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Effects of the PAF antagonists BN50726 and BN50739 on arrhythmogenesis and extent of necrosis during myocardial ischaemia/reperfusion in rabbits

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1 The effects of two novel platelet activating factor (PAF) antagonists BN50726 and BN50739 on arrhythmias, haemodynamics and extent of necrosis during myocardial ischaemia and reperfusion were investigated in anaesthetized rabbits subjected to coronary artery ligation.

2 BN50739 reduced heart rate prior to coronary artery occlusion ($P < 0.005$) but had no other significant haemodynamic effects at this time. BN50739 and BN50726 did not significantly alter heart rate or blood pressure during 30 min of ischaemia or 30 min of reperfusion, compared to control hearts.

3 BN50739 and BN50726 had no effect on the incidence of arrhythmias during ischaemia. BN50726 significantly reduced the incidence of reperfusion ventricular fibrillation compared to controls (0% v 40%, $P < 0.05$), and improved survival (80% v 39%, $P < 0.05$). Similar trends were observed with BN50739.

4 BN50726 reduced the extent of necrosis compared to control hearts ($18 \pm 2\%$ v $30 \pm 3\%$, $P < 0.01$). A similar trend was observed with BN50739.

5 These results demonstrate that PAF antagonism with BN50726 attenuates reperfusion-induced arrhythmias and preserves myocardium in the early phase of ischaemia, independently of haemodynamic effects.

Keywords: Platelet activating factor; ischaemia; reperfusion; haemodynamics; arrhythmias; necrosis; infarction

Introduction

Clinical and experimental studies suggest that platelet activation contributes to myocardial necrosis during ischaemia and reperfusion (Mehta & Mehta, 1979; Mikhailidis *et al.*, 1987; Rösen *et al.*, 1987; Wainwright *et al.*, 1989; Chakrabarty *et al.*, 1991a). Release of platelet activating factor (PAF) from the ischaemic myocardium has been demonstrated experimentally and in man (Lotner *et al.*, 1980; Annable *et al.*, 1985; Zimmerman *et al.*, 1985; Montrucchio *et al.*, 1986; 1989; Sisson *et al.*, 1987). Experimental studies have shown that PAF increases myocardial necrosis and arrhythmogenesis (Mickelson *et al.*, 1988; Chakrabarty *et al.*, 1991a) during myocardial ischaemia and that it has direct myocardial cellular electrophysiological and arrhythmogenic effects (Flores & Sheridan, 1990). Recent studies have shown that PAF antagonists have opposite actions, reducing the incidence of arrhythmias and preventing necrosis during ischaemia and reperfusion (Wainwright *et al.*, 1989; Chakrabarty *et al.*, 1991a; Koltai *et al.*, 1991a). Such findings suggest that PAF could play a significant role in the progression and complications of myocardial ischaemia. While PAF antagonism has been shown to be effective during experimental myocardial ischaemia it is not clear whether it will be beneficial clinically at improving survival and reducing morbidity.

Although several PAF antagonists are available, relatively high concentrations are required *in vivo* and *in vitro* to antagonize the effects of PAF. BN50739 (6-(2-chlorophenyl)-9-[2- (3,4-dimethoxyphenyl) thio]-1-thioxoethyl) -7,8,9,10-tetrahydro-1-methyl]-4H-pyrido [4'3':4,5] thieno [3,2-f] [1'2'4] triazolo [4,3-a] [1,4] diazepine) and BN50726 (6-(2-chlorophenyl)-9- (hexadecylsulphonyl) -7,8,9,10-tetrahydro-1-methyl-4H-pyrido [4'3':4,5] thieno [3,2-f] [1'2'4] triazolo [4,3-a] [1,4] diazepine) are two novel synthetic PAF antagonists which have been developed recently and shown to be more potent than other compounds in antagonizing the effects of PAF (Yue *et al.*, 1990). Koltai *et al.* (1991a) recently reported that BN50739 reduced the incidence of ventricular arrhythmias during myocardial ischaemia in rat isolated hearts in the absence of platelets, but relatively little is known about the effects of BN50739 and BN50726 *in vivo*. To gain further information, we performed experiments designed to examine the haemodynamic, antiarrhythmic and anti-necrotic effects of these PAF antagonists during regional myocardial ischaemia and reperfusion in anaesthetized open chest rabbits.

Methods

New Zealand White rabbits (2.5–3.0 kg) were maintained at 21–22°C, $50 \pm 5\%$ humidity and 12/12 hourly light/dark cycles for 3 to 5 days prior to study. Animals were fed on R14 'high fibre without grass' meal and water *ad libitum*. All experiments were carried out in a single laboratory maintained at 21–22°C throughout the year between the hours of 9 am and 6 pm.

Surgical procedures

Anaesthesia was induced with alphaxalone (9 mg kg^{-1}, i.v.) and maintained with pentobarbitone (25 mg kg^{-1}). Tracheostomy and tracheal intubation were performed and animals were ventilated with room air by means of a mechanical pump (CF Palmer (London) Ltd., Model No. 16/24) at a fixed rate of 45 strokes min^{-1} and a tidal volume of 25–30 ml producing an arterial P_{O_2} of 96 mmHg, P_{CO_2} of 36 mmHg and pH of 7.36. Body temperature was monitored with an oesophageal thermistor and maintained at a constant level by an overhead heating lamp. The right carotid artery was cannulated to monitor the arterial pressure using a Lec-

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tromed transducer (No. 3552; Lectromed Ltd., Letchworth). ECG leads were connected to the four limbs and the signals amplified using a Lectromed ECG amplifier. Arterial pressure and ECG signals were continuously recorded on a Lectromed Multitrace 4 chart recorder.

A thoracotomy was performed via a left parasternal incision between the fourth and fifth ribs. The heart was exposed by incising the pericardium and was then supported in a pericardial cradle. The left ventricular branch of the circumflex artery was identified (Flores *et al.*, 1984) and a loose 2/0 polyester ligature was placed around it adjacent to its origin. Each preparation was allowed to stabilize for 45 min before coronary artery occlusion. Regional ischaemia was produced with a snare made of polyethylene tubing 2.5 cm in length and 3 mm in diameter which was threaded over the suture and clamped firmly in place for 30 min. Reperfusion was induced by releasing the clamp. Following 30 min of reperfusion the animals were killed with an overdose of anaesthetic. The hearts were removed for measurement of the extent of myocardial damage.

Drug administration

BN50739 and BN50726 were obtained from Institut Henri Beaufour, Le Plessis Robinson, France. Both compounds, at a dose of 5 mg kg^{-1}, were dissolved in 0.5 ml of dimethylsulphoxide (DMSO, Sigma Chemicals Co., Poole, Dorset) and made up to 5 ml with distilled water and given intravenously through the marginal ear vein 10 min before coronary occlusion. Ten animals received BN50739, with ten receiving BN50726. Eighteen animals received vehicle alone and served as controls. Unequal numbers were studied so as to offset the greater survival in the treated group and provide similar numbers of hearts for infarct size measurement.

Arrhythmia analysis

The ECG recordings were analysed according to the guidelines of the Lambeth Conventions (Walker *et al.*, 1988). The incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) were noted during ischaemia and reperfusion. Only those animals that remained in sinus rhythm throughout ischaemia, or had spontaneously reverted from a ventricular arrhythmia to sinus rhythm by the end of the ischaemic period, were reperfused. Animals which remained in sinus rhythm until the end of reperfusion, or spontaneously reverted so that they were in sinus rhythm at the end of reperfusion, were regarded as survivors. VT was defined as a run of four or more consecutive ventricular premature beats.

Infarct size measurement

Infarct size measurements were made only on survivors. Following the 30 min of reperfusion each heart was removed and immediately placed in a 10% KCl solution to induce rapid asystole. Thereafter, the heart was cut into four sections from base to apex, each approximately 2 mm thick. The sections were washed in cold normal saline and placed in freshly prepared 0.5% w/v nitrobluetetrazolium (Sigma Chemical Co., Poole, Dorset) dissolved in phosphate buffer at pH 7.4 at 37°C for 10 min. They were then washed in water and placed in formaldehyde solution for fixation overnight. The sections were photographed with a reference grid on 35 mm colour slides and their projected images were used to measure the areas of infarction (absence of blue staining) and non-infarction (blue staining) by computerized planimetry, as previously described (Chakrabarty *et al.*, 1991a). The volumes of each section were calculated as the product of the planimetered areas and the section thickness. The volume of infarction was calculated as a percentage of each section and the total left ventricular volume.

Statistical analysis

Haemodynamic parameters are presented as means ± standard error of the mean. Results were compared by Student's paired *t* test within groups and Student's unpaired *t* test between groups. Arrhythmia analysis was performed by Chi-squared analysis and infarct size analysis was performed by Student's unpaired *t* test. Statistical significance was accepted if $P < 0.05$.

Results

Haemodynamic changes

Figures 1 and 2 show the haemodynamic changes that occurred in the three groups. No significant differences in blood pressure were observed prior to coronary artery occlusion. Coronary artery occlusion significantly reduced blood pressure in both the control and the BN50739 groups (from 98 ± 2 mmHg to 86 ± 3 mmHg after 5 min of ischaemia, $P < 0.001$ in control hearts, and from 95 ± 3 mmHg to 83 ± 4 mmHg, $P < 0.01$, after 5 min in BN50739 treated hearts). A similar trend was observed in the BN50726 group but this failed to reach statistical significance (from 99 ± 3 mmHg to 88 ± 5 mmHg after 5 min of ischaemia). No significant differences in blood pressure were observed between the groups during ischaemia or reperfusion. BN50739 reduced heart rate from 260 ± 9 beats min^{-1} to 237 ± 8 beats min^{-1} ($P < 0.01$) prior to coronary artery occlusion, but BN50726 was without effect. No significant differences in heart rate were observed between the groups during ischaemia or reperfusion.

Arrhythmias

Figure 3 illustrates the percentage incidence of VF during ischaemia and reperfusion for the control and treated groups. No significant differences in the incidence of VT during ischaemia were observed between the control group and either of the treated groups: 7/18 (39%) of control rabbits, 1/10 (10%) of rabbits treated with BN50739 and 1/10 (10%)

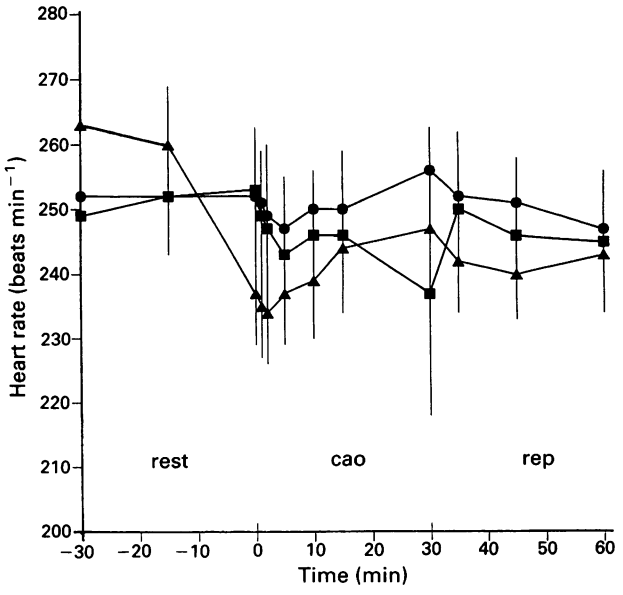

Figure 1 Changes in heart rate in control (●), BN50739 (▲), and BN50726 (■)-treated rabbits before coronary occlusion (rest), during occlusion (cao) and during reperfusion (rep). BN50739 reduced heart rate prior to coronary occlusion. No significant differences were observed between the groups during ischaemia or reperfusion.

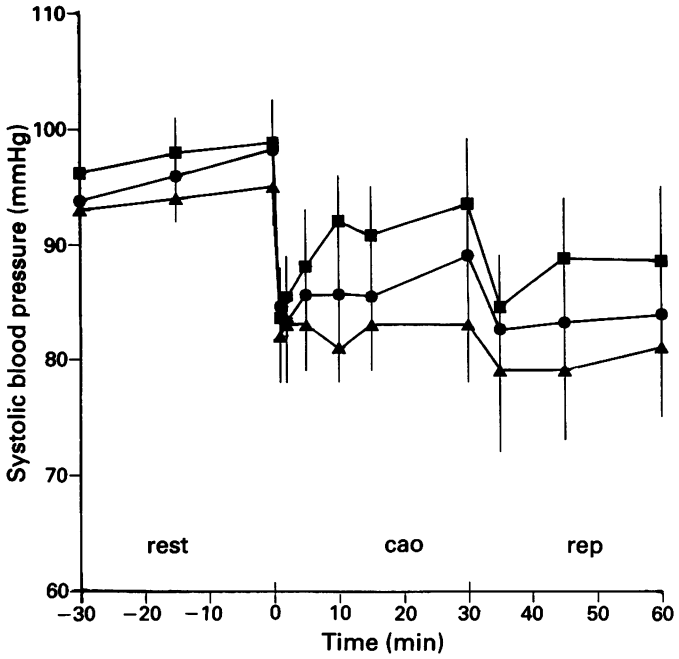

Figure 2 Changes in systolic blood pressure in control (●), BN50739 (▲) and BN50726 (■)-treated rabbits before coronary artery occlusion (rest), during occlusion (cao) and during reperfusion (rep). Blood pressure fell significantly following ligation in the control and BN50739 treated groups, a similar trend was seen in the BN50726-treated group but this did not reach significance. No significant differences were observed between the groups prior to, during or following ischaemia.

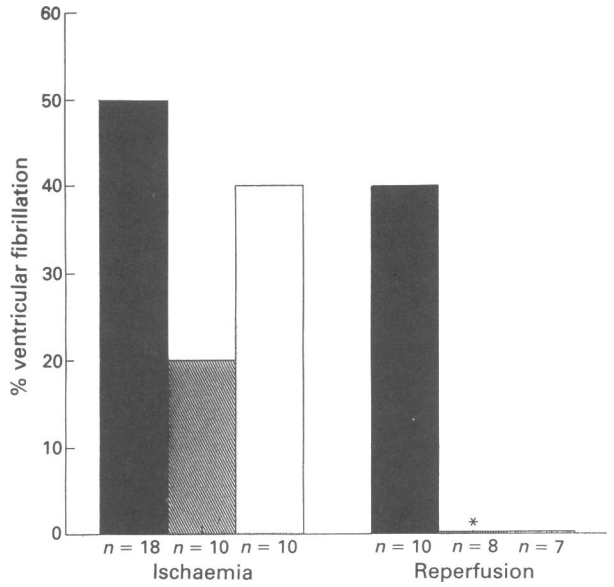

Figure 3 Effects of BN50726 (hatched column) and BN50739 (open column) on the incidence of ventricular fibrillation (VF) during ischaemia and reperfusion. BN50726 significantly reduced the incidence of VF during reperfusion compared to control hearts (solid column).
*$P < 0.05$ v the control group.

of rabbits treated with BN50726. Similarly, no significant differences in the incidence of VF during ischaemia were observed between control and treated groups: 9/18 (50%) of control rabbits, 4/10 (40%) for the BN50739-treated group and 2/10 (20%) for the BN50726-treated group. Although 9/18 control hearts developed VF during ischaemia, one of these recovered spontaneously so that 10 control rabbits survived ischaemia. Of the 10 rabbits treated with BN50739,

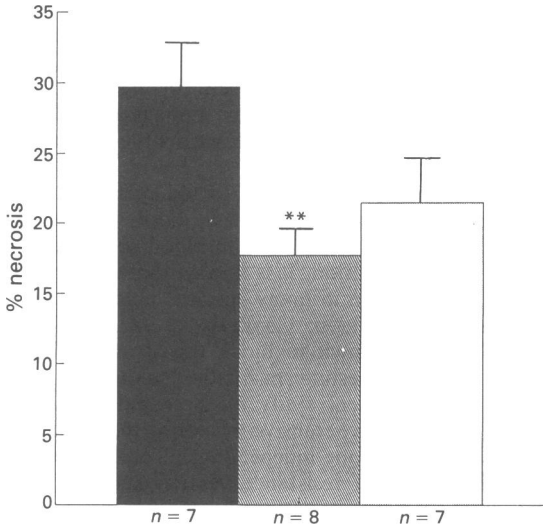

Figure 4 Effects of BN50726 (hatched column) and BN50739 (open column) on the extent of myocardial necrosis expressed as a percentage of total left ventricular volume. BN50726 significantly reduced the extent of necrosis compared to control hearts.
**$P < 0.01$ v control group.

7 survived ischaemia and 8 of the 10 rabbits treated with BN50726 survived ischaemia and went on to reperfusion. Death during ischaemia was due to terminal VF. No significant differences in the incidence of VT during reperfusion were observed between the control group and either of the treated groups: 4/10 (40%) of controls, 1/7 (14%) of the BN50739-treated group and 2/8 (25%) of the BN50726-treated group. BN50726 significantly reduced the incidence of VF occurring during reperfusion compared to the control group (0/8 [0%] v 4/10 [40%], $P < 0.05$). A similar trend was observed in the BN50739 treated group, but this just failed to reach significance (0/7 [0%] v 4/10 [40%]).

Administration of BN50726 significantly increased the number of rabbits surviving to the end of reperfusion compared to untreated rabbits (8/10 [80%] BN50726 v 7/18 [39%] controls, $P < 0.05$). BN50739 tended to improve survival but this just failed to reach statistical significance (7/10 [70%] BN50739 v 7/18 [39%] controls).

Infarct size

Figure 4 illustrates the extent of myocardial necrosis, as a percentage of total left ventricular volume, observed in control and treated groups. Pretreatment with BN50726 reduced the extent of necrosis compared to the control group from $29.61 \pm 3.17\%$ to $17.85 \pm 1.92\%$ ($P < 0.01$). A similar trend was observed for BN50739 but failed to reach statistical significance ($29.61 \pm 3.17\%$ v $21.41 \pm 3.10\%$).

Discussion

This study demonstrated that PAF antagonism with BN50726 significantly reduced the incidence of ventricular fibrillation during reperfusion, compared to control hearts. This was associated with a reduction in the extent of necrosis and a reduction in mortality. BN50739 produced similar trends but these just failed to reach statistical significance. These antiarrhythmic and anti-necrotic effects appeared to be independent of haemodynamic changes.

We have previously used this anaesthetized, open chest rabbit model to investigate the effects, on arrhythmogenesis and necrosis, of modulating PAF activity during myocardial ischaemia (Chakrabarty *et al.*, 1991a). Following coronary artery ligation in the rabbit, there is a significant fall in blood

pressure, high incidence of ventricular arrhythmias and a substantial volume of myocardial necrosis. In this model the volume of necrosis tends to be greatest at the apex, which is due to the coronary artery chosen for ligation. The left ventricular branch of the circumflex supplies a consistent and large portion of the left ventricle with little collateral flow (Flores et al., 1984).

Despite the ability of BN50726 to reduce the incidence of arrhythmias and reduce necrosis, both BN50726 and BN50739 failed to prevent the fall in blood pressure observed following coronary ligation. It is known that exogenous PAF can induce hypotension in many species, including the rabbit and rat (Yue et al., 1990; Chakrabarty et al., 1991a) and BN50739 has been shown to block this in rats (Yue et al., 1990). Although the mechanism for the hypotensive action of PAF is unclear, a recent study by Yamanaka et al. (1992) revealed a biphasic hypotensive response mediated by two different mechanisms, one independent of prostaglandins and involving dilatation of resistance vessels, and the other involving venodilatation and mediated by prostaglandins. Previous studies have shown that PAF antagonists can attenuate the hypotension following coronary occlusion (BN52021, SDZ63-675 in the rabbit; Montrucchio et al., 1990; Chakrabarty et al., 1991a), however, other studies have failed to show such an effect (BN52021, SRI63441 and CV3988 in the dog; Wainwright et al., 1989; Maruyama et al., 1990). This may indicate that other mechanisms, e.g. loss of regional contractility and cardiac output following an abrupt reduction in flow, are involved. Platelets from different species are however known to respond to PAF differently (McManus et al., 1981; Cargill et al., 1983) and it appears that there may be different PAF receptors on different cells in the same species (Stewart & Dusting, 1988).

The fall in heart rate with injection of BN50739 is difficult to explain and has not been reported before with other antagonists (Wainwright et al., 1989; Montrucchio et al., 1990; Chakrabarty et al., 1991a). PAF is known to cause a bradycardia as well as other haemodynamic effects such as vasoconstriction, hypotension, decreased contractility and decreased cardiac output (Chakrabarty et al., 1991a) and it may be that BN50739 has partial agonist activity although there were no other haemodynamic changes, with its injection, to support this. The structure of BN50739 also allows for potential free radical scavenging activity (Koltai et al., 1991b).

The antiarrhythmic properties of BN50726 that were observed in this study and the similar trend seen with BN50739 give further evidence for a role of PAF in mediating arrhythmogenesis during myocardial ischaemia, and correlate well with other reports using PAF antagonists (Wainwright et al., 1989; Yue et al., 1990; Koltai et al., 1991a; Chakrabarty et al., 1991a). The exact mechanism through which PAF mediates these effects is not clear. Direct myocardial actions, which are known to be arrhythmogenic (Tamargo et al., 1985; Stahl et al., 1988; Flores & Sheridan, 1990) may be important, together with the activation of platelets and/or white cells (Wainwright et al., 1989; Chakrabarty et al., 1991a). Koltai et al. (1991a) recently commented on the complexity of the actions of PAF in the whole animal. Interactions between PAF and cytokines may promote cellular necrosis, and PAF may also alter calcium influx and enhance sodium/hydrogen exchange (Koltai et al., 1991a) producing cellular electrophysiological effects which alter the susceptibility to arrhythmogenesis. The relative ability of PAF antagonists to prevent these effects may be important in determining their efficacy in preventing arrhythmogenesis and reducing or delaying the extent of necrosis.

Comparison of results obtained in this study with our earlier investigation of the effects of PAF antagonism using BN52021 at a dose of 10 mg kg^{-1} (Chakrabarty et al., 1991a) reveals that BN50726 and BN50739 reduced the extent of myocardial necrosis following coronary ligation to a similar extent, but with a dose of 5 mg kg^{-1}, compared to that seen with BN52021 and pretreatment with platelet antiserum. Whereas the antiarrhythmic potency of BN50739 has been shown to be six times that of BN52021 (Koltai et al., 1991b), no comparable data are available for BN50726 (M. Koltai, personal communication). Yue et al. (1990) compared the ability of BN50739, BN50726 and BN52021 to antagonize PAF-induced 5-hydroxytryptamine release from rabbit platelets and reported IC_{50} values of 3.67 ± 0.20 nM, 5.40 ± 1.68 nM and $14\,900 \pm 1\,600$ nM respectively. Little information is available about the effects of these compounds *in vivo*, although a preliminary report by Schaer et al. (1990) described beneficial effects of BN50739 against reperfusion injury at a dose of 5 mg kg^{-1} in dogs. Although we did not measure risk zone in these experiments, we have previously shown that the risk zone represents a consistent and highly reproducible percentage of the left ventricular volume when the left ventricular branch of the circumflex artery is occluded (Chakrabarty et al., 1991b).

In conclusion, these results provide further evidence that modification of the effects of platelet activating factor has important benefits in the early stages of acute myocardial infarction. Further studies to investigate the mode of action of these agents, and to investigate their potential role in clinical practice should be useful in providing a clearer understanding of the importance of PAF in mediating the effects observed.

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