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Effects of a combination of metoprolol and dazmegrel on myocardial infarct size in rats

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1 The effects of acute pretreatment with metoprolol, dazmegrel and a combination of these two drugs has been examined on myocardial infarct size in rats. Ischaemic damage was assessed 4 h after coronary artery occlusion in anaesthetized rats and after 48 h of ischaemia in conscious rats. Infarct size was measured histochemically (by using periodic-acid-Schiff diastase reaction for glycogen) and by standard histological examination (haematoxylin and eosin stain).

2 There was some evidence of protection of the myocardium by metoprolol following 4 h of ischaemia (determined histologically) but this was not apparent 48 h after occlusion.

3 When given alone, dazmegrel had no significant effects on infarct size assessed by either method. A clear reduction in the extent of glycogen depletion and histological damage was observed with the combination of metoprolol and dazmegrel 48 h after the onset of ischaemia. This protection was seen to occur in the horizontal plane of the heart, preventing the extension of the infarct towards the posterior wall of the left ventricle and showing some salvage of the epicardial surfaces.

Introduction

It is well established that the balance between oxygen supply and demand is a vital determinant of the integrity of the ischaemic myocardium (Maroko *et al.*, 1971). Thus, agents which either decrease myocardial oxygen demand or increase myocardial oxygen supply would be expected to be beneficial in myocardial ischaemia. Catecholamines are released early during myocardial ischaemia and by their positive inotropic and chronotropic actions result in an increase in the oxygen requirements of the myocardium. Indeed, β-adrenoceptor blocking agents, which block the cardiac responses of released catecholamines, protect the myocardium when given before the onset of ischaemia (e.g. Reimer *et al.*, 1973; 1976). Such protection has been associated with a decrease in oxygen demand by the ischaemic myocardium. Similarly, thromboxane A_2, released primarily from platelets (Hamberg & Samuelsson, 1974) may accumulate locally within the coronary circulation, particularly during ischaemic states (Smith *et al.*, 1980). Thromboxane A_2 constricts blood vessels (Ellis *et al.*, 1976), induces platelet aggregation (Hamberg *et al.*, 1975) and increases the permeability of lysosomal membranes (Schrör *et al.*, 1980) and may thus be involved in mediation of the extension of ischaemic myocardial damage by exacerbating the effects of ischaemia and contributing to the inflammatory processes. Further, experimental studies in cats with inhibitors of thromboxane synthesis have shown a protection of the ischaemic myocardium (Smith *et al.*, 1979; Burke *et al.*, 1983a,b,).

In a previous study we found that a combination of a β-adrenoceptor blocking agent and a thromboxane synthetase inhibitor reduces the severity of the ventricular arrhythmias which follow coronary artery ligation in conscious rats (Leprán *et al.*, 1984). The aim of this study was to investigate whether the antiarrhythmic effect is accompanied by a reduction in the extent of damage after permanent coronary artery occlusion. We chose metoprolol as the β-blocker and dazmegrel as the thromboxane synthetase inhibitor (Parry *et al.*, 1982; Coker, 1984), and these were administered in doses that have previously been shown to be antiarrhythmic in rats (Wainwright & Parratt, 1985).

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Methods

Studies on infarct size after 4 h of ischaemia

Myocardial infarction was induced in male Sprague-Dawley rats (200–300 g), anaesthetized with sodium pentobarbitone (Sagatal) 60 mg kg^{-1}, by the method described by Selye *et al.* (1960) and by Clark *et al.* (1980). Briefly, the rats were ventilated on room air, a left thoracotomy was performed at the fourth or fifth intercostal space and the pericardium opened to allow access to the heart. The heart was then exteriorized and a 6/0 braided silk suture attached to a reverse cutting needle (Ethicon 812) was passed through the myocardium and around the left coronary artery 3–4 mm from its origin. The rats were then allowed to stabilize for 15 min before coronary artery occlusion. Four hours after occlusion the rats were killed by an injection of air and the hearts excised.

Studies on infarct size after 48 h of occlusion

For the purpose of obtaining 48 h myocardial infarcts, coronary artery occlusion was performed in conscious rats by use of the method described by Leprán *et al.* (1979). Male Sprague-Dawley rats were lightly anaesthetized with ether and a thoracotomy performed at the fifth intercostal space. The heart was exteriorized and a silk ligature was passed around the left coronary artery 3–4 mm from the origin, one end of the ligature being taken through the wall and the other through the lumen of a small polythene tube which was inserted in the thoracic cavity. The chest was then closed and the animal allowed to recover. Artificial ventilation was not required as the chest was open for only a total of 40–50 s. Several days after preliminary surgery the coronary artery was occluded by tightening the loose ends of the ligature. Forty-eight hours after occlusion the animals were re-anaesthetized, exsanguinated and the hearts excised.

Processing of tissues

After excision, hearts were either perfusion fixed with 4% formol saline for 10 min (followed by immersion fixation for 24 h) or frozen rapidly in hexane cooled to −60°C and stored at −70°C. Two 10 μm thick serial sections were cut at 0.5 mm intervals from the point of ligation to the apex of the heart. The sections were mounted and stained, one with haematoxylin and eosin (H & E) for standard histological assessment of myocardial damage, the other stained for glycogen (periodic-acid-Schiff-diastase reaction) as a histochemical method of infarct size determination. The following measurements were made from each sampling point by computer aided planimetry: (1) cross-sectional area of left ventricular myocardium; (2) cross-sectional area of left ventricular myocardium showing morphological damage with H & E stain; (3) cross sectional area of left ventricular myocardium showing depletion of glycogen.

From these measurements the percentage of the area of left ventricular myocardium involved in these changes was calculated and eventually expressed as a % of the left ventricular volume. All measurements were done in a randomized and blind fashion. Some qualitative observations of the characteristics of damage were made when the tissue was viewed under the light microscope.

Drug treatments

In both the 4 and 48 h infarct studies there were 4 treatment groups. The control group consisted of a group of animals given saline injection and the three other groups were given either metoprolol (2 mg kg^{-1}), dazmegrel (5 mg kg^{-1}) or a combination of metoprolol (2 mg kg^{-1}) and dazmegrel (5 mg kg^{-1}). All doses were administered intravenously 15 min before coronary artery occlusion and were repeated 2 h after the onset of ischaemia. In the 4 h infarct study a group of sham-operated rats was also included; this involved using the same procedure as that described

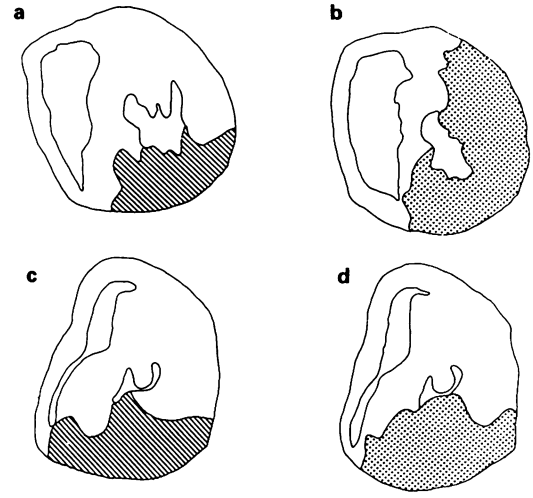

Figure 1 Tracings of transvers sections from control rat hearts. (a) Section of rat heart 4 h after occlusion stained with haematoxylin and eosin (H & E). The shaded area represents the zone of morphological damage. (b) Section taken 4 h after occlusion and stained for glycogen. The section was taken from the same heart as that shown in (a). The shaded area shows depletion of glycogen which is more widely distributed than the morphological damage. (c) Section of rat heart after 48 h of ischaemia stained with H & E. The shaded areas represent the area of morphological damage which has extended to the area of glycogen depletion at this time (d).

Table 1 The effects of acute pretreatment with metoprolol, dazmegrel and a combination of the two drugs on myocardial infarct size after 4 h of ischaemia

Group	n	*% of left ventricular volume showing*	
		Morphological damage	*Glycogen depletion*
Control	10	17 ± 3	—
Metoprolol	10	9 ± 3**	—
Sham operated	10	< 1%	—
Control	8	23 ± 4	41 ± 6
Dazmegrel	8	25 ± 5	41 ± 7
Metoprolol + dazmegrel	8	22 ± 4	47 ± 4

** $P<0.01$ compared with control group. Values are expressed as mean ± s.e.mean of n observations.

above except that the coronary artery was not ligated and no drugs were given.

Results

Qualitative changes in the myocardium during ischaemia

Four hours after the onset of ischaemia, sections stained with H & E showed morphological damage consisting of waviness, thinning and separation of cardiac muscle fibres (as a result of accumulation of interstitial fluid) and occasional areas of haemorrhage, presumably due to localized breakdown of the vascular wall. This area of damage was contained within a more widely distributed zone of glycogen depletion seen at this time. However, at 48 h, the sections stained with H & E showed that the total area of glycogen depletion was frankly necrotic and planimetric determination demonstrated that the necrotic zone corresponded quantitatively, as well as topographically, to the area of glycogen loss. Figure 1 shows tracings of transverse serial sections from control hearts after 4 and 48 h ischaemia stained with H & E or for glycogen.

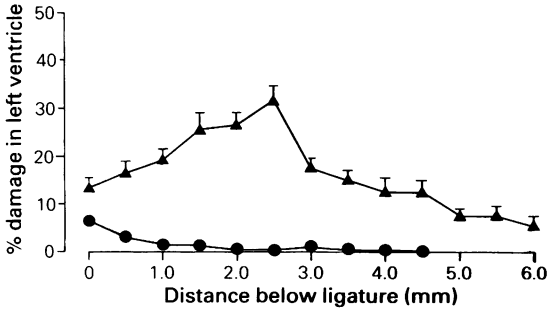

Figure 2 Profile of morphological damage after 4 h of ischaemia, with distance from the point of ligation in sham-operated rats (●) and control ligated rats (▲).

The effects of drug intervention on the extent of myocardial damage determined after 4 h of ischaemia

Infarct size assessed by both methods in control rats and in rats pretreated with metoprolol, dazmegrel or the drug combination is shown in Table 1. Pretreatment with metoprolol produced a significant reduction in the extent of damage to the myocardium, assessed by H & E stain, the area of damage being reduced by approximately 40–50%. In this group of rats the damage was not assessed histochemically.

Dazmegrel, given alone or in combination with metoprolol had no effects on the extent of damage assessed by either H & E stain or glycogen depletion. In the group of sham-operated rats, on which the entire operative procedure had been performed except for the tying of the ligature, there was very little myocardial damage compared to the control (ligated) group and any damage was restricted to the area in which the ligature was placed (Figure 2).

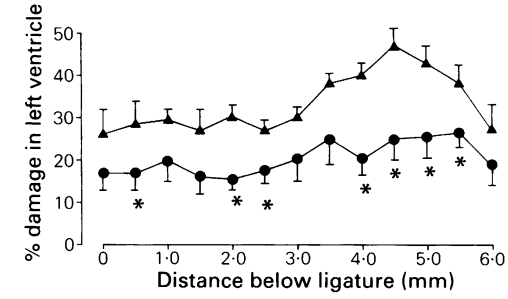

Figure 3 Profile of morphological damage to the left ventricle after 48 h of ischaemia in control rats (▲) and rats pretreated with a combination of metoprolol (2 mg kg^{-1}) and dazmegrel (5 mg kg^{-1}) (●). * $P<0.05$ compared to corresponding sampling site in controls.

Table 2 The effects of metoprolol, dazmegrel and the drug combination on myocardial infarct size after 48 h of ischaemia

Group	n	*% of left ventricular volume showing*	
		Morphological damage	*Glycogen depletion*
Control	8	34±5	35±5
Metoprolol	8	27±5	17±5**
Dazmegrel	8	30±5	31±5
Metoprolol + dazmegrel	8	21±5**	22±5**

* $P<0.05$; ** $P<0.01$ compared with control group. Values are expressed as mean ± s.e.mean of n observations.

The effects of drug intervention on the extent of myocardial damage determined after 48 h of ischaemia

Table 2 shows the effects of pretreatment with metoprolol, dazmegrel or the drug combination on the extent of histological damage and glycogen depletion in the myocardium after 48 h of ischaemia. Neither metoprolol nor dazmegrel alone reduced the area of necrosis assessed by staining with H & E but metoprolol significantly reduced the area of glycogen depletion observed. The combination of metoprolol and dazmegrel, however, caused a significant reduction in estimated infarct size assessed by both H & E and glycogen depletion. Figure 3 illustrates the profile of histological damage with distance from the point of ligation and from this it appears that the reduction in the area of damage with the drug combination occurred uniformly from the base to the apex of the heart, rather than being localized in the vertical plane of the heart. In an attempt to demonstrate where this protection had occurred a composite diagram was constructed using serial sections to show the area of damage in serial sections from the base to the apex of a control heart and a heart from a rat pretreated with metoprolol and dazmegrel (Figure 4). The diagram shows that the reduction in the damage occurred in the horizontal plane, that is the extension of the infarct to the posterior wall of the left ventricle had been reduced by metoprolol and dazmegrel administration. The damage had also been restricted to the sub-endocardium rather than extending transmurally.

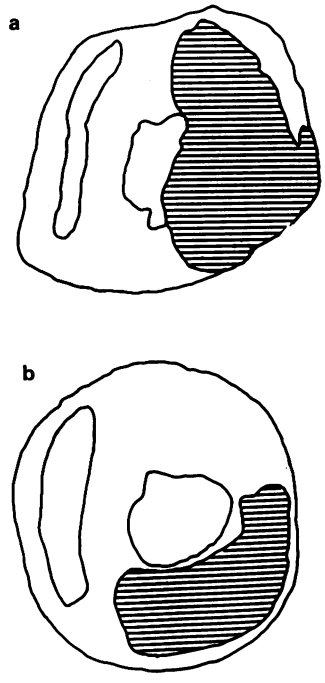

Figure 4 Composite diagram of the area of damage in serial sections from the base to the apex of the heart from a control rat (a) and from a rat pretreated with a combination of metoprolol and dazmegrel (b). Damage was assessed by haematoxylin and eosin stain 48 h after coronary artery occlusion. Notice there is epicardial salvage and the infarct has 'shrunk'.

Discussion

The results of this study have shown that is is possible to reduce the extent of myocardial damage following acute myocardial ischaemia in rats by drug intervention. Pretreatment with a thromboxane synthetase inhibitor (dazmegrel) did not protect against the development of myocardial damage assessed at either 4 or 48 h after the onset of ischaemia. Metoprolol showed some protection of the myocardium after 4 h of ischaemia but there was no reduction in infarct size at 48 h. The most marked improvement in the status of the ischaemic myocardium was with the combination of metoprolol and dazmegrel.

Coronary artery occlusion resulted in an area of damage to the myocardium, which was found to be in the early stages of development after 4 h of ischaemia, and progressed to complete injury over 48 h. The characteristics of damage and time-course of develop-

ment observed in this study were similar to those described by Fishbein *et al.* (1980). However, to determine the true effects of drug intervention on myocardial infarct size it would seem necessary to assess damage when the process of injury is complete, i.e. after 48 h in the study, as metoprolol was shown to protect the myocardium at an early stage in infarction but not when the process was complete. There is thus an important difference between simply delaying the processes through which the damage occurs and actually reducing the severity of ischaemic injury. An alternative explanation for the apparent protection with metoprolol after 4 h of ischaemia is that the infarcts in the metoprolol and corresponding control group were slightly smaller than the other groups in the study and it may therefore be easier to reduce the size of a larger infarct. Alternatively, the loss of effect with metoprolol after 48 h may reflect the necessity for continued dosing with the β-blocker. Repeated dosing over 48 h may sustain the protective effect. This would require further work.

Previous studies with β-adrenoceptor blocking agents on myocardial infarct size have produced varying results. From experiments performed in rats, Campbell *et al.* (1984) showed that prolonged administration of oxprenolol had no effect on infarct size assessed by enzyme depletion, although the analysis was performed at a time when oxprenolol was exerting an antiarrhythmic effect. In contrast, Koike *et al.* (1981) found a dose-related reduction in myocardial injury measured by loss of creatine phosphokinase (CPK) following administration of bucumolol. The majority of experiments performed in dogs have shown β-blockers to have a beneficial effect on the myocardium. For example, propranolol pretreatment has been shown to delay cell death following temporary occlusion (Reimer *et al.*, 1973; 1976), and also to protect when given *after* the onset of ischaemia (Rasmussen *et al.*, 1977; Jesmok *et al.*, 1978). Most authors have attributed the protective effects of β-blockers in ischaemia to an effect on oxygen supply and demand, changes in the balance of which may either increase or decrease the area of ischaemic injury (Maroko *et al.*, 1971). Studies with propranolol (e.g. Maroko & Braunwald, 1973; Sommers & Jennings, 1972), oxprenolol (Pelides *et al.*, 1972) and practolol and timolol (Libby *et al.*, 1973) have all shown a decrease in ischaemic injury in dogs associated with a decrease in oxygen demand of the myocardium.

Studies of the effects of thromboxane synthetase inhibitors on the ischaemic myocardium have mostly been performed in cats. A decrease in myocardial damage, assessed by changes in CPK levels in the ischaemic area, has been observed with imidazole (Smith *et al.*, 1980), CGS 13080 (Burke *et al.*, 1983a) and OKY-1581 (Burke *et al.*, 1983b). These protective effects are not believed to be due to a reduction in oxygen demand but to removal of the vasoconstrictor actions of thromboxane A_2 in the ischaemic region or to inhibition of the cytolytic actions of thromboxane A_2 since it increases the permeability of lysosomal membranes (Schrör *et al.*, 1980) and may thus contribute to the inflammatory processes during ischaemia.

Alternatively, inhibition of thromboxane synthesis may divert endoperoxides to form other prostaglandins, such as PGI_2, which has been shown to be cardioprotective in ischaemia (Ogletree *et al.*, 1979).

As described above, we have found no protection with either a β-adrenoceptor blocking agent or an inhibitor of thromboxane synthesis against myocardial damage following 48 h of ischaemia in rats. The discrepancies between results obtained with the same types of drugs in different animal models of ischaemia, i.e. cats and dogs, as opposed to rats may be related in part to the different methodologies employed to determine the extent of infarction. However, perhaps more importantly the results may be influenced by inter-species variations in the anatomy of the coronary circulation. Cats and dogs in general have a well developed collateral circulation, whereas rats have been reported to have a coronary collateral blood flow of only 6% (Winkler *et al.*, 1984). If resting collateral flow is so low in this species, how is it possible to achieve any myocardial salvage in rats as seen in the present study? It is possible that the protection seen with the combination of metoprolol and dazmegrel was not a reduction of damage in the ischaemic region *per se*, but rather a prevention of the extension of the damage beyond the 'boundary' of the ischaemic area by leakage of substances which are normally released during ischaemia (such as catecholamines and thromboxane) into areas of the left ventricular wall which will still have an adequate blood supply. This may also help to explain why only the drug combination was protective; it may be necessary to inhibit simultaneously the effects of more than one potentially damaging endogenous agent before significant salvage is achieved.

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