Effect of duration of ischaemia on reduction of myocardial infarct size by inhibition of neutrophil accumulation using an anti-CD18 monoclonal antibody

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1 Neutrophil accumulation is a characteristic feature of the inflammatory response in myocardial tissue which has undergone a period of ischaemia. The aim of this study was to examine whether inhibition of myocardial neutrophil infiltration, using an antibody to the CD18 leukocyte adhesion molecule, was effective in reducing infarct size in anaesthetized rabbits.

2 Anaesthetized rabbits underwent coronary artery occlusion (CAO) for periods of 30 or 45 min followed by reperfusion for 3 h. Animals were treated intravenously 10 min prior to reperfusion with IB4, a monoclonal antibody to CD18 (1 mg kg⁻¹) or saline (1 ml kg⁻¹). In one group undergoing 45 min CAO, a control antibody, OKMI (1 mg kg⁻¹) was given.

3 Following either 30 or 45 min of CAO, administration of IB4 resulted in a <75% inhibition in neutrophil accumulation in the area at risk myocardium (AR) compared with control animals.

4 With the 30 min occlusion period, IB4 significantly reduced myocardial infarct size, 27.2 ± 3.2% vs 67.4 ± 5.6% in the saline control group ($n = 5$ $P < 0.01$). In contrast, IB4 did not reduce infarct size following a 45 min period of ischaemia.

5 In the same animals administration of IB4 significantly inhibited oedema formation in skin elicited by intradermal administration of the neutrophil chemoattractant f-Met-Leu-Phe, but had no effect on coronary microvascular plasma protein leakage in the AR.

6 Our results indicate that infiltrating neutrophils exacerbate tissue injury following a relatively short, 30 min period of myocardial ischaemia in the rabbit. However, protection with IB4 was no longer seen if the period of CAO was extended to 45 min. The results in this model suggest neutrophils are not a major determinant of tissue injury following more than a very short period of ischaemia.

Keywords: Myocardial infarction; neutrophils; CD11/CD18; IB4; oedema formation

Introduction

It is well established that neutrophil infiltration is a characteristic feature of myocardial infarcts (Mallory *et al.*, 1939). More recently it has been reported in a number of experimental studies that myocardial infarct size is reduced by interventions which deplete circulating neutrophils (Romson *et al.*, 1983; Mullane *et al.*, 1984). Protection has also been observed by use of other agents such as ibuprofen and prostacyclin, which suppress neutrophil infiltration into infarcted myocardium (Romson *et al.*, 1982; Simpson *et al.*, 1987). As a result of these studies it has been suggested that neutrophils may extend the area of tissue damage through injury of viable myocytes. However, in some studies no myocardial protection was observed using anti-neutrophil interventions such as ibuprofen or leukocyte filters (Reimer *et al.*, 1985; Chatelain *et al.*, 1987) and consequently there is still controversy over this hypothesis.

An essential prerequisite for neutrophil accumulation at sites of inflammation is an initial adherence of the leukocytes to the endothelium, which involves the expression of adhesion molecules on the surface of both cell types. In the case of leukocytes the CD11/CD18 β_2 integrins have been implicated in this process (Harlan *et al.*, 1985; Lo *et al.*, 1989). Monoclonal antibodies to the leukocyte CD18 antigen have been shown to inhibit neutrophil accumulation at sites of inflammation (Arfors *et al.*, 1987). We have previously shown that pretreatment of ¹¹¹In-neutrophils with a monoclonal antibody to the CD18 adhesion molecule, significantly reduces their accumulation in skin sites injected with chemoattractants (Nourshargh *et al.*, 1989) and in ischaemic/reperfused rabbit myocardium (Williams *et al.*, 1990). In this study we have examined the effect of systemic administration of IB4, a monoclonal antibody to CD18 (Wright *et al.*, 1983), on myocardial infarct size in anaesthetized rabbits using two different periods of ischaemia.

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Methods

Coronary artery occlusion and reperfusion

New Zealand White rabbits, anaesthetized with intravenously administered sodium pentobarbitone (60 mg kg⁻¹) were used for this study. The trachea was cannulated to allow positive pressure ventilation with room air. Arterial blood pressure was monitored from a cannula in the carotid artery and a lead I electrocardiogram (ECG) from subcutaneous limb leads. The heart was exposed by a left thoracotomy at the 5th intercostal space. A silk ligature was positioned around a branch of the left main coronary artery and both ends passed through a short length of polythene tubing. Following a stabilisation period of 15 min, the coronary artery was occluded by pulling on the suture and clamping the ends against the tubing. After a period of either 30 or 45 min coronary artery occlusion (CAO), tension on the ligature was released to allow reperfusion of the myocardium for a further 3 h. At the end of this time, 500 units of heparin was administered intravenously as an anti-coagulant. The experiment was then terminated by anaesthetic overdose. The heart was rapidly removed and cannulated via the aorta to allow retrograde perfusion of the coronary circulation. Intravascular blood was washed out with saline. The coronary artery

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was reoccluded with the original ligature and the heart perfused with Monastral blue dye to stain the normal myocardium. Small samples of myocardium were taken from the normal zone (NZ) and area at risk (AR) and stored at -20°C prior to assay for myeloperoxidase as an index of neutrophil accumulation. The rest of the heart was frozen prior to staining for infarct size (Thornton *et al.*, 1990).

Measurement of infarct size

The heart, while still frozen was sectioned transversely into 2 mm thick slices from apex to base. Each slice was weighed and traced onto transparency film, carefully demarcating normal (stained with Monastral blue) and ischaemic (unstained) regions. The heart sections were then incubated, in the dark in 1% triphenyltetrazolium chloride (TTC) in phosphate-buffered saline (PBS, pH 7.4) for 30 min at 37°C . The viable tissue stained dark red while the infarcted tissue appeared pale brown. This differential staining was enhanced by storing the heart in 1% formalin overnight. The slices were subsequently retraced to delineate the regions of viable and infarcted tissue. Computerised planimetry was used to determine the areas of risk and infarction on each slice. Measurement of AR and infarct areas were carried out blind.

Measurement of myeloperoxidase concentration

Measurement of myeloperoxidase (MPO) activity was carried out according to a previously described method (Rubin *et al.*, 1990). Myocardial samples were weighed and then homogenized in 0.02 M NaPO_4 buffer (pH 4.7) containing 0.1 M NaCl and 0.015 M Na_2EDTA . Homogenates were centrifuged at 20,000 g for 15 min at 4°C and the supernatant, containing the majority of the haemoglobin, discarded. The pellets were homogenized a second time in 0.05 M NaPO_4 with 0.5% hexadecyltrimethylammonium-bromide (HETAB) and freeze/thawed in liquid nitrogen three times. After a second centrifugation at 20,000 g for 15 min at 4°C , the supernatant containing the majority of the MPO was taken for assay. This was carried out using the following: 1.6 mM tetramethylbenzidine, 0.3% H_2O_2 , 12% dimethyl formamide and 40% Dulbecco's PBS made up in 0.08 M NaPO_4 , pH 5.4. The change in absorbance was measured at 690 nm. One unit of MPO was defined as the amount of enzyme reducing $1\text{ }\mu\text{mol}$ of $\text{H}_2\text{O}_2\text{ min}^{-1}$.

Measurement of plasma protein leakage in myocardial tissue and skin

Plasma protein leakage was measured as the local accumulation of intravenously administered ^{125}I -labelled albumin (5 μCi), given 10 min prior to coronary artery occlusion. Samples of myocardium were taken from the NZ and the AR, weighed and the radioactivity measured in a gamma counter. The effect of treatment with IB4 was also examined on plasma protein leakage in skin, following intradermal injection of f-Met-Leu-Phe (FMLP) and bradykinin in the same animals. These two inflammatory mediators cause plasma exudation through mechanisms which are neutrophil-dependent and neutrophil-independent respectively (Wedmore & Williams, 1981). The mediators were injected in a volume of 0.1 ml into the shaved flank of the rabbit, just after reperfusion. Treatments were given in replicates of six. FMLP was given at a dose of 5×10^{-11} mol per site and bradykinin at a dose of 1×10^{-10} mol per site. Control sites received saline. At the end of the experiment, a blood sample was taken for preparation of plasma. The skin was removed and the injection sites excised with a 17 mm punch. Plasma and skin samples were then counted in a gamma counter. Results are expressed in terms of volume of plasma exudate and are calculated by dividing the number of ^{125}I counts in tissue samples by the number in $1\text{ }\mu\text{l}$ of plasma.

Drug treatment

IB4 is a murine IgG₂ monoclonal antibody directed against an epitope on the common β -chain (CD18) of the leukocyte adhesion complex (Wright *et al.*, 1983) and has been shown to bind to rabbit neutrophils (Tuomanen *et al.*, 1989). The antibody was administered intravenously at a dose of 1 mg kg^{-1} since this dose has been shown to suppress neutrophil accumulation in rabbit skin elicited by zymosan-activated serum (Lundberg & Wright, 1990). In order to investigate if the deleterious effects of neutrophils were exerted during the reperfusion phase, treatment was administered 10 min before the end of the occlusion period. The effect of IB4 on infarct size was compared following CAO for 30 ($n = 5$) or 45 min ($n = 6$). Control animals received 1 ml kg^{-1} saline ($n = 5$, 30 min CAO or $n = 6$, 45 min CAO). One group of animals undergoing 45 min CAO was treated with a control antibody, OKM1 (1 mg kg^{-1} , i.v., $n = 7$). OKM1 is also a murine IgG₂ antibody but it binds to CD11b on the leukocyte surface without inhibiting binding of these cells to endothelial cells (Wallis *et al.*, 1986). Animals were assigned randomly to control or treatment groups.

Measurement of circulating neutrophil numbers

Arterial blood samples were taken into 0.01 M EDTA at the following time points: pre-occlusion, prior to antibody administration and at 60, 120 and 180 min of reperfusion. The total leukocyte count and the % of neutrophils present were determined with a haemocytometer and by differential staining respectively. From these values the number of circulating neutrophils was calculated.

Materials

Monastral blue dye, TTC, HETAB, tetramethylbenzidine, bradykinin and FMLP were obtained from Sigma Chemical Company, Dorset. ^{125}I -labelled human serum albumin was obtained from Amersham International, Buckinghamshire. Sodium pentobarbitone was obtained from RMB Animal Health Ltd, Essex. IB4 and OKM1 were provided by Dr E. MacIntyre, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, U.S.A.

Data analysis

Results are expressed as the mean $\pm$ s.e.mean of n observations. Statistical analysis was carried out by analysis of variance.

Results

Occlusion of the left main coronary artery was confirmed by changes in the colour of the distal portion of the artery and of the epicardial surface. Characteristic alterations in the ECG, which are indicative of myocardial ischaemia, were seen in all animals. Of the 29 animals that entered the study, two suffered irreversible ventricular fibrillation during CAO (one each in the saline and OKM1 groups) and one animal receiving OKM1 developed profound hypotension during reperfusion.

Myeloperoxidase measurements

In control animals receiving saline, CAO for either a 30 or 45 min period followed by 3 h of reperfusion, resulted in a marked increase in the concentration of myeloperoxidase activity in AR myocardium compared with the NZ (Figure 1). This increase in MPO activity in the AR was not affected by administration of the control antibody, OKM1. However, administration of IB4 resulted in a substantial and significant

Figure 1 The concentration of myeloperoxidase (MPO) in normal zone (NZ, open columns) and area at risk (AR, cross-hatched columns) myocardium from animals undergoing 30 or 45 min coronary artery occlusion (CAO) and 3 h reperfusion. Animals were treated with saline (1 ml kg⁻¹, *n* = 5), OKM1 (1 mg kg⁻¹, *n* = 5) or IB4 (1 mg kg⁻¹, *n* = 5, 30 min CAO and *n* = 6, 30 min CAO). **P* < 0.05; ***P* < 0.01 treated vs control.

reduction in this response following both 30 and 45 min periods of CAO (Figure 1).

Myocardial infarct size

The AR was expressed as a % of the area of the left ventricle and infarct size was expressed as a % of the AR. Following the 30 min period of CAO, IB4 significantly reduced myocardial infarct size, 27.2 ± 3.2% compared with the saline control group, 67.4 ± 5.6% (Figure 2, *n* = 5, *P* < 0.01). There was no difference in the size of the AR for these two groups, 38.9 ± 3.4 and 32.0 ± 4.0% respectively. In contrast, infarct size resulting from a 45 min period of CAO, was not significantly reduced by the administration of IB4, 58.3 ± 6.7% (*n* = 6) compared with that of animals receiving either saline, 81.8 ± 8.7% (*n* = 5) or the control antibody, OKM1, 73.6 ± 7.3% (*n* = 5, Figure 2). The AR in the IB4 group, 59.9 ± 6.3% did not differ from that in the OKM1, 53.9 ± 7.9% and saline-treated groups, 46.8 ± 7.6%.

Plasma protein leakage in ischaemic/reperfused myocardium

In the control group, myocardial plasma volume increased by 254% from 54.6 ± 7.0 μl g⁻¹ in the NZ to 192.7 ± 25.3 μl g⁻¹ in the AR (*P* < 0.01, *n* = 5, Figure 3a) following a period of 30 min CAO and 3 h reperfusion. Administration of IB4 had no effect on this oedema formation (Figure 3), the plasma volume in the AR, 170.9 ± 35.3 μl g⁻¹ being 307% greater than that in the NZ, 46.2 ± 9.6 μl g⁻¹ (*P* < 0.01, *n* = 5). IB4 was similarly without effect on myocardial oedema formation following the longer 45 min period of CAO (results not shown).

Plasma protein leakage in response to intradermal administration of inflammatory mediators

Both FMLP and bradykinin elicited local oedema formation when injected into skin. The plasma volume of FMLP-injected sites was significantly reduced from 24.0 ± 1.2 μl in control animals compared with 14.8 ± 2.2 μl g⁻¹ in rabbits treated with IB4 (Figure 3b, *n* = 5, *P* < 0.01). The values in the IB4-treated group were indistinguishable from those in saline-injected sites. In contrast, plasma protein leakage in response to the directly-acting mediator, bradykinin was unaffected by administration of IB4.

Figure 2 Infarct size as a % of the area at risk following 30 or 45 min coronary artery occlusion (CAO) and 3 h reperfusion in animals treated with saline (1 ml kg⁻¹, open columns, *n* = 5), OKM1 (1 mg kg⁻¹, cross-hatched columns, *n* = 5) or IB4 (1 mg kg⁻¹, filled columns, *n* = 5, 30 min CAO and *n* = 6, 45 min CAO). ***P* < 0.01 IB4 vs control.

Figure 3 Plasma volume of (a) normal zone (NZ) and area at risk (AR) myocardium following 30 min coronary artery occlusion (CAO) and 3 h reperfusion and of (b) skin injected with saline, bradykinin (BK, 1 × 10⁻¹⁰ mol per site) or FMLP (5 × 10⁻¹¹ mol per site) in animals receiving saline (open columns) or IB4 (1 mg kg⁻¹, cross-hatched columns, *n* = 5). **P* < 0.01 IB4 vs control.

Table 1 Number of circulating neutrophils in control and IB4-treated animals undergoing coronary artery occlusion (CAO) and reperfusion

Treatment	n	Pretreatment	No. of neutrophils ml ⁻¹	
			1 h reperfusion	3 h reperfusion
Saline (30' CAO)	5	1.6 ± 0.5	1.8 ± 0.3	3.4 ± 0.7
IB4 (30' CAO)	5	1.0 ± 0.2	2.7 ± 0.8	3.9 ± 1.2
Saline (45' CAO)	5	1.8 ± 0.5	2.4 ± 0.6	2.4 ± 0.8
OKM1 (45' CAO)	5	1.3 ± 0.2	1.6 ± 0.6	2.4 ± 0.6
IB4 (45' CAO)	6	0.7 ± 0.2	1.9 ± 0.5	2.0 ± 0.6

Table 2 Systemic haemodynamic measurements in control and IB4-treated animals

Treatment	n	Mean arterial blood pressure (mmHg)			Heart rate (beats min ⁻¹)		
		Pre-occlusion	30' CAO	3 h REP	Pre-occlusion	30' CAO	3 h REP
Saline (30' CAO)	5	70 ± 7	73 ± 8	71 ± 6	230 ± 9	225 ± 14	180 ± 17*
IB4 (30' CAO)	5	74 ± 6	71 ± 4	68 ± 3	248 ± 7	231 ± 3	232 ± 15
Saline (45' CAO)	5	62 ± 3	65 ± 3	65 ± 3	212 ± 12	208 ± 8	203 ± 7
OKM1 (45' CAO)	5	68 ± 2	68 ± 4	64 ± 5	230 ± 13	224 ± 10	215 ± 14
IB4 (45' CAO)	6	72 ± 2	70 ± 2	71 ± 3	229 ± 9	222 ± 9	217 ± 7

Values are means ± s.e.mean.

**P* < 0.05 3 h REP vs Pre-occlusion.

Circulating neutrophil numbers

The number of circulating neutrophils in each group is shown in Table 1. Administration of IB4 did not result in a significant alteration in the number of circulating neutrophils. The control antibody, OKM1 was also without effect on this parameter. None of the experimental groups differed in the number of neutrophils present in blood samples at any of the measurement time points.

Blood pressure and heart rate measurements

Blood pressure and heart rate measurements for the control and treated groups are shown in Table 2. Neither of these parameters was altered during the course of the experiment in any of the treatment groups with the exception of heart rate in one of the control groups, which was significantly lower at 3 h of reperfusion.

Incidence of ventricular arrhythmias

Ventricular ectopic beats were observed in most rabbits during either CAO or reperfusion, although the incidence of the more severe arrhythmias was low in all groups. There was no significant difference in the incidence of arrhythmias in any of the treatment groups.

Discussion

In this study we have examined the effect of administration of an antibody to the leukocyte CD18 adhesion molecule on both neutrophil accumulation and infarct size following myocardial ischaemia and reperfusion. In addition, we have also examined whether the outcome of this intervention is dependent on the duration of the ischaemic period.

Measurement of MPO activity is well established as a method for the quantification of neutrophil accumulation in myocardial tissue (Bednar *et al.*, 1985; Mullane *et al.*, 1985). In saline-treated rabbits both the 30 and 45 min periods of CAO resulted in a substantial increase in the MPO concentration in the AR, indicating neutrophil accumulation. Whilst the control antibody, OKM1 did not affect the increase in MPO concentration, administration of IB4 resulted in a marked suppression of this response following both ischaemic periods. Administration of IB4 did not, however, affect

the number of circulating neutrophils. The small residual increase in MPO activity in IB4-treated animals may reflect non-specific trapping of neutrophils.

Administration of IB4 towards the end of the 30 min period of CAO also resulted in a significant reduction in myocardial infarct size, suggesting that infiltrating neutrophils have a deleterious effect on reperfused tissue. When activated, neutrophils release proteolytic enzymes and free radicals (Weiss, 1989) and these toxic agents may well cause further damage to myocytes within the AR. However, extending the duration of ischaemia to 45 min resulted in a loss of the protective effect of IB4 on infarct size. The concentration of MPO measured in the AR of IB4-treated animals was, however, comparable for both occlusion periods. These results suggest that although infiltrating neutrophils contribute to myocyte injury following a relatively short ischaemic period of 30 min, the role of these leukocytes in reperfusion injury appears to be less important following a longer 45 min period of ischaemia. A possible reason for this is that the extent of direct ischaemic injury to myocytes following 45 min CAO in rabbit heart may be so great that further exacerbation by activated neutrophils is not detectable.

Previous studies examining the role of neutrophils in myocardial reperfusion injury have given mixed results. Whereas administration of anti-neutrophil serum has been reported to reduce myocardial infarct size in some experimental studies (Romson *et al.*, 1983; Jolly *et al.*, 1986; Simpson *et al.*, 1988a) it was ineffective in others (Jolly *et al.*, 1986; Chatelain *et al.*, 1987). Administration of monoclonal antibodies to leukocyte adhesion molecules has likewise been reported to reduce infarct size in some studies (Simpson *et al.*, 1988b; Ma *et al.*, 1991); however, Tanaka *et al.* (1993) recently reported that IB4 was ineffective in a canine model of acute myocardial infarction. The majority of these studies were carried out using dogs which have a relatively high, but variable, component of collateral blood flow in the myocardium. The absence of measurements of myocardial blood flow in many of these studies has been suggested as a possible reason for the contradictory results reported in the literature (Reimer *et al.*, 1989) since there could be differences in the level of collateral blood flow in control and treated groups. This was not considered to be a problem in our study since rabbits have been shown to have negligible coronary collateral blood flow (Maxwell *et al.*, 1987).

In agreement with our findings that IB4 was only effective with a short period of ischaemia, other studies in which

protection was reported with anti-neutrophil interventions were characterized by a shorter duration of coronary artery occlusion (Litt *et al.*, 1989; de Lorgeril *et al.*, 1989; Simpson *et al.*, 1990), compared with most of those reporting no effect (Reimer *et al.*, 1985; Chatelain *et al.*, 1987). Furthermore, Jolly *et al.* (1986) have previously reported that protection with anti-neutrophil antiserum in a canine model of acute myocardial infarction was lost when the duration of ischaemia was extended from 90 min to 4 h. Thus, there appears to be a difference between dogs and rabbits regarding the duration of ischaemia which can be tolerated. In species such as dogs in which there is a relatively high collateral blood flow, it is likely that the window of opportunity for use of anti-neutrophil interventions will be longer than in those with low collateral flow such as rabbits.

In most of the studies carried out using anti-neutrophil interventions, including that of Jolly *et al.* (1986), treatment was instituted prior to CAO. An important feature of our study is that administration of IB4 was made towards the end of the ischaemic period. Protection afforded by IB4 therefore appears to be exerted during the reperfusion period. Treatment just prior to reperfusion has also been shown to reduce myocardial infarct size in cats following administration of an anti-CD18 antibody (Ma *et al.*, 1991) and in dogs with anti-leukocyte filters (Litt *et al.*, 1989).

Administration of IB4 completely inhibited plasma protein leakage in response to intradermal injection of the neutrophil-dependent mediator FMLP whilst that elicited by brady-

kinin, a neutrophil-independent mediator, was unaffected. However, IB4 treatment did not reduce the oedema formation seen in the AR myocardium. This is in agreement with previous findings with this model, in which depletion of circulating neutrophils using either a polyclonal anti-neutrophil serum or a nitrogen mustard was found to be ineffective in reducing oedema formation in ischaemic/reperfused myocardium (Williams *et al.*, 1990). This oedema formation appears therefore to be due either to the release of neutrophil-independent mediators of increased microvascular permeability, or to direct injury to the vascular endothelium.

In conclusion, we have found that administration of IB4 prior to reperfusion significantly reduces myocardial infarct size following 30 min coronary artery occlusion in the anaesthetized rabbit. However, this protective action was lost when a longer 45 min occlusion period was used. This suggests that infiltrating neutrophils do exacerbate myocardial tissue injury following ischaemia and reperfusion but, in terms of potential therapy targeted at the neutrophil, the window of opportunity may be limited to relatively short periods of ischaemia.

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References

- ARFORS, K.-E., LUNDBERG, C., LINDBOM, L., LUNDBERG, K., BEATTY, P.G. & HARLAN, J.M. (1987). A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. *Blood*, **69**, 338–340.
- BEDNAR, M., SMITH, B., PINTO, A. & MULLANE, K.M. (1985). Nafazatrom-induced salvage of ischemic myocardium in anesthetized dogs is mediated through inhibition of neutrophil function. *Circ. Res.*, **57**, 131–141.
- CHATELAIN, P., LATOUR, J.-G., TRAN, D., LE LORGERIL, D., DUPRAS, G. & BOURASSA, M. (1987). Neutrophil accumulation in experimental myocardial infarcts: relation with extent of injury and effect of reperfusion. *Circulation*, **75**, 1083–1090.
- DE LORGERIL, M., BASMADJIAN, A., LAVALLEE, M., CLEMENT, R., MILLETTE, D., ROUSSEAU, G. & LATOUR, J.-G. (1989). Influence of leukopenia on collateral flow, reperfusion flow, reflow ventricular fibrillation, and infarct size in dogs. *Am. Heart J.*, **117**, 523–532.
- HARLAN, J.M., KILLEN, P.D., SENEAL, F., SCHWARTZ, B.R., YEE, E.K., TAYLOR, R.F., BEATTY, P.G., PRICE, T. & OCHS, H.D. (1985). The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium in vitro. *Blood*, **66**, 167–178.
- JOLLY, S.R., KANE, W.J., HOOK, B.G., ABRAMS, G.D., KUNKEL, S.L. & LUCCHESI, B.R. (1986). Reduction of myocardial infarct size by neutrophil depletion: effect of duration of occlusion. *Am. Heart J.*, **112**, 682–690.
- LITT, M.R., JEREMY, R.W., WEISMAN, H.F., WINKELSTEIN, J.A. & BECKER, L.C. (1989). Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. *Circulation*, **80**, 1816–1827.
- LO, S.K., DETMERS, P.A., LEVIN, S.M. & WRIGHT, S.D. (1989). Transient adhesion of neutrophils to endothelium. *J. Exp. Med.*, **169**, 1779–1793.
- LUNDBERG, C. & WRIGHT, S.D. (1990). Relation of the CD11/CD18 family of leukocyte antigens to the transient neutropenia caused by chemoattractants. *Blood*, **76**, 1240–1245.
- MA, X.-L., TSAO, P.S. & LEFER, A.M. (1991). Antibody to CD-18 exerts endothelial and cardiac protective effects in myocardial ischemia and reperfusion. *J. Clin. Invest.*, **88**, 1237–1243.
- MALLORY, G.K., WHITE, P.D. & SALCEDO-SALGAR, J. (1939). The speed of healing of myocardial infarction. A study of the pathologic anatomy in seventy-two cases. *Am. Heart J.*, **18**, 647–671.
- MAXWELL, M.P., HEARSE, D.J. & YELLON, D.M. (1987). Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.*, **21**, 737–746.
- MULLANE, K.M., KRAEMER, R. & SMITH, B. (1985). Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J. Pharmacol. Methods*, **14**, 157–167.
- MULLANE, K.M., READ, N., SALMON, J.A. & MONCADA, S. (1984). Role of leukocytes in acute myocardial infarction in anesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.*, **228**, 510–522.
- NOURSHARGH, S., RAMPART, M., HELLEWELL, P.G., JOSE, P.J., HARLAN, J.M., EDWARDS, A.J. & WILLIAMS, T.J. (1989). Accumulation of ¹¹¹In-neutrophils in rabbit skin in allergic and non-allergic inflammatory reactions in vivo: inhibition by neutrophil pretreatment in vitro with a monoclonal antibody recognising the CD18 antigen. *J. Immunol.*, **142**, 3193–3198.
- REIMER, K.A., JENNINGS, R.B., COBB, F.R., MURDOCK, R.H., GREENFIELD, J.C., BECKER, L.C., BULKLEY, B.R., HUTCHINS, G.M., SCHWARTZ, R.P., BAILEY, K.R. & PASSAMANI, E.R. (1985). Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. *Circ. Res.*, **56**, 651–665.
- REIMER, K.A., MURRY, C.E. & RICHARD, V.J. (1989). The role of neutrophils and free radicals in the ischemic-reperfused heart: why the confusion and controversy? *J. Mol. Cell Cardiol.*, **21**, 1225–1239.
- ROMSON, J.L., HOOK, B.G., KUNKEL, S.L., ABRAMS, G.D., SCHORK, M.A. & LUCCHESI, B.R. (1983). Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation*, **67**, 1016–1023.
- ROMSON, J.L., HOOK, B.G., RIGOT, V.H., SCHORK, M.A., SWANSON, D.P. & LUCCHESI, B.R. (1982). The effect of ibuprofen on accumulation of indium-111-labelled platelets and leukocytes in experimental myocardial infarction. *Circulation*, **66**, 1002–1011.
- RUBIN, B.B., SMITH, A., LIAUW, S., ISENMAN, D., ROMASCHIN, A.D. & WALKER, P.M. (1990). Complement activation and white cell sequestration in postischemic skeletal muscle. *Am. J. Physiol.*, **259**, H525–H531.

- SIMPSON, P.J., FANTONE, J.C., MICKELSON, J.K., GALLAGHER, K.P. & LUCCHESI, B.R. (1988a). Identification of a time window for therapy to reduce experimental canine myocardial injury: suppression of neutrophil activation during 72 hours of reperfusion. *Circ. Res.*, **63**, 1070–1079.
- SIMPSON, P.J., MITSOS, S.E., VENTURA, A., GALLAGHER, K.P., FANTONE, J.C., ABRAMS, G.D., SCHORK, M.A. & LUCCHESI, B.R. (1987). Prostacyclin protects ischemic reperfused myocardium in the dog by inhibition of neutrophil activation. *Am. Heart J.*, **113**, 129–137.
- SIMPSON, P.J., TODD III, R.F., FANTONE, J.C., MICKELSON, J.K., GRIFFIN, J.D. & LUCCHESI, B.R. (1988b). Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (Anti-Mol, Anti-CD11b) that inhibits leukocyte adhesion. *J. Clin. Invest.*, **81**, 624–629.
- SIMPSON, P.J., TODD, R.F., MICKELSON, J.K., FANTONE, J.C., GALLAGHER, K.P., LEE, K.A., TAMURA, Y., CRONIN, M. & LUCCHESI, B.R. (1990). Sustained limitation of myocardial reperfusion injury by a monoclonal antibody that alters leukocyte function. *Circulation*, **81**, 226–237.
- TANAKA, M., BROOKS, S.E., RICHARD, V.J., FITZHARRIS, G.P., STOLER, R.C., JENNINGS, R.B., ARFORS, K.-E. & REIMER, K.A. (1993). Effect of anti-CD18 antibody on myocardial neutrophil accumulation and infarct size after ischemia and reperfusion in dogs. *Circulation*, **87**, 526–535.
- THORNTON, J., STRIPLIN, S., LIU, G.S., SWAFFORD, A., STANLEY, A.W.H., VAN WINKLE, D.M. & DOWNEY, J.M. (1990). Inhibition of protein synthesis does not block myocardial protection afforded by preconditioning. *Am. J. Physiol.*, **259**, H1822–H1825.
- TUOMANEN, E.I., SAUKKONEN, K., SANDE, S., CIOFFE, C. & WRIGHT, S.D. (1989). Reduction of inflammation, tissue damage and mortality in bacterial meningitis in rabbits treated with monoclonal antibodies against adhesion-promoting receptors of leukocytes. *J. Exp. Med.*, **170**, 959–968.
- WALLIS, W.J., HICKSTEIN, D.D., SCHWARTZ, B.R., JUNE, C.H., OCHS, H.D., BEATTY, P.G., KLEBANOFF, S.J. & HARLAN, J.M. (1986). Monoclonal antibody-defined functional epitopes on the adhesion-promoting glycoprotein complex (CDw18) of human neutrophils. *Blood*, **67**, 1007–1013.
- WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289**, 646–650.
- WEISS, S.J. (1989). Tissue destruction by neutrophils. *N. Engl. J. Med.*, **320**, 365–375.
- WILLIAMS, F.M., COLLINS, P.D., TANNIERE-ZELLER, M. & WILLIAMS, T.J. (1990). The relationship between neutrophils and increased microvascular permeability in a model of myocardial ischaemia and reperfusion in the rabbit. *Br. J. Pharmacol.*, **100**, 729–734.
- WRIGHT, S.D., RAO, P.E., VAN VOORHIS, W.C., CRAIGMYLE, L.S., IIDA, K., TALLE, M.A., WESTBERG, E.F., GOLDSTEIN, G. & SILVERSTEIN, S.C. (1983). Identification of the C3bi receptor on human monocytes and macrophages by using monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 5699–5703.

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