$ in the treated group (NS).

Haemodynamics

Table 1 shows the values for the heart rate and arterial blood pressure for the 2 groups over the experimental period. Pentoxifylline had no significant effect on heart rate, systolic or diastolic arterial blood pressure.

Regional myocardial blood flow

Table 2 shows the regional myocardial blood flow

Table 1 The effect of pentoxifylline on heart rate, systolic and diastolic blood pressure in dogs

Time (h)	0	0.5	1.5	6
Heart rate (b.p.m.)				
Control	161 ± 6	144 ± 7	149 ± 7	145 ± 10
Pentoxifylline	155 ± 5	149 ± 6	143 ± 5	127 ± 7
Systolic BP (mmHg)				
Control	143 ± 5	141 ± 6	132 ± 6	127 ± 7
Pentoxifylline	134 ± 6	123 ± 6	130 ± 5	128 ± 5
Diastolic BP (mmHg)				
Control	121 ± 3	121 ± 3	109 ± 5	105 ± 5
Pentoxifylline	111 ± 6	104 ± 7	105 ± 7	106 ± 5

measured pre- and post-randomization in the control and pentoxifylline groups. Both groups show markedly reduced regional myocardial blood flow across the left ventricular wall supplied by the left anterior descending coronary artery throughout the observation period. There were no significant differences between the groups at any time.

Discussion

The results of the present study demonstrate that pentoxifylline does not change the amount of necrosis as a function of the area at risk, in a canine model of acute myocardial infarction. To our knowledge no previous study has investigated the effect of pentoxifylline on anatomical infarct size. In a previous study in anaesthetized cats subjected to coronary artery ligation (Gallenkemper *et al.*, 1984), pentoxifylline did not significantly affect the loss of creatine kinase from left ventricles, although, there was a significant reduction in the standard limb lead ST segment elevation.

In the present study, pentoxifylline had no effect on heart rate or arterial blood pressure; this is in agreement with other studies. With more elaborate haemodynamic evaluation in dogs it has been shown that pentoxifylline increases cardiac output and cardiac work and decreases total peripheral resistance without changes in heart rate and arterial blood pressure resulting in an increase in total oxygen consumption (Komarek *et al.*, 1977).

In the present study no change in ischaemic or non-ischaemic coronary blood flow was observed with pentoxifylline. Other studies investigating the effects of pentoxifylline on changes of normal blood flow distribution in rats have found that this agent can increase blood flow in various tissues including heart and skeletal muscle (Vetterlein *et al.*, 1979). The reason for this discrepency with our study is unknown.

To date, no clinical studies on the effect of pentoxifylline on acute myocardial ischaemia or infarction have been published. However, the treatment of intermittent claudication by pentoxifylline is well established. The erythrocyte flexibility of patients with intermittent claudication is severely reduced (Reid *et al.*, 1976), and it has been shown that pentoxifylline can improve the *in vitro* erythrocyte flexibility. The acute administration of 800 mg pentoxifylline increased muscular oxygen tension of patients with intermittent claudication (Ehrly, 1983). Studies have shown that patients treated with pentoxifylline can

Table 2 Regional myocardial blood flow (ml min^{-1} g^{-1}) of ischaemic and non-ischaemic areas of the control and pentoxifylline treated groups of dogs

	Isc-epi	*Isc-mid*	*Isc-endo*	*Nor-epi*	*Nor-mid*	*Nor-endo*
			Post-occlusion/pretreatment			
Control ($n = 10$)	0.28 ± 0.09	0.12 ± 0.04	0.07 ± 0.02	0.86 ± 0.11	1.12 ± 0.14	1.18 ± 0.12
Treated ($n = 10$)	0.20 ± 0.07	0.08 ± 0.03	0.05 ± 0.02	0.80 ± 0.11	0.89 ± 0.13	0.97 ± 0.17
			Post-occlusion/1 h post-treatment			
Control ($n = 6$)	0.28 ± 0.10	0.14 ± 0.05	0.06 ± 0.03	0.87 ± 0.11	0.99 ± 0.17	1.00 ± 0.17
Treated ($n = 8$)	0.30 ± 0.06	0.13 ± 0.04	0.07 ± 0.02	0.78 ± 0.12	0.88 ± 0.05	0.96 ± 0.08
			Post-occlusion/5.5 h post-treatment			
Control ($n = 7$)	0.26 ± 0.08	0.06 ± 0.02	0.05 ± 0.01	0.89 ± 0.15	1.01 ± 0.16	1.01 ± 0.15
Treated ($n = 8$)	0.15 ± 0.04	0.06 ± 0.02	0.03 ± 0.01	0.77 ± 0.15	0.70 ± 0.11	0.79 ± 0.11

Isc = ischaemic, Nor = normal; epi = epicardium; mid = midmyocardium; endo = endocardium.

walk further before the onset of pain compared to pretreatment, and that this can be associated with an increase in muscle oxygen tension (Porter *et al.*, 1982).

It is tempting to compare the use of pentoxifylline in intermittent claudication with the analogous situation of coronary artery atherosclerosis. There are obvious similarities between these conditions, the most important being the progressive build up of atherosclerotic lesions. Whether pentoxifylline proves to be of benefit to patients with severe coronary atherosclerosis remains to be seen.

The extrapolation of data from anaesthetized dogs with non-diseased coronary arteries and normal erythrocyte flexibility to that of the patient with severe coronary stenosis requires considerable caution. However, the results of the present study suggest that this agent does not acutely display infarct limiting properties nor does it enhance collateral blood flow in the canine model of myocardial infarction.

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The animals used in this study were maintained in accordance with the guidelines of the Committee on Animal Studies of Wayne State University School of Medicine and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, revised 1985).

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