Br. J. Pharmac. (1985), **86**, 229–234

The effects of metoprolol and dazmegrel, alone and in combination, on arrhythmias induced by coronary artery occlusion in conscious rats

Istvan Leprán*, James R. Parratt, Laszlo Szekeres*, & Cherry L. Wainwright[1]

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, and the Institute of Pharmacology*, University Medical School of Szeged, Szeged, Hungary

1 The effects of metoprolol and the thromboxane synthetase inhibitor dazmegrel, alone and in combination, were examined in a model of coronary artery occlusion in conscious rats.

2 In a dose (2 mg kg^{-1}), intravenously, that resulted in a marked bradycardia (of 50–80 beats min^{-1}) metoprolol did not influence the incidence or severity of the ventricular arrhythmias that occur in the first 20 min following occlusion, nor did it improve survival (assessed at both 20 min and 16 h). In a dose (5 mg kg^{-1}), intravenously, that in another conscious rat model involving tissue hypoperfusion inhibited thromboxane production, dazmegrel also did not modify ischaemic arrhythmias or survival.

3 In contrast, metoprolol and dazmegrel (2 mg kg^{-1} and 5 mg kg^{-1} i.v.) when given together prior to coronary artery occlusion, produced a significant reduction in mortality both at 20 min and 16 h (e.g. from 60–75% in the control, metoprolol alone and dazmegrel alone groups and only 25% in the combined-treatment group). This was due to a decrease in the incidence of terminal ventricular fibrillation.

4 The results suggest that a combination of a β-adrenoceptor blocking drug with a drug that inhibits thromboxane synthesis may offer more protection against ischaemia-induced ventricular fibrillation than either drug used alone. They suggest a role for both catecholamines and thromboxane in the genesis of ischaemia-induced ventricular fibrillation.

Introduction

Results from experimental studies of acute myocardial ischaemia have shown that β-adrenoceptor blocking agents reduce the incidence and duration of the serious ventricular arrhythmias that follow coronary artery occlusion (reviewed by Fitzgerald, 1982). However, results from secondary prevention trials have shown that, although there is a significant decrease in sudden cardiac death, a significant number of patients given a β-blocker do not survive a subsequent infarction (Andersen *et al.*, 1979; Hjalmarson *et al.*, 1981). Assuming that at the time of death such patients had adequate plasma and tissue levels of the β-blocker, it would appear that factors other than catecholamine release contribute to the genesis of life-threatening arrhythmias early in ischaemia. There is experimental evidence for this. Studies performed in anaesthetized greyhounds have demonstrated the early release of thromboxane A_2 from the ischaemic region of the myocardium following coronary artery occlusion (Coker *et al.*, 1981; 1982) and drugs which inhibit the synthesis of thromboxane A_2, such as dazmegrel (Coker, 1984) and RO-22-4679 (Huddleston *et al.*, 1983) have been shown to protect against ischaemia-induced arrhythmias in dogs.

In a recent study we have shown that a combination of a β-adrenoceptor blocking agent (metoprolol) and a thromboxane synthetase inhibitor (dazmegrel) (Parry *et al.*, 1982) possesses antiarrhythmic properties against arrhythmias induced by coronary artery occlusion in anaesthetized rats (Parratt & Wainwright, 1983; Wainwright & Parratt, 1985). Because of the possibility that anaesthetics might complicate the incidence and severity of arrhythmias arising soon after the onset of ischaemia (Au *et al.*, 1979; MacLeod *et al.*, 1983) the present study was designed to evaluate the effects of these drugs, alone and in combination, against the early ischaemic arrhythmias which result from coronary artery occlusion in conscious rats. A preliminary account of these studies was presented to

[1]Author for correspondence.

the British Pharmacological Society in April, 1984 (Leprán et al., 1984).

Methods

The experiments were performed in the Institute of Pharmacology, Szeged. Male Sprague-Dawley CFY rats (170–200 g body weight) were subjected to a preliminary operation as described by Selye et al. (1960) and modified by Leprán et al. (1979). Under light ether anaesthesia a thoracotomy was performed at the fourth intercostal space and the heart was exposed by applying light pressure on the thorax. A 6/0 braided silk suture was passed through the wall of a small polythene tube, around the left coronary artery at its origin and back through the lumen to the tube. The thorax was closed and the tube left within the thoracic cavity between the heart and the chest wall. The loose ends of the ligature were fixed subcutaneously outside the thorax. Artificial ventilation was not required as the chest was open for less than 1 min.

Six to seven days after preliminary surgery the rats were lightly anaesthetized with ether and the loose ends of the ligature were exposed. At the same time bipolar ECG leads were implanted in the skin covering the chest wall and linked to an electrocardiagraph by a flexible lead. The animals were allowed to recover for several hours before metoprolol ($2\,mg\,kg^{-1}$), dazmegrel ($5\,mg\,kg^{-1}$), the drug combination (the drugs were given consecutively) or saline were administered via the tail vein. The doses were chosen (a) to provide a substantial degree of β-adrenoceptor blockade (Wainwright & Parratt, 1985) and (b) to inhibit thromboxane synthesis stimulated by impaired tissue perfusion (Furman et al., 1984).

Assessment of post-occlusion arrhythmias and statistical analysis

In both models the severity of the arrhythmias was assessed in the 0–20 min post-occlusion period by noting survival, the incidence and duration of ventricular fibrillation and of ventricular tachycardia (which, for the purpose of this study was defined as heart rate exceeding 500 beats min^{-1}) and of other types of arrhythmias including premature extrasystoles, sinus bradycardia and atrioventricular block.

Comparisons between results from different groups of data were made using Student's t test for unpaired data (independent t test). For comparisons of incidences of events the Chi Square test or Fisher's Exact probability test were used. All results were considered to be significant at probability level of $P < 0.05$.

The presence of an infarct was confirmed at the end of the experiment by incubating heart slices in nitro blue tetrazolium. Any 'missed occlusions' were excluded from the study.

Drugs

Dazmegrel and metoprolol were generously provided by Pfizer Central Research (Sandwich, Kent) and by Ciba-Geigy (Horsham) respectively.

Results

The effects of metoprolol, dazmegrel, the drug combination and of coronary artery occlusion on heart rate in conscious rats

The results are shown in Table 1, Metoprolol, either alone or as part of combination therapy with dazmegrel, reduced resting heart rate by a mean of 50–80 beats min^{-1}. This indicates a marked degree of β-adrenoceptor blockade; in anaesthetized rats this dose ($2\,mg\,kg^{-1}$) of metoprolol causes a 10 fold displacement of the cardiac responses to isoprenaline (Wainwright & Parratt, 1985). In general, this drug-induced bradycardia was maintained well into the occlusion period. It was also of some interest that the reduction in heart rate appeared to be more pronoun-

Table 1 The effects of saline (control), metoprolol ($2\,mg\,kg^{-1}$), dazmegrel ($5\,mg\,kg^{-1}$) a combination of the two drugs, and of coronary artery occlusion on heart rate in conscious rats

		Prior to occlusion	*Time (min) after occlusion*			
	n		1	5	10	15
Control	37	397 ± 14	403 ± 19	382 ± 22	381 ± 19	364 ± 16
Metoprolol	10	341 ± 7*	356 ± 18*	365 ± 25	355 ± 5	350 ± 10
Dazmegrel	10	419 ± 8	459 ± 13	390 ± 10	315 ± 75	345 ± 25
Metoprolol plus dazmegrel	12	316 ± 8*	328 ± 15*	297 ± 23*	330 ± 19*	300 ± 17*

Values are expressed as mean ± s.e.mean of n observations.
*$P < 0.05$ compared to corresponding saline (control) group

ced (and prolonged) when metoprolol was given together with dazmegrel than when given alone (Table 1). Coronary artery occlusion itself produced only a transient tachycardia.

The effects of metoprolol, dazmegrel and the drug combination on the incidence and severity of post-occlusion ventricular arrhythmias and on mortality

In control (saline-treated) rats, occlusion of the left coronary artery resulted in arrhythmias which started soon after the onset of ischaemia. After 20 min of occlusion the mortality in the control group of conscious rats was 60%; the usual cause of death was ventricular fibrillation, although a few animals died from pronounced bradycardia leading to asystole. The effects of pretreatment with metoprolol (2 mg kg^{-1}), dazmegrel (5 mg kg^{-1}) and the drug combination on survival following occlusion of the coronary artery are illustrated in Figure 1. Neither metoprolol nor dazmegrel alone had any effect on mortality at either 20 min or 16 h post-occlusion. However, administration of a combination of metoprolol and dazmegrel resulted in a marked improvement in survival at 20 min due mainly to a reduction in the number of animals dying in ventricular fibrillation. There was also a significant reduction in mortality at 16 h (Figure 1).

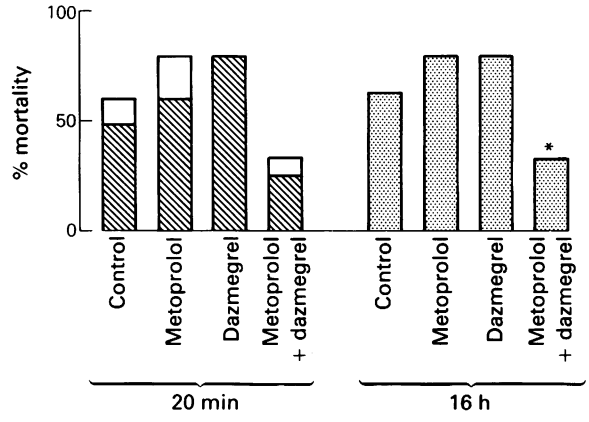

Figure 1 Mortality, at both 20 min and 16 h, in conscious rats subjected to coronary artery occlusion. The rats were pretreated with saline, metoprolol, dazmegrel or the drug combination. The hatched columns show the percentage incidence of mortality resulting from fibrillation, the open columns the incidence of mortality resulting from asystole and the stippled columns the total mortality at 16 h. Animals alive at this time were regarded as survivors.

The incidence of the different types of arrhythmias seen after occlusion of the coronary artery in the four treatment groups is shown in Table 2. Administration of either drug alone had no effect on the incidence of ventricular fibrillation, ventricular tachycardia or premature extrasystoles. The combination of metoprolol and dazmegrel produced no significant change in either the total incidence of ventricular fibrillation or ventricular tachycardia but there was a significant increase in the occurrence of premature ventricular beats.

Figure 2 shows the time course of arrhythmias both in animals that survived or that died. Dazmegrel significantly reduced the time to the first appearance of ectopic activity, whereas metoprolol, and the drug combination, produced no change in the time of appearance of the first arrhythmias, the time to death or to termination of arrhythmias. The total duration of arrhythmic activity was not altered by any of the treatments (41 ± 19 s in the control group, 35 ± 12 s in the dazmegrel group and 43 ± 14 s in the combination group).

Discussion

The results of this investigation show that pretreatment with a combination of metoprolol and dazmegrel increases survival following occlusion of a coronary artery close to its origin in conscious rats and this was

Table 2 The incidence of arrhythmias in conscious rats subjected to coronary artery occlusion and pretreated with saline (control group), metoprolol, dazmegrel or the drug combination

		% animals showing:		
Group	n	*ventricular fibrillation*	*ventricular tachycardia*	*ectopic beats*
Control	37	62	70	53
Metoprolol	10	70	70	80
Dazmegrel	10	90	60	70
Metoprolol and Dazmegrel	12	50	58	92*

*$P < 0.05$

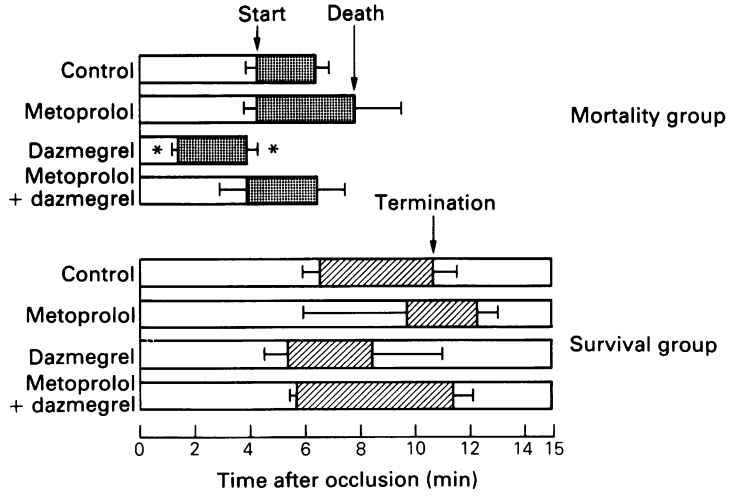

Figure 2 The time course of arrhythmias following coronary artery occlusion in conscious rats pretreated with saline (control), metoprolol, dazmegrel or the drug combination. Animals have been divided into two groups; those that died as a result of coronary artery occlusion and those that survived the procedure. The shaded area represents the period during which the arrhythmias occurred. Mortalities were 21 out of 37 in the control group; 8 out of 10 in the metoprolol and dazmegrel groups and 4/12 in the drug combination group.
*$P < 0.05$.

associated with a decrease in the incidence of terminal ventricular fibrillation.

Previous studies in which conscious models of myocardial infarction in rats were used have not shown the protection with β-blocking drugs that is characteristic of these drugs in anaesthetized animals. For example, Botting *et al.* (1983) were unable to demonstrate protection against arrhythmias in conscious rats with either acute or chronic β-blockade with propranolol in doses that have been shown to be antiarrhythmic in anaesthetized rats (Campbell & Parratt, 1983). The validity of anaesthetized animal coronary artery occlusion models has been questioned by Fozzard (1975) and it appears that the type and depth of anaesthesia is decisive to the appearance and severity of arrhythmias following coronary artery occlusion (Au *et al.*, 1979; MacLeod *et al.*, 1983). In a comparative study between the anaesthetized and conscious rat models, Kane *et al.* (1980) found that the type and time course of arrhythmias were similar in both groups (although there was increased mortality in the conscious model) and that arrhythmias in both models were protected by the membrane stabilizing drug Org 6001; higher doses were however required for antiarrhythmic effects in conscious rats. Similar observations have also been made with meptazinol, a partial agonist at opioid receptors (Fagbemi *et al.*, 1983). In the present studies, metoprolol alone, in a dose that profoundly reduced heart rate and which is markedly effective in the anaesthetized rat model (Wainwright & Parratt, 1985) did not, itself, reduce the severity or incidence of early ischaemic arrhythmias; neither did it reduce mortality (Figure 1). This result is thus in accord with the previous studies in this model outlined above and is discussed in detail in those papers.

The thromboxane synthetase inhibitor dazmegrel was also unable to protect against life-threatening arrhythmias when given alone. There is little published literature on the influence of such drugs on ischaemic arrhythmias in rats although there are some studies in conscious rats using cyclo-oxygenase inhibitors. Aspirin, alone and in combination with prostacyclin, has been demonstrated to be antiarrhythmic in the early (0–30 min) period following coronary artery occlusion (Johnston *et al.*, 1983), as have high doses of sulphinpyrazone, indomethacin and salicylates (Leprán *et al.*, 1983). There is a good deal of evidence implicating thromboxane in the genesis of reperfusion arrhythmias (Coker *et al.*, 1982; Coker, 1984), but the role of thromboxane in ischaemic arrhythmias has not yet been clarified, although the thromboxane mimetic U46619 has been shown to produce arrhythmias in dogs (Mehta *et al.*, 1982). The results shown here would suggest that although thromboxane may be involved it is not alone of major importance.

From the results of this study it would appear that metoprolol and dazmegrel do not protect against life-threatening arrhythmias when given separately but do when the two sub-optimal doses of the drugs were

combined. The mechanism of this protective action is unknown but could imply that both noradrenaline and thromboxane, when released together during the early stages of ischaemia, have detrimental effects on the myocardium. One possible mechanism of this protection might be inhibition of platelet aggregation (an early consequence of coronary artery occlusion) since there is recent clinical evidence that, *in vitro*, combining aspirin (acting presumably by thromboxane synthetase inhibition) and the α and β-adrenoceptor blocking drug labetalol produces a significantly greater inhibition of platelet aggregation than when either drug is given alone (Greer *et al.*, 1985).

Such a drug combination would have the added advantage of protecting against reperfusion arrhythmias, should they occur.

References

ANDERSON, M.P., FREDERICKSEN, J., JURGENSEN, H.J., PEDERSEN, F. & BECHSGAARD, P. (1979). The effects of alprenolol mortality among patients with definite or suspected acute myocardial infarction. *Lancet*, **ii**, 865–868.

AU, T.L., COLLINS, G.A., MacLEOD, B.A. & WALKER, M.J.A. (1979). Actions of nitroglycerin and propranolol in rats after coronary artery ligation. *Pharmacologist*, **21**, 274, 4680A.

BOTTING, J.H., JOHNSTON, K.M., MacLEOD, B.A. & WALKER, M.J.A. (1983). The effect of modification of sympathetic activity on responses to ligation of a coronary artery in the conscious rat. *Br. J. Pharmac.*, **79**, 265–271.

CAMPBELL, C.A. & PARRATT, J.R. (1983). The effect of β-adrenoceptor blocking agents, with differing ancillary properties, on the arrhythmias resulting from acute coronary artery ligation in anaesthetised rats. *Br. J. Pharmac.*, **79**, 939–946.

COKER, S.J. (1984). Further evidence that thromboxane excaberates arrhythmias: effects of UK 38485 during coronary artery occlusion and reperfusion in anaesthetised greyhounds. *J. molec. cell. Cardiol.*, **16**, 633–641.

COKER, S.J., PARRATT, J.R., LEDINGHAM, I.McA. & ZEITLIN, I.J. (1981). Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. *Nature, New Biol.*, **291**, 323–324.

COKER, S.J., PARRATT, J.R., LEDINGHAM, I.McA. & ZEITLIN, I.J. (1982). Evidence that thromboxane contributes to ventricular fibrillation induced by reperfusion of the ischaemic myocardium. *J. molec. cell. Cardiol.*, **14**, 483–485.

FAGBEMI, O., KANE, K.A., LEPRÁN, I., PARRATT, J.R. & SZEKERES, L. (1983). Antiarrhythmic actions of meptazinol, a partial agonist at opiate receptors, in acute myocardial ischaemia. *Br. J. Pharmac.*, **78**, 455–460.

FITZGERALD, J.D. (1982). The effects of β-adrenoceptor blocking drugs on early arrhythmias in experimental and clinical myocardial ischaemia. In *Early Arrhythmias resulting from Myocardial Ischaemia: Mechanisms and Prevention by Drugs*. ed. Parratt, J.R. pp. 295–315. London: MacMillan.

FOZZARD, H.A. (1975). Validity of myocardial infarction models. *Circulation*, **51–52** (suppl. III), 131–146.

FURMAN, B.L., McKECHNIE, K. & PARRATT, J.R. (1984). Failure of drugs which selectively inhibit thromboxane synthesis to modify endotoxin shock in conscious rats. *Br. J. Pharmac.*, **82**, 289–294.

GREER, I.A., WALKER, J.J., CALDER, A.A. & FORBES, C.D. (1985). Aspirin with an adrenergic or calcium-channel blocking agent as new combination therapy for arterial thrombosis. *Lancet*, **i**, 351–352.

HJALMARSON, A., ELMFELDT, D., HERLITZ, J., HOLMBERG, S., MALEK, I., NYBERG, G., RYDEN, L., SWEDBERG, K., VEDIN, A., WAAGSTEIN, F., WALDENSTROM, A., WALDENSTROM, J., WEDEL, H., WILHELMSEN, L. & WILHELMSSON, C. (1981). Effect on mortality of metoprolol in acute myocardial infarction. *Lancet*, **ii**, 823–827.

HUDDLESTON, C.B., LUPINETTI, F.M., LAWS, K.H., COLLINS, J.C., CLANTON, J.A., HAWIGER, J.J., OATES, J.A. & HAMMON, J.W. (1983). The effects of RO-22-4679, a thromboxane synthetase inhibitor, on ventricular fibrillation induced by coronary artery occlusion in conscious dogs. *Circulation Res.*, **52**, 608–613.

JOHNSTON, K.M., MacLEOD, B.A. & WALKER, M.J.A. (1983). Effects of aspirin and PGI_2 on arrhythmias resulting from coronary artery ligation and on infarct size. *Br. J. Pharmac.*, **78**, 29–38.

KANE, K.A., LEPRÁN, I., McDONALD, F.M., PARRATT, J.R. & SZEKERES, L. (1980). The effects of prolonged oral administration of a new antidysrhythmic drug (ORG 6001) on coronary artery ligation dysrhythmias in conscious and anaesthetised rats. *J. cardiovasc. Pharmac.*, **2**, 411–423.

LEPRÁN, I., KOLTAI, M. & SZEKERES, L. (1983). Effect of non-steroidal anti-inflammatory drugs in experimental myocardial infarction in rats. *Eur. J. Pharmac.*, **69**, 235–238.

LEPRÁN, Z., PARRATT, J.R., SZEKERES, L. & WAINWRIGHT, C.L. (1984). Antiarrhythmic effects of a metoprolol-thromboxane synthesis inhibitor combination in conscious rats. *Br. J. Pharmac.*, **82**, 225P.

LEPRÁN, I., SIEGMUND, W. & SZEKERES, L. (1979). The method for acute coronary occlusion in closed chest rats. *Acta physiol. Acad. Sci. hung.*, **53**, 190.

MacLEOD, B.A., AGEREAU, P. & WALKER, M.J.A. (1983). Effects of halothane anaesthesia compared with fentanyl anaesthesia and no aneasthesia during coronary artery ligation in rats. *Anaesthesiology*, **58**, 44–52.

MEHTA, J., NICHOLS, W., MEHTA, P. & CONTI, C.R. (1982). Thromboxane and prostacyclin in systemic and coronary vascular beds following endoperioxide analog infusion. *Am. J. Cardiol.*, **49**, Abstract 1014.

PARRATT, J.R. & WAINWRIGHT, C.L. (1983). β-adrenoceptor blockade, with and without a thromboxane synthetase inhibitor on ischaemic arrhythmias. *J. molec. cell. Cardiol.*, **15**, Suppl. 1, Abstract 346.

PARRY, M.J., RANDALL, M.J., HAWKERWOOD, E., CROSS, P.E. & DICKINSON, R.P. (1982). Enhanced production of prostacyclin in blood after treatment with selective thromboxane synthetase inhibitor, UK-38, 485. *Br. J. Pharmac.*, **77**, 547P.

SELYE, H., BAJUSZ, E., GRASSO, E. & MENDELL, P. (1960). Simple techniques for the surgical occlusion of coronary vessels in the rat. *Angiology*, **11**, 398–407.

WAINWRIGHT, C.L. & PARRATT, J.R. (1985). The effect of β-adrenoceptor blockade and of thromboxane synthetase inhibition, alone and in combination, on arrhythmias resulting from myocardial ischaemia. *Adv. Myocardiol.*, **6**, 573–583.

(*Received March 7, 1985.*
Revised May 8, 1985.
Accepted May 16, 1985.)