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The relationship between neutrophils and increased microvascular permeability in a model of myocardial ischaemia and reperfusion in the rabbit

[1]Frances M. Williams, Paul D. Collins, *Marianne Tannière-Zeller & Timothy J. Williams

Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY and *University of Bourgogne, Dijon, France

1 ^{111}In-labelled neutrophils and ^{125}I-labelled albumin were used to measure neutrophil accumulation and microvascular plasma protein leakage in the ischaemic/reperfused myocardium of anaesthetized rabbits.

2 A period of 30 min coronary artery occlusion followed by 3 h reperfusion resulted in an increase in both ^{111}In and ^{125}I counts in the area at risk (AR) of the myocardium.

3 Pretreatment of ^{111}In-neutrophils *in vitro* with monoclonal antibody 60.3 directed against the CD18 antigen on neutrophils, followed by intravenous administration, significantly suppressed their accumulation into the AR myocardium.

4 Depletion of circulating neutrophils by use of anti-neutrophil serum or mustine hydrochloride did not affect plasma protein leakage into the AR myocardium.

5 Administration of the platelet activating factor (PAF) antagonist WEB 2086 (10 mg kg^{-1}, i.v.) had no effect on the accumulation of ^{111}In-neutrophils or on plasma protein leakage in the AR myocardium.

Introduction

Myocardial tissue which has undergone a period of ischaemia displays characteristic signs of an acute inflammatory response (Sommers & Jennings, 1964; Lucchesi & Mullane, 1986). Among the early events characterizing this response are neutrophil infiltration (Fishbein *et al.*, 1978; Engler *et al.*, 1983; Mullane *et al.*, 1984) and oedema formation (Sommers & Jennings, 1964; Reimer & Jennings, 1979; Mullane *et al.*, 1984). Reports that depletion of circulating neutrophils is associated with a reduction in myocardial infarct size (Romson *et al.*, 1983; Mullane *et al.*, 1984) have stimulated increased interest in this aspect of the inflammatory response. However, the amount of oedema formation has also been shown to influence the size of the infarct (Reimer & Jennings, 1979). Thus interventions which affect oedema formation may also have an apparent effect on infarct size.

The evidence that neutrophils have a deleterious effect on infarcted myocardium comes from experimental studies showing a protective effect when agents such as anti-neutrophil serum (Romson *et al.*, 1983), ibuprofen (Romson *et al.*, 1982) and prostacyclin (Simpson *et al.*, 1987) are used. An alternative approach is to block the neutrophil surface adhesion glycoprotein complex, CD11b/CD18. There is evidence that this complex is involved in the adherence of the neutrophil to the vascular endothelium (Pohlman *et al.*, 1986; Wallis *et al.*, 1986), which is the first stage in local neutrophil accumulation in response to inflammatory stimuli. A monoclonal antibody 60.3, which binds to an epitope on CD18, has been found to be effective in inhibiting extravascular migration of neutrophils in response to chemoattractants when given systemically (Arfors *et al.*, 1987) or when used to pretreat ^{111}In-neutrophils *in vitro* before intravenous injection into recipients (Rampart & Williams, 1988; Nourshargh *et al.*, 1989). In the study described here we have used the latter method to examine whether this adhesion glycoprotein is important in the infiltration of neutrophils into ischaemic/reperfused myocardium of anaesthetized rabbits.

In a model of intestinal ischaemia and reperfusion, increased microvascular permeability was found to be dependent on circulating neutrophils (Hernandez *et al.*, 1987). We have previously shown that plasma protein leakage in response to certain chemoattractants such as the complement-derived peptide C5a, leukotriene B_4 (LTB_4) and N-formyl-methionyl-leucyl-phenylalanine (FMLP) is also dependent on circulating neutrophils (Wedmore & Williams, 1981a). In the study described here we have used a rabbit model of myocardial ischaemia and reperfusion to examine whether a similar relationship between neutrophils and oedema can be demonstrated in the heart using anti-neutrophil serum or mustine hydrochloride to cause systemic neutrophil depletion. In addition we have also considered whether platelet activating factor (PAF), which causes oedema formation by a neutrophil-independent mechanism (Wedmore & Williams, 1981b) could have a role in this situation by using the potent PAF antagonist WEB 2086.

Methods

^{111}In-labelling of rabbit neutrophils

The method used for labelling neutrophils was as previously described (Rampart & Williams, 1988). Briefly the method was as follows. Male specific pathogen-free, New Zealand White rabbits (2.5–3.5 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Blood was collected into acid citrate dextrose and red blood cells removed by centrifuge-facilitated sedimentation with hydroxyethylstarch (3% final concentration). Leukocytes were separated from platelets by further centrifugation and removal of platelet rich plasma. The white blood cell pellets were resuspended in a minimum volume of autologous citrated plasma and applied to a two layer discontinuous Percoll-plasma gradient (3 ml of 50% Percoll layered over 3 ml of 69% Percoll). Neutrophils (>90% pure) were harvested from the 50–69% Percoll interface. Cells (3.5–5.0×10^7 in 1.2–1.5 ml) were incubated for 15 min at room temperature with $^{111}InCl_3$ (50–200 μCi) chelated with 2-mercaptopyridine-N-oxide (40 μg in 0.1 ml of 50 mM phosphate buffered saline). Labelled cells were then washed three times in autologous plasma and resuspended in a volume of 3 ml of plasma for intravenous injection into recipient animals.

[1] Author for correspondence.

Coronary artery occlusion and reperfusion in anaesthetized rabbits

Male specific pathogen-free, New Zealand White rabbits (2.5–3.5 kg) were anaesthetized initially with Hypnorm (0.3 ml kg^{-1}, i.m) followed by diazepam (0.5 mg kg^{-1}, i.v). Anaesthesia was maintained thereafter with sodium pentobarbitone. Animals were intubated and received positive pressure ventilation with room air (32 strokes min^{-1}, 21 ml stroke volume). A central ear artery was cannulated to allow the measurement of arterial blood pressure and a lead I electrocardiogram recorded from surface electrodes. A left thoracotomy was performed at the 5th intercostal space and the heart exposed through the pericardium. A silk ligature (mersilk 6/0) was positioned around a branch of the left circumflex coronary artery, and a piece of polythene tubing tied down over the artery. The occlusion was maintained for 30 min after which the ligature was cut and reperfusion allowed for 3 h. In the case of sham occlusions the ligature remained in place but was not tied.

Measurement of ^{111}In-neutrophil accumulation and plasma protein leakage in ischaemic/reperfused myocardium

Oedema formation and neutrophil accumulation were measured as the accumulation of ^{125}I-human serum albumin and ^{111}In-labelled neutrophils respectively. ^{125}I-albumin was administered intravenously 15 min prior to coronary artery occlusion at a dose of 15 μCi when ^{111}In was present, or 5 μCi in its absence. ^{111}In-neutrophils were also administered intravenously, 15 min before occlusion.

At the end of the reperfusion period an arterial blood sample was collected, for measurement of ^{111}In and ^{125}I counts in blood and plasma. The coronary artery was reoccluded and Evans blue dye (2 ml kg^{-1} of a 2.5% solution in saline) administered intravenously, thereby staining perfused myocardium blue and leaving the area at risk unstained. Animals were killed with an overdose of sodium pentobarbitone and the heart removed. The left ventricle was dissected free and sliced transversely into 6 sections of equal thickness. Each section was divided into normal tissue (NZ) and area at risk (AR) on the basis of staining with Evans blue dye. The tissue samples were weighed and the ^{125}I and ^{111}In counts measured in a multiwell gamma counter (LKB 1260 multigamma 2). The ratio of ^{111}In counts per neutrophil was calculated and from this the number of labelled cells per g tissue determined. The volume of plasma in tissue samples was determined by dividing the number of ^{125}I counts by those in 1 μl of plasma. Data are expressed as the % increase in the plasma volume and the number of ^{111}In-neutrophils per g of tissue in the AR compared to the NZ in each animal. Results are shown as the mean % increase $\pm$ s.e.mean for each group. From the blood sample taken at the end of the experiment the % of circulating ^{111}In-neutrophils and the % of ^{111}In plasma counts present were determined. In the control groups at the end of the reperfusion period 27.4 $\pm$ 4.1% (mean $\pm$ s.e.mean) of the injected ^{111}In was circulating with blood cells. The % of total ^{111}In counts in the blood which were present in plasma was 6.1 $\pm$ 1.0. The size of the AR as a % of the left ventricle was also determined in each experiment as

$$\frac{\text{weight of unstained tissue}}{\text{weight of left ventricle}} \times 100$$

^{111}In-labelling of rabbit red blood cells

Experiments were carried out to determine whether ischaemia and reperfusion of the myocardium resulted in an alteration of intravascular blood volume. Red blood cells labelled with ^{111}In were administered at the end of the reperfusion period. The isolation and labelling procedure was as follows. A 9 ml blood sample was collected into acid citrate dextrose 2 h after reperfusion. Centrifugation at 300 *g* followed by a wash of the pellet in phosphate-buffered saline, resulted in a suspension of red blood cells >95% pure. These were incubated with ^{111}In as described above. The cells were then washed to remove unbound ^{111}In, resuspended in autologous plasma and administered intravenously 10 min before the end of the experiment.

Monoclonal antibody 60.3 pretreatment

^{111}In-labelled neutrophils were washed twice, resuspended in 1.5 ml of autologous citrated plasma and incubated for 25 min either in the presence or absence of the murine monoclonal antibody 60.3 (132 μg per 4 $\times$ 10^7 cells). Neutrophils (test and controls) were subsequently washed once more in plasma, resuspended in plasma in a volume of 3 ml and injected intravenously.

Depletion of circulating neutrophils

Neutrophil depletion was carried out by two different methods, either pretreatment with mustine hydrochloride or administration of an anti-neutrophil serum. Mustine hydrochloride (1.75 mg kg^{-1}), or saline in the case of controls, was administered intravenously and animals used 4 days later for coronary artery occlusion and reperfusion experiments when circulating numbers of neutrophils were <6% of normal.

A polyclonal antiserum to rabbit neutrophils was raised in sheep by a method adapted from Knicker & Cochrane (1965). The IgG fraction was prepared by use of caprylic acid (Steinbuch & Audran, 1969), followed by precipitation with ammonium sulphate and extensive dialysis against saline. This was administered intravenously (0.5 ml kg^{-1}) 20 min before coronary artery occlusion and the dose repeated at 90 min intervals. Neutrophil counts were reduced to <10% of normal using this procedure. Control animals received the IgG fraction of a pre-immune sheep serum. Blood samples were taken prior to treatment with mustine or anti-neutrophil serum and at intervals during the experimental procedure in order to monitor numbers of circulating neutrophils and other leukocytes. Both of these techniques abolished local oedema responses induced by intradermal injection of chemoattractants as previously described (Wedmore & Williams, 1981a).

Treatment with the PAF antagonist WEB 2086

Ten minutes prior to occlusion WEB 2086, 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a]-[1,4]-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone (10 mg kg^{-1}) or in the case of controls the solvent (acidified saline pH 6) was administered intravenously. Upon initiation of coronary artery occlusion a constant infusion of WEB 2086 (1.3 mg kg^{-1} h^{-1}) or an equivalent volume of saline was started and maintained until the end of the experiment. At the end of some experiments, from both treated and control groups, PAF (1 μg) was administered as a bolus intravenously and blood pressure monitored for 1 min. In a separate experiment intravenous WEB 2086 (10 mg kg^{-1}) was tested against ^{125}I-albumin leakage (30 min) induced by intradermally-injected (dorsal skin) PAF and bradykinin, each with a potentiating dose of PGE_2.

Statistical analysis

Experimental results are expressed as the mean $\pm$ s.e.mean. Fisher's exact probability test was used for the analysis of difference in the incidence of arrhythmias. Paired or unpaired Student's *t* test or analysis of variance were used as appropriate to analyse differences within groups. A *P* value of < 0.05 was considered significant.

Materials

Bradykinin, FMLP, PGE_2 and 2-mercaptopyridine N-oxide were purchased from Sigma Chemical Company, Poole, Dorset. Evans blue dye was purchased from BDH, Poole, Dorset. Hydroxyethyl starch (Hespan) was purchased from American Hospital Supply, Didcot, Oxfordshire. Percoll was purchased from Pharmacia Ltd, Milton Keynes, Buckinghamshire. $^{111}InCl_3$ and ^{125}I-human serum albumin were purchased from Amersham International, Amersham, Buckinghamshire. PAF was purchased from Bachem, Bubendorf, Switzerland. Mustine hydrochloride was purchased from the Boots Company, Nottingham, Nottinghamshire.

Results

Measurement of neutrophil accumulation and plasma protein leakage in ischaemic/reperfused myocardium

The aim of this study was to examine neutrophil accumulation and plasma protein leakage in myocardial tissue in response to a period of ischaemia followed by reperfusion. These were measured as the % increase in ^{111}In-neutrophils and ^{125}I-albumin in the area at risk myocardium (AR) compared with normal myocardium (NZ). For this study a period of 30 min coronary artery occlusion followed by reperfusion for 3 h was selected, which resulted in a substantial increase in both ^{111}In-neutrophils and ^{125}I-albumin in the AR (Figure 1). In sham occluded controls in which the AR was similar ($20.5 \pm 2.6\%$ vs $20.9 \pm 1.1\%$ in sham and test animals respectively, $n = 5$) there was no increase in ^{111}In or ^{125}I counts compared with the NZ (Figure 1). In histological sections (not shown) it could be seen that neutrophils were present within the vasculature and in the extravascular space.

In order to validate the model it was necessary to preclude alterations in the intravascular volume from contributing to these changes in the AR. Red blood cells labelled with ^{111}In were administered intravenously at the end of the reperfusion phase, whilst ^{125}I-albumin was used to measure plasma protein leakage as before. Whilst there was an increase in ^{125}I-albumin in the AR of $140 \pm 34\%$ the intravascular volume as measured by ^{111}In-red blood cells was decreased by $22 \pm 6\%$. This indicates that the increased ^{125}I counts observed in the AR are due to leakage of ^{125}I-albumin from coronary microvessels and not to an increase in intravascular blood volume.

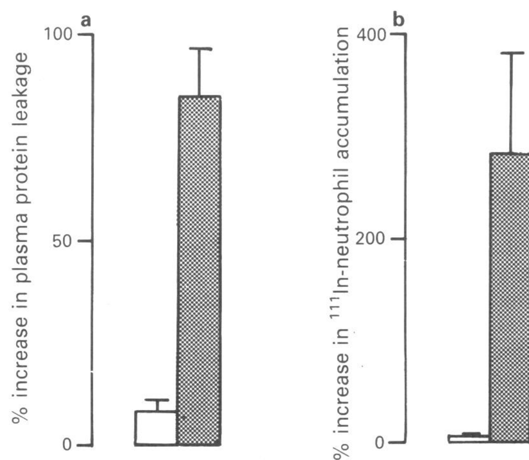

Figure 1 Percentage increase (±s.e.mean shown by vertical bars) in (a) plasma protein leakage and (b) ^{111}In-neutrophil accumulation into the area at risk compared with normal myocardium following 30 min coronary artery occlusion and 3 h reperfusion. In 'sham' occluded animals (open columns) the ligature was not tied; animals undergoing 30 min occlusion (stippled columns); $n = 5$ for both groups.

Role of adhesion glycoproteins in neutrophil infiltration into ischaemic/reperfused myocardium

The effect of a monoclonal antibody directed against the CD18 antigen of leukocytes was examined to see if neutrophil infiltration into ischaemic myocardium was dependent on the CD11/CD18 adhesion glycoprotein complex. For these experiments neutrophils labelled with ^{111}In were incubated with the monoclonal antibody prior to intravenous injection into the recipient animal. The number of ^{111}In-neutrophils (mean ± s.e.mean) administered was $3.8 \pm 0.3 \times 10^7$ control and $4.1 \pm 0.3 \times 10^7$ 60.3-treated cells. Treatment of ^{111}In-neutrophils with MoAb 60.3 did not affect their ability to circulate: the mean number of labelled cells per ml of blood at the end of the experiment was $3.3 \pm 0.8 \times 10^4$ in controls vs. $3.1 \pm 0.6 \times 10^4$ in test animals. The AR myocardium was also similar for the control and MoAb 60.3 groups ($20.9 \pm 2.0\%$ vs. $20.1 \pm 1.1\%$ respectively). However the accumulation of ^{111}In-neutrophils in the AR myocardium was markedly attenuated following incubation with MoAb 60.3 (Figure 2, $P < 0.01$). Antibody treatment of cells had no effect on plasma protein leakage in the myocardium (labelled cells account for only about 3% of the circulating neutrophils).

Relationship between neutrophil accumulation and microvascular plasma protein leakage in the ischaemic myocardium

Two methods of neutrophil depletion were employed, pretreatment with mustine or acute administration of anti-neutrophil serum. Both procedures abolished oedema formation induced by chemoattractants injected intradermally (results not shown). The number of circulating neutrophils was markedly reduced in both treatment groups (Table 1). However, administration of anti-neutrophil serum or mustine was without effect on the leakage of plasma protein in the AR myocardium (Table 1). Thus neutrophils do not appear to be involved in plasma protein leakage in myocardium following ischaemia and reperfusion.

Effect of a PAF antagonist WEB 2086 on plasma protein leakage and ^{111}In-neutrophil accumulation

Since the oedema formation did not appear to be a neutrophil-mediated event we investigated a mediator of

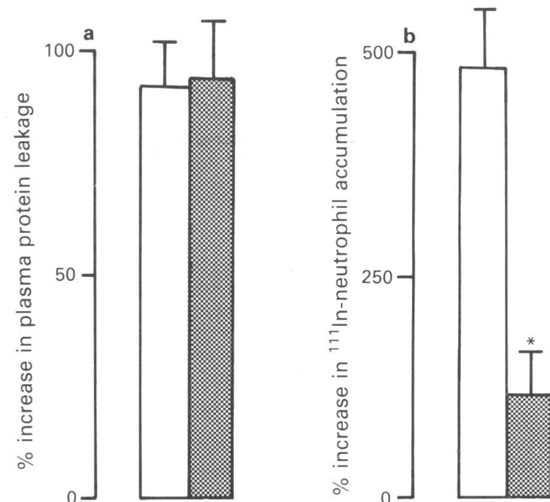

Figure 2 Percentage increase (± s.e.mean shown by vertical bars) in (a) plasma protein leakage and (b) ^{111}In-neutrophil accumulation in the area at risk compared with normal zone following 30 min coronary artery occlusion and 3 h reperfusion. ^{111}In-neutrophils were incubated either in the presence (stippled columns) or absence (open columns) of the monoclonal antibody 60.3 before administration *in vivo*; $n = 5$ for both groups.

Table 1 Effect of anti-neutrophil serum and mustine on white blood cell (wbc), neutrophil counts (neut) and % increase in ^{125}I-labelled albumin in AR myocardium

Treatment	*Pre-occlusion* wbc ml^{-1} (× 10^6)	*Pre-occlusion* neut ml^{-1} (× 10^6)	*Reperfusion* (3 h) wbc ml^{-1} (× 10^6)	*Reperfusion* (3 h) neut ml^{-1} (× 10^6)	*% increase in* ^{125}*I-albumin*
Pre-immune serum	4.62 ± 1.10	1.84 ± 0.62	5.15 ± 0.71	3.39 ± 0.63	78 ± 9
Anti-neutrophil serum	1.48 ± 0.27*	0.11 ± 0.03*	2.41 ± 0.27**	0.35 ± 0.10**	80 ± 25
Saline control	4.31 ± 1.03	1.17 ± 0.23	5.30 ± 0.57	3.07 ± 0.42	91 ± 13
Mustine	1.71 ± 0.10*	0.07 ± 0.02**	2.49 ± 0.77*	0.19 ± 0.12**	90 ± 16

$n = 5$ rabbits/group. * $P < 0.05$; ** $P < 0.01$ for pre-immune serum vs anti-neutrophil serum, or saline vs mustine at the appropriate time.

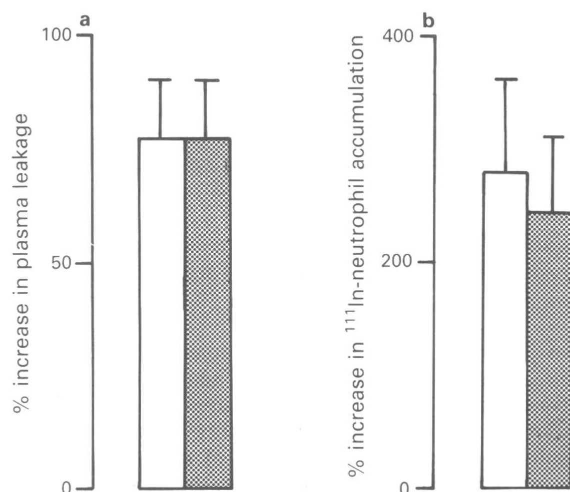

Figure 3 Percentage increase (± s.e.mean shown by vertical bars) in (a) plasma protein leakage and (b) ^{111}In-neutrophil accumulation in the area at risk compared with normal zone following 30 min coronary artery occlusion and 3 h reperfusion. Animals received 10 mg kg^{-1} WEB 2086 then an infusion of 1.3 mg kg^{-1} h^{-1} (stippled columns), or the solvent (acidified saline) in the case of controls (open columns); $n = 6$ for both groups.

increased microvascular permeability that acts independently of neutrophils in the rabbit, i.e. PAF. For these studies a recently developed, potent PAF antagonist WEB 2086 was used. The AR for the two groups of rabbits was similar (22.2 ± 2.8% for controls vs. 24.5 ± 2.1% for the group receiving WEB 2086). As shown in Figure 3, WEB 2086 had no effect on plasma protein leakage in the AR myocardium. Accumulation of ^{111}In-neutrophils into the AR was likewise unaffected by this treatment (Figure 3). The dose of WEB 2086 used inhibited plasma protein leakage in the skin in response to intradermal PAF (Table 2). Furthermore administration of 1 μg PAF i.v. at the end of the experiment caused a fall in blood pressure of 21 ± 3 mmHg in controls compared with 2 ± 1 mmHg in animals receiving WEB 2086 ($n = 3$), indicating that adequate blockade of PAF receptors was still present. The results suggest that PAF is not involved in either oedema formation or neutrophil accumulation in the model employed.

Table 2 The effect of WEB 2086 (10 mg kg^{-1}, i.v.) on leakage of ^{125}I-albumin in response to platelet activating factor (PAF) or bradykinin (BK) in rabbit skin

Dose of mediator (mol per site)	*% change in* ^{125}*I-albumin* *Pre-treatment*	*Post-treatment*
PAF 10^{-11}	+100	−17
PAF 10^{-10}	+200	−18
PAF 10^{-9}	+293	+13
BK 10^{-10}	+496	+332

Results are expressed as the % change in ^{125}I-labelled albumin counts in skin sites in response to intradermal administration in a volume of 0.1 ml of PAF or BK compared with that caused by saline prior to or following administration of WEB 2086. Prostaglandin E_2 (3 × 10^{-10} mol per site) was co-administered with all mediators. Results are expressed as the % increase over baseline for 6 sites.

Table 3 Blood pressure and heart rate in rabbits undergoing 30 min coronary artery occlusion followed by reperfusion

Time	*Blood pressure* (mmHg)	*Heart rate* (beats min^{-1})
Pre-occlusion	72 ± 4	255 ± 9
Occlusion (3 min)	56 ± 5*	260 ± 25
Reperfusion (30 min)	60 ± 6*	265 ± 25
Reperfusion (3 h)	55 ± 7	309 ± 32

Values are mean ± s.e.mean.
$n = 5$ rabbits/group; * $P < 0.05$.

Blood pressure and heart rate measurements and disturbances of cardiac rhythm

Occlusion of the left circumflex coronary artery resulted in a fall in mean arterial blood pressure from 72 ± 4 mmHg to 56 ± 5 mmHg ($n = 5$, $P < 0.05$) but did not affect heart rate (Table 3). Blood pressure and heart rate measurements were comparable in all control and treatment groups. The incidence of severe ventricular arrhythmias was fairly low in all of the control groups, i.e. the incidence of ventricular fibrillation ranged from 14%–37.5%. This reflects the relatively small size of the AR. There was no significant alteration in the incidence of arrhythmias with any of the treatments used.

Discussion

Myocardial tissue which has undergone acute infarction exhibits a number of features which are characteristic of an acute inflammatory response, including tissue oedema, an early neutrophil infiltration and a later monocyte/lymphocyte infiltration (Mallory *et al.*, 1939; Sommers & Jennings, 1964; Fishbein *et al.*, 1978). In this study we have used a model of temporary coronary artery occlusion and reperfusion to examine two early features of the response, tissue oedema and neutrophil infiltration. Initial studies indicated that a 30 min period of coronary artery occlusion followed by 3 h of reperfusion elicited a substantial increase in ^{111}In-neutrophils and ^{125}I-albumin in the AR, which was not a consequence of increased intravascular volume.

Incubation of ^{111}In-neutrophils with MoAb 60.3 proved highly effective in suppressing their accumulation into myo-

cardium with ischaemic/reperfusion injury. Previous studies have shown that this antibody binds to a functional epitope of the CD18 glycoprotein inhibiting functions such as chemotaxis, aggregation and stimulated adhesion to endothelial monolayers *in vitro* (Harlan *et al.*, 1985; Wallis *et al.*, 1986). Accumulation of neutrophils in response to intradermal administration of inflammatory mediators is also inhibited by systemic administration of this antibody (Arfors *et al.*, 1987). Neutrophils incubated with MoAb 60.3 *in vitro* and then administered intravenously *in vivo* also fail to accumulate in response to intradermally-injected chemotactic mediators (Rampart & Williams, 1988; Nourshargh *et al.*, 1989). We have used this *in vitro* incubation procedure in the present study. The reduced accumulation of ^{111}In-neutrophils into ischaemic/reperfused myocardium was not a result of any effect of the antibody on the ability of the cells to circulate. Our results therefore demonstrate that accumulation of neutrophils in infarcted myocardium is dependent on the CD18 adhesion glycoprotein. The mechanisms by which CD18 modulates neutrophil adhesion have not been fully elucidated so far. It has been shown that increased expression of CD18 is not necessary for increased neutrophil adhesion *in vitro* (Vedder & Harlan, 1988; Buyon *et al.*, 1988; Philips *et al.*, 1988) or neutrophil accumulation in the skin *in vivo* (Nourshargh *et al.*, 1989). Our results support these findings, since *in vitro* incubation with MoAb 60.3 would only be expected to block basally expressed CD18 and not interfere with further expression *in vivo*.

The mechanisms involved in increased coronary microvascular permeability in infarcted myocardium have not been determined. It has been suggested, however, that neutrophils may be involved (Engler *et al.*, 1986). This was based on the observation that the increase in myocardial tissue water was correlated with the density of neutrophils present in the tissue following a period of ischaemia and reperfusion. We were interested in exploring this possibility further in the light of studies in a skin model of inflammation, in which tissue oedema in response to chemoattractant mediators was found to be dependent on circulating neutrophils (Wedmore & Williams, 1981a). Treatment of the ^{111}In-neutrophils *in vitro* with MoAb 60.3 had no effect on plasma protein leakage in AR myocardium. However, these cells only contribute approximately 3% to the recipients' total circulating neutrophil population and would therefore have negligible effect on function. In order to examine whether there was a relationship between neutrophils and oedema formation in the heart, the effect of systemic depletion of circulating neutrophils was examined in this model. Leakage of plasma proteins into the AR myocardium was completely unaffected by neutrophil depletion. These findings indicate that a neutrophil-dependent mediator of increased vascular permeability is not involved in this model. These results differ from those obtained with a model of intestinal ischaemia and reperfusion in the cat (Hernandez *et al.*, 1987). In that study increased microvascular permeability was found to be dependent on circulating neutrophils. Apart from the difference in species and tissue there are other differences which might possibly contribute e.g. duration of ischaemia and reperfusion.

There are also certain mediators of increased vascular permeability which are neutrophil-independent. One of these is PAF (Wedmore & Williams, 1981b). The presence of this phospholipid has been detected following reperfusion of the ischaemic isolated heart of the rabbit (Montrucchio *et al.*, 1989). Furthermore, a PAF antagonist, CV6209, has been reported to attenuate the increased vascular permeability induced by global ischaemia in the rat isolated perfused heart (Stahl *et al.*, 1988). In the present study, however, pretreatment with a potent and selective PAF antagonist, WEB 2086, had no effect on either plasma protein leakage or on ^{111}In-neutrophil accumulation into AR. The dose of compound used was, however, sufficient to abolish plasma protein leakage in the skin in response to intradermally-injected PAF and also the hypotensive response following intravenous administration of PAF. From these results we conclude that it is unlikely that PAF has a role in mediating either plasma protein leakage or neutrophil accumulation in response to ischaemia in this model.

In conclusion, we have shown that a period of ischaemia followed by reperfusion in rabbit myocardium results in plasma protein leakage which is independent of circulating neutrophils and unaffected by systemic administration of a PAF antagonist WEB 2086. Neutrophil infiltration into the ischaemic myocardium is also unaffected by the PAF antagonist but is markedly attenuated by incubation of the cells with MoAb 60.3 prior to administration *in vivo*. Our future studies are aimed at determining the mediators responsible for neutrophil accumulation and whether protein leakage from coronary microvessels results from the effects of chemical mediators or from endothelial damage induced directly by ischaemia.

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