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Role of oxygen radicals and arachidonic acid metabolites in the reverse passive Arthus reaction and carrageenin paw oedema in the rat

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1 The role of arachidonic acid metabolites and oxygen radicals in carrageenin-induced rat paw oedema and dermal reverse passive Arthus reaction (RPA) have been investigated.

2 Indomethacin (10 mg kg^{-1}, p.o.) inhibited carrageenin paw oedema when administered 30 min before, but not 2 h after carrageenin. BWB70C (10 mg kg^{-1}, p.o.), a selective inhibitor of 5-lipoxygenase, had no effect whether administered before or after carrageenin. Administration of both indomethacin and BWB70C had no greater anti-inflammatory effect than indomethacin alone.

3 BW755C (20 mg kg^{-1}, p.o.), which inhibits the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism, or superoxide dismutase-polyethylene glycol conjugate (SOD-PEG, 3000 u, i.v.) inhibited carrageenin paw oedema whether administered either 30 min before, or 2 h after carrageenin.

4 Pretreatment with dexamethasone (0.1 mg kg^{-1}) or colchicine (2 mg kg^{-1}), likewise suppressed carrageenin paw oedema.

5 BW755C (25–100 mg kg^{-1}, p.o.) dose-dependently reduced plasma leakage in the RPA, whereas indomethacin (5 mg kg^{-1}, p.o.) or BWB70C either alone or in combination, did not.

6 SOD-PEG (300–3000 u, i.v.) dose-dependently inhibited plasma leakage in the RPA. In addition, the iron chelator and peroxyl radical scavenger, desferrioxamine (200 mg kg^{-1}, s.c.) also inhibited plasma leakage.

7 Pretreatment with dexamethasone (0.1 mg kg^{-1}) or colchicine (1 mg kg^{-1}) reduced the plasma leakage in RPA, whereas MK-886 (10 mg kg^{-1}) had no effect.

8 These results indicate an important role for oxygen radicals but not arachidonic acid metabolites in the maintenance of carrageenin paw oedema and the plasma leakage in RPA. Furthermore, the results suggest that the anti-inflammatory actions of BW755C can be dissociated from its effects on arachidonic acid metabolism and are attributed to its anti-oxidant activity.

Keywords: Carrageenin oedema; reverse passive Arthus reaction; neutrophil-dependent plasma leakage; cyclo-oxygenase; lipoxygenase; 5-lipoxygenase inhibitor; BW755C; oxygen radicals

Introduction

There is considerable evidence from both *in vitro* and *in vivo* studies that oxygen-derived radicals play a role in acute inflammation. Reactive oxygen molecules generated enzymically or from activated phagocytes, damage mammalian cells, including endothelial cells (for review, Fantone & Ward, 1982; Ward, 1991). Administration of oxygen radical generating systems into the lung, knee joint or foot of experimental animals leads to increases in vascular permeability, cellular infiltration and tissue damage (for review, Schraufstatter *et al.*, 1987). Furthermore, the anti-oxidant enzyme, superoxide dismutase (SOD) has anti-inflammatory activity in models of acute inflammation (Oyanagui, 1976).

Arachidonic acid metabolites of the cyclo-oxygenase and lipoxygenase enzymes have also been implicated as mediators of acute inflammation. The vasodilator prostaglandins produce erythema and hyperalgesia (for review, see Higgs *et al.*, 1984), while the potent actions of the 5-lipoxygenase product, leukotriene B_4 (LTB_4) on polymorphonuclear leucocytes (PMN's) may contribute to their accumulation and activation at sites of inflammation (for review, see Lewis *et al.*, 1990). Furthermore, in the presence of vasodilator prostaglandins, LTB_4 produces a neutrophil-dependent increase in vascular permeability (Wedmore & Williams, 1981).

The role of arachidonic acid metabolites has been investigated in a number of models of acute inflammation. Thus carrageenin-induced paw oedema in the rat has been used extensively to determine the activity of non-steroid anti-inflammatory or aspirin-like drugs which inhibit prostaglandin synthesis (Higgs *et al.*, 1983). The experimental anti-inflammatory compound BW755C, a dual cyclo-oxygenase/lipoxygenase inhibitor, also inhibits carrageenin-induced paw oedema (Higgs *et al.*, 1979). Oxygen radicals have also been implicated in models of acute inflammation in which there is a neutrophil-dependent increase in vascular permeability (for review, see Fantone & Ward, 1982). However, the role of oxygen radicals in acute inflammation in relation to the activity of arachidonic acid metabolites remains unclear. In the present study the role of both oxygen radicals and arachidonate metabolites have been investigated in carrageenin-induced paw oedema and in the dermal reverse passive Arthus reaction (RPA) in the rat.

Methods

Carrageenin paw oedema

Male Wistar rats (140–190 g) which had been fasted overnight (18 h) received a subplantar injection in the right hind paw of carrageenin (100 µl of 1% suspension in 0.85% saline). Paw thickness was measured from ventral to dorsal

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surfaces, with dial callipers (Mitutoyo), immediately prior to carrageenin injection and then at hourly intervals from 1–6 h afterwards. Oedema was expressed as the increase in paw thickness (in mm) measured after carrageenin injection compared to the pre-injection value for individual animals.

Drugs were administered orally in 0.25% methyl cellulose (celacol) or intravenously (dose volume of 5 ml kg^{-1}), either 30 min before or 2 h after carrageenin, unless otherwise stated. Doses were selected from previous studies (indomethacin, Higgs *et al.*, 1983; BWB70C, Salmon *et al.*, 1989 and unpublished data; BW755C, Higgs *et al.*, 1979; Salmon, 1986; MK-886, Gillard *et al.*, 1989).

Reverse passive Arthus reaction

Male Wistar rats (225–275 g) which had been fasted overnight were anaesthetized with halothane (2%, 3 l – oxygen min^{-1}). Antigen (3 mg rabbit IgG), mixed with ^{125}I-labelled human serum albumin (HSA; 0.5 μCi) and Evans blue (2.5% in saline), was injected via the tail vein (1 ml). Immediately afterwards, either antibody (50 μg goat anti-rabbit IgG) or saline was injected intra-dermally (50 μl, 12 × 0.4 mm needle), in triplicate according to a random plan, to a previously shaved area of dorsal skin. The rats were allowed to recover from the anaesthesia.

Rats were re-anaesthetized with halothane 90 min after challenge and, following laparotomy, blood (1 ml) was drawn from the abdominal aorta into tri-sodium citrate (0.315% final concentration). Plasma was separated by centrifugation (10,000 *g*; 2 min). The rats were killed by cervical dislocation, the dorsal skin removed and injection sites (blue areas) and two untreated sites punched out with a cork borer (17 mm). Radioactivity in skin samples and duplicate plasma samples (100 μl) was counted (Nuclear Enterprises – NE1600 gamma counter). Plasma leakage was calculated as μl of plasma.

BW755C, BWB70C and indomethacin were administered orally 1 h before and MK-886, 4 h before induction of the RPA response, (suspended in 0.5% methyl cellulose, dosing volume of 1 ml kg^{-1}). Colchicine and dexamethasone were administered subcutaneously 2 h before and desferrioxamine 1 h before induction of the RPA reaction. Superoxide dismutase-polyethylene glycol conjugate (SOD-PEG) was injected i.v. (in saline, 2 ml kg^{-1}) via the tail vein 1 min before initiation of RPA. Control animals received the vehicle via the relevant route of administration.

Myeloperoxidase activity

Myeloperoxidase (MPO), a haemoprotein which is located in azurophil granules of neutrophils where it plays a role in bacterial killing, has been used as an enzyme marker of neutrophil infiltration in various tissues (Bradley *et al.*, 1982). In the present study, MPO was measured by a method similar to that described by Bradley *et al.* (1982).

Skin sites were finely chopped with scissors and homogenized (Ultra turrax 45 s) in 3 ml of 0.5% hexadecyltrimethyl-ammonium bromide (HTAB) in 50 mM potassium phosphate buffer (pH 6). Aliquots (1 ml) were frozen (on cardice) and thawed (immersion in warm water, 37°C) three times. Following centrifugation (10,000 *g* for 20 min) the supernatant (200 μl) was mixed in a cuvette with 2.8 ml of 50 mM phosphate buffer (pH 6) containing 0.167 mg ml^{-1} O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide at 37°C and the change in absorbance at 460 nm measured immediately (Guildford Response spectrophotometer). The MPO activity is expressed as the number of rat peritoneal neutrophils (glycogen elicited and purified as described by McCall *et al.*, 1989) containing an equivalent amount of MPO.

Plasma SOD activity

SOD activity was measured spectrophotometrically in diluted plasma (1:2) as inhibition of ferricytochrome C reduction by O_2^-. Ferricytochrome C (20 mM) was incubated (37°C) with an O_2^- generating system (5 mu xanthine oxidase and 100 μM hypoxanthine, 37°C) and increases in absorbance at 550 nm measured (Guildford Response spectrophotometer). The amount of O_2^--scavenging activity in the plasma was expressed as units of SOD activity.

Statistical analysis

Results are expressed as mean ± s.e.mean of (*n*) rats. Where appropriate, statistical significance was calculated by Student's *t* test for unpaired data (two tailed) or one way analysis of variance (ANOVA) and Bonferroni corrected *P* value for multiple comparisons. The level of statistical significance was taken as $P<0.05$. In time course experiments (Figures 1–5) analysis by Student's *t* test was made at one time point (3 h), being representative of the drug-induced change.

Materials

BWB70C ((E)-N-3-[3-(4-fluorophenoxy)phenyl]-1(**R,S**)-methyl-prop-2-en-l-yl-*N*-hydroxyurea), (Salmon *et al.*, 1989) and BW755C (3-amino-l-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline) (Higgs *et al.*, 1979), MK-886(3-[1-(4-chlorobenzyl)-3-*t*-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid) (Gillard *et al.*, 1989) were synthesized by Wellcome Research Laboratories (Beckenham). Dexamethasone (Decadron) was obtained from Merk, Sharpe and Dohme (Hertfordshire) and desferrioxamine (Desferal) from Ciba Laboratories (Horsham). Methyl cellulose was obtained from Courtaulds Fine Chemicals (Derby), O-dianisidine di-hydrochloride and hexadecyltrimethyl-ammonium bromide from Aldrich Chemical Co. Ltd., (Dorset), Halothane BP from RPM Animal Health Ltd., and ^{125}I-labelled human serum albumin (2.5 μCi mg^{-1} protein) from Amersham International plc (Amersham). All other reagents and antibodies were obtained from Sigma Chemical Co. Ltd. (Poole, Dorset).

Results

Carrageenin paw oedema

Cyclo-oxygenase and 5-lipoxygenase inhibitors A subplantar injection of carrageenin in control rats induced an increase in paw thickness over 6 h (Figure 1). Pretreatment with indomethacin (10 mg kg^{-1}, p.o.) 30 min before carrageenin prevented the increase in paw thickness between 2 and 6 h ($P<0.001$ at 3 h, $n=5$, Figure 1a), while treatment with indomethacin (10 mg kg^{-1}, p.o.) 2 h after carrageenin had no effect on paw oedema (Figure 1b). The selective 5-lipoxygenase inhibitor, BWB70C (10 mg kg^{-1}, p.o.) had no effect on paw thickness whether administered 30 min before or 2 h after carrageenin (Figures 1a and b). Pretreatment with a combination of indomethacin (10 mg kg^{-1}, p.o.) and BWB70-C (10 mg kg^{-1}, p.o.) inhibited the increase in paw thickness produced between 1 and 6 h ($P<0.01$ at 3 h, $n=5$, Figure 1a) but had no inhibitory effect when administered 2 h after carrageenin (Figure 1b).

BW755C Pretreatment (30 min) with BW755C (20 mg kg^{-1}, p.o.) inhibited oedema formation produced between 2 and 6 h after carrageenin (Figure 2a). Furthermore, treatment with BW755C (20 mg kg^{-1}, p.o.) 2 h after injection of carrageenin also inhibited the increases in paw thickness over the subsequent 4 h, while producing an apparent reversal of oedema formation (Figure 2b).

SOD-PEG SOD-PEG (3000 u, i.v.) inhibited the increase in paw thickness when given either before (30 min) or 2 h after carrageenin (Figure 3). The inhibition produced by SOD-PEG when given after carrageenin was dose-dependent with

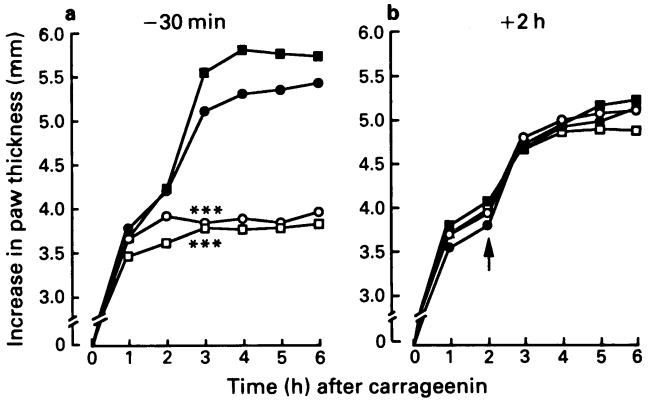

Figure 1 Effect of indomethacin and BWB70C on carrageenin-induced paw oedema administered either (a) 30 min before or (b) 2 h after carrageenin, as indicated by an arrow: (■) celacol; (□) indomethacin (10 mg kg^{-1}, p.o.); (●) BWB70C (10 mg kg^{-1}, p.o.); (○) indomethacin + BWB70C. Results are shown as the mean increase in paw thickness (mm) of 5 rats, statistical significance from the celacol group was determined after 3 h (***$P<0.001$). For clarity s.e.mean are not included, but are less than 5% of the mean for all points.

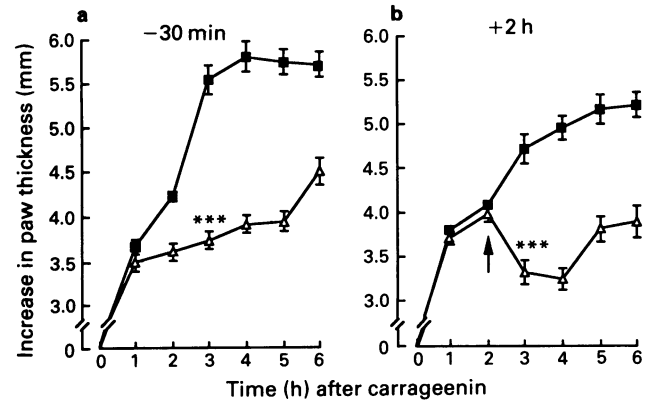

Figure 2 Inhibition of carrageenin-induced paw oedema by BW755C, administered either (a) 30 min before or (b) 2 h after carrageenin, as indicated by an arrow; (■) celacol; (△) BW755C (20 mg kg^{-1}, p.o.). Results expressed as the increase in paw thickness (mm) are shown as mean ± s.e.mean of 5 rats; statistical significance from the celacol group was determined after 3 h (***$P<0.001$).

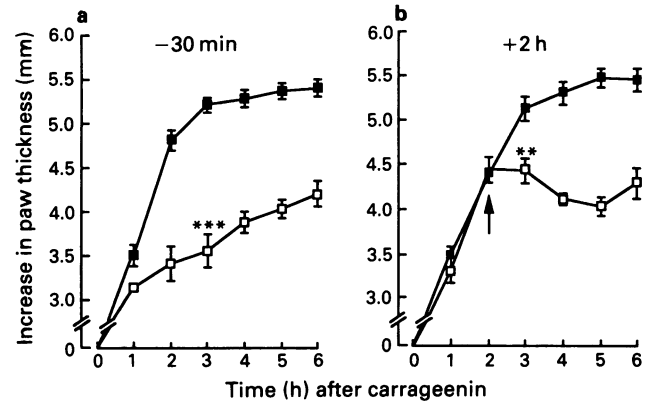

Figure 3 Inhibition of carrageenin-induced paw oedema by superoxide dismutase-polyethylene glycol conjugate (SOD-PEG) administered either (a) 30 min before or (b) 2 h after carrageenin, as indicated by an arrow; (■) saline; (□) SOD-PEG (3 000 u, i.v.). Results expressed as the increase in paw thickness (mm) are shown as mean ± s.e.mean of 6 rats; statistical significance from the saline group was determined after 3 h (***$P<0.001$).

a reduction in paw thickness between 1 and 4 h at doses of 300, 1 000 and 3 000 u of 47 ± 8%, 59 ± 7% and 83 ± 7% respectively ($n = 6$). Furthermore, the high dose of SOD-PEG (3 000 u) caused an apparent reversal of the oedema (Figure 3b).

Plasma SOD-like activity from untreated rats was equivalent to 24 u ml^{-1} SOD ($n = 2$). Immediately after an intravenous bolus injection of SOD-PEG (1 500 u) the activity in plasma rose to 195 ± 10 u ml^{-1} of SOD-like activity which decreased to 137 ± 6 u ml^{-1} after 3 h and 140 ± 5 u ml^{-1} after 6 h ($n = 3$).

Dexamethasone Preatment with dexamethasone (0.1 mg kg^{-1}, i.v.) completely suppressed the increase in paw thickness between 2 and 6 h after carrageenin (Figure 4a). Given 2 h after carrageenin, the glucocorticoid also reduced oedema in a time-dependent manner (Figure 4b).

Colchicine The inhibitor of microtubule activity, colchicine, was used to determine the role of leucocytes in the oedema response to carrageenin. Colchicine (2 mg kg^{-1}, s.c.) inhibited the increase in paw thickness induced by carrageenin. The degree of inhibition was dependent upon the time of pretreatment prior to challenge (Figure 5).

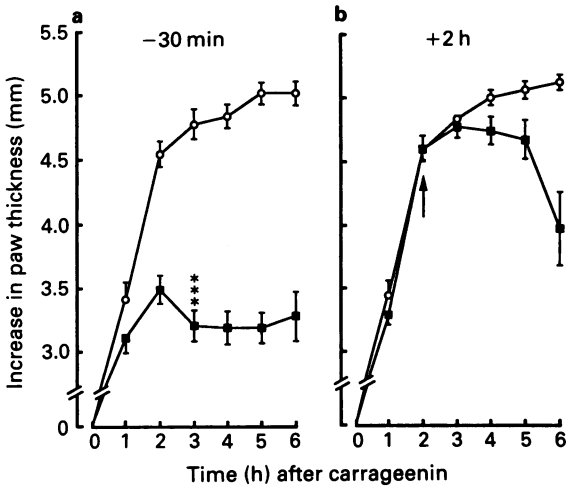

Figure 4 Effect of dexamethasone on carrageenin-induced paw oedema, administered either (a) 30 min before or (b) 2 h after carregeenin, as indicated by an arrow; (○) saline; (■) dexamethasone (0.1 mg kg^{-1}, i.m.). Results expressed as the increase in paw thickness (mm) are shown as mean ± s.e.mean of 6 rats; statistical significance from the saline group was determined after 3 h (***$P<0.001$).

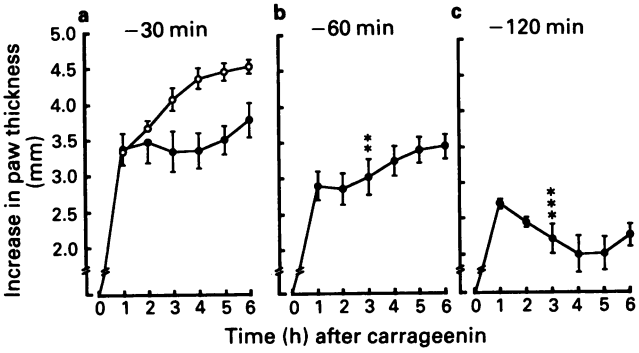

Figure 5 Effect of colchicine on carrageenin-induced paw oedema, administered (a) 30 min, (b) 60 min and (c) 120 min before carrageenin; (○) saline; (●) colchicine 2 mg kg^{-1}, s.c. Results expressed as the increase in paw thickness (mm) are shown as mean ± s.e.mean of 6 rats; statistical significance from the saline group was determined after 3 h (**$P<0.01$, ***$P<0.001$).

Dermal reverse passive Arthus reaction

The induction of the RPA response, by intravenous antigen and intradermal antibody, resulted in a substantial increase in plasma leakage within 90 min (68 ± 6 μl plasma/site, $n = 30$, $P < 0.001$ compared to saline-injected sites). Dermal administration of antibody (50 μg) in the absence of antigen ($n = 3$) or saline injection (50 μl) produced minimal plasma leakage (< 10 μl, $n = 20$).

Table 1 The effect of inhibitors of arachidonic acid metabolism on plasma leakage in the rat dermal reverse passive Arthus (RPA) reaction

Compound	Dose (mg kg^{-1})	Route	Pretreatment time	% control plasma leakage	n
Indomethacin	5	p.o.	1 h	103 ± 17	(8)
BWB70C	10	p.o.	1 h	149 ± 10	(8)
	100	p.o.	1 h	105 ± 24	(3)
Indomethacin + BWB70C	5 10	p.o.	1 h	106 ± 11	(5)
MK-886	10	p.o.	4 h	136 ± 11	(4)
Dexamethasone	0.1	s.c.	2 h	9 ± 1**	(4)

Drugs were administered prior to induction of the RPA reaction as shown by the pretreatment time. Results are expressed as percentage of the plasma leakage in RPA sites from control rats as mean ± s.e.mean of (n) rats. The data are from three separate experiments and statistical significance of inhibition was calculated by comparison with the appropriate control group before normalization to percentage control (**$P < 0.01$).

Cyclo-oxygenase and 5-lipoxygenase inhibition Pretreatment (1 h) with indomethacin (5 mg kg^{-1}, p.o.) or BWB70C (10 mg kg^{-1}, p.o.), either alone or in combination, did not reduce the plasma leakage in the RPA sites (Table 1). A higher dose of BWB70C (100 mg kg^{-1}, p.o.) or MK-886 (10 mg kg^{-1}, p.o., 4 h prior to challenge) also failed to inhibit plasma leakage (Table 1).

BW755C Pretreatment (1 h) with BW755C (25–100 mg kg^{-1}, p.o.) produced a dose-dependent reduction in plasma leakage in RPA sites (Figure 6a). At the highest dose (100 mg kg^{-1}, p.o.) the plasma leakage in RPA sites was not significantly different from that produced by the saline injected sites ($P < 0.001$; $n = 5$).

SOD-PEG and desferrioxamine SOD-PEG (300–3 000 u, i.v. 1 min prior to challenge) dose-dependently reduced plasma leakage in RPA sites (Figure 6b), producing a 53 ± 9% inhibition ($P < 0.01$; $n = 4$–8) of plasma leakage at the highest dose (3 000 u, i.v.) The vehicle (PEG 200 nmol, i.v.) had no significant effect on plasma leakage in RPA sites (73 ± 10 μl; $n = 7$) compared to RPA sites in rats receiving only saline (64 ± 9 μl; $n = 4$). The iron chelator and peroxyl radical scavenger, desferrioxamine (200 mg kg^{-1}, s.c. 1 h prior to challenge) also significantly inhibited plasma leakage (44 ± 12% inhibition, $n = 6$, $P < 0.01$).

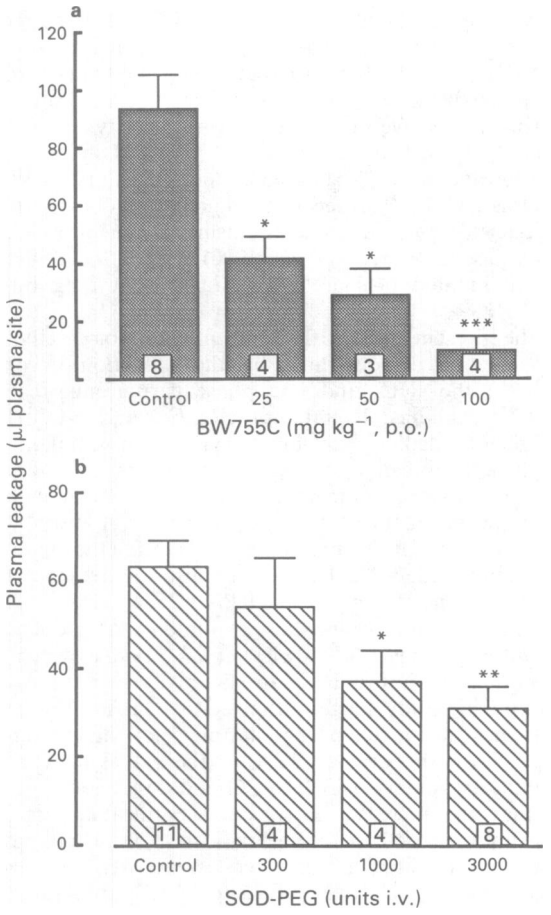

Figure 6 Dose-dependent inhibition of plasma leakage in the rat dermal reverse passive Arthus (RPA) reaction by (a) BW755C (25–100 mg kg^{-1}) and (b) superoxide dismutase-polyethylene glycol conjugate (SOD-PEG, 300–3 000 u). Results expressed as μl plasma leakage per site are shown as mean ± s.e.mean of n (number in column) rats, where statistically significant difference from control animals is shown as *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

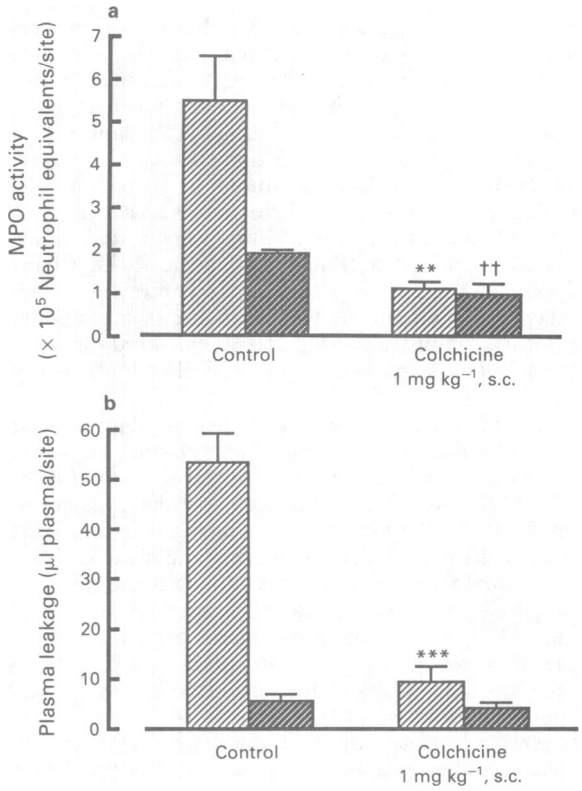

Figure 7 Effect of colchicine (1 mg kg^{-1}, s.c.) on (a) neutrophil infiltration, measured by myeloperoxidase (MPO) activity, (b) plasma leakage, in reverse passive Arthus reaction (RPA) (light columns) and saline (dark columns) injected sites. MPO levels were expressed as neutrophil equivalents, from values obtained from a standard curve constructed with known numbers of rat peritoneal neutrophils. Results are shown as mean ± s.e.mean of 7–8 rats. Statistical significance compared to the RPA response in control rats is shown as **$P < 0.01$, ***$P < 0.001$ and compared to the saline injected site in control rats as ††$P < 0.01$.

Dexamethasone Administration of dexamethasone (0.1 mg kg^{-1}, s.c.) 2 h before induction of the RPA reaction significantly reduced plasma leakage (91 ± 1% inhibition, $n = 4$; $P < 0.01$; Table 1).

Colchicine Colchicine (1 mg kg^{-1}, s.c. 2 h prior to challenge) inhibited neutrophil accumulation in the RPA reaction by 80 ± 4% ($P < 0.01$; $n = 7-8$), as measured by MPO activity (Figure 7a). The MPO activity in the saline-injected sites was also lower in the colchicine-treated group than in the controls. The decreased MPO activity in colchicine-treated animals is unlikely to be due to a direct inhibitory activity of colchicine on the enzyme or assay system, as colchicine (10 μg ml^{-1}) had no effect on MPO activity from rat peritoneal neutrophils *in vitro* (data not shown). In a parallel experiment (Figure 7b) colchicine administration significantly inhibited plasma leakage in RPA sites by 83 ± 5% ($P < 0.001$; $n = 7$).

Discussion

The present study provides strong evidence for an important role of oxygen radicals in the plasma leakage produced during the dermal reverse passive Arthus reaction (RPA) and in the oedema induced by carrageenin in the rat paw. Furthermore, the data suggest that arachidonic acid cyclo-oxygenase (CO) and 5-lipoxygenase (5-LO) products do not contribute to the increases in plasma leakage in the dermal RPA or the later phase of the carrageenin paw oedema.

Carrageenin paw oedema has been used extensively to evaluate non-steroid anti-inflammatory drugs and the activity of these compounds is closely related to their potency as inhibitors of CO (Higgs *et al.*, 1983). Indeed, in the present study pretreatment with indomethacin abolished the oedema induced by carrageenin. However, administration of indomethacin 2 h after carrageenin had no effect on paw oedema, confirming previous studies by Holsapple & Yim (1984). In the present study, indomethacin also failed to reduce the plasma leakage produced in the dermal RPA, confirming previous studies where indomethacin and other non steroid anti-inflammatory drugs were found either to have weak or no effect (Pflum & Graeme, 1979; Chang & Otterness, 1981; Carter *et al.*, 1982). In contrast, prostaglandins may play a role in the RPA response in the rabbit skin since local administration of PGE_2 enhanced, and indomethacin reduced, plasma leakage (Hellewell & Williams, 1987).

At the inflammatory site where there is damaged endothelium, vasodilators may potentiate plasma leakage and hence oedema formation by increasing blood flow (Williams & Peck, 1977). In the present study, plasma leakage in rat dermal RPA and the later phase of carrageenin paw oedema appeared to be independent of prostaglandins, and therefore other vasodilators may be involved, for example nitric oxide (NO). Indeed, recent studies have indicated a role for NO in substance P- and carrageenin-induced oedema in the rat (Hughes *et al.*, 1990; Ialenti *et al.*, 1992) and in endotoxin-induced vascular permeability changes in the intestine (Boughton-Smith *et al.*, 1992).

The plasma leakage induced in the dermal RPA and in the later phase of the carrageenin-induced paw oedema has been proposed to be neutrophil-dependent (Humphrey, 1955). This mechanism of action is supported in the present study by the inhibition with colchicine (an inhibitor of microtubule activity that prevents leucocyte motility) of neutrophil infiltration and plasma leakage in dermal RPA and carrageenin-induced paw oedema. Although LTB_4 is thought to be a major mediator of neutrophil accumulation and plasma leakage in man and rabbit (for review, see Higgs *et al.*, 1984), both the selective 5-LO inhibitor BWB70C and the 5-lipoxygenase activating protein (FLAP) inhibitor MK-886 failed to reduce the plasma leakage in the dermal RPA at doses that markedly inhibit leukotriene synthesis *in vivo* (Salmon *et al.*, 1989; Gillard *et al.*, 1989). BWB70C was also without effect on the carrageenin-induced paw oedema, confirming previous studies with other selective 5-LO inhibitors (Higgs *et al.*, 1988). Although Namiki *et al.* (1986) reported that LTB_4 was chemotactic for rat neutrophils *in vitro*, others have failed to demonstrate such an effect (Kreisle *et al.*, 1985, Kopp *et al.*, 1985). Moreover, binding studies suggest that rat neutrophils lack the low affinity LTB_4 receptor which is thought to mediate chemotaxis (Kreisle *et al.*, 1985), which therefore may explain the lack of activity of 5-LO inhibitors in these neutrophil-dependent models of inflammation.

The 'dual inhibitor' of CO and LO, BW755C, reduced paw oedema when administered either before or after carrageenin. In addition, BW755C produced a dose-dependent inhibition of plasma exudation in the dermal RPA. The effect of BW755C given before carrageenin may be mediated via CO inhibition since pretreatment with indomethacin was also effective. However, the anti-inflammatory effect of BW755C when given after carrageenin and in the RPA was independent of 5-LO and CO as the combination of BWB70C and indomethacin failed to inhibit these responses. BW755C also inhibits the 12-LO and 15-LO pathways of arachidonic acid metabolism (Randall *et al.*, 1980). Since these enzymes would not be inhibited by the combination of BWB70C and indomethacin at the doses used, the anti-inflammatory activity of BW755C may have been due to inhibition of 12-LO or 15-LO. However, the anti-inflammatory activity of BW755C may also be mediated by its anti-oxidant activity (Marnett *et al.*, 1982; Pekoe *et al.*, 1982). This mechanism is supported by the ability of both SOD-PEG, and desferrioxamine to attenuate vascular leakage in dermal RPA. Also, SOD-PEG, like BW755C, reduced oedema when administered either before or after carrageenin. SOD has previously been shown to have anti-inflammatory activity in both carrageenin paw oedema (Oyanagui, 1976) and in rat dermal RPA (Petrone *et al.*, 1980; McCormick *et al.*, 1981; Warren *et al.*, 1987; 1990). Furthermore, Fligiel *et al.* (1984) reported that desferrioxamine attenuated plasma leakage in rat dermal RPA, although Warren *et al.* (1990) found that desferrioxamine attenuated plasma leakage in the rat lung but not dermal RPA.

In the present study, the anti-inflammatory activity of dexamethasone demonstrated by the reduction in plasma leakage in the RPA and late phase carrageenin reactions cannot be explained by inhibition of CO and 5-LO. However, glucocorticoids, via inhibition of phospholipase A_2 through induction of lipocortin, will also inhibit 12-LO and 15-LO pathways of arachidonic acid metabolism and also prevent the production of PAF (for review, Flower, 1989), which may account for the activity of dexamethasone in the present study. Indeed a PAF antagonist suppressed oedema formation in the rabbit dermal RPA (Hellewell & Williams, 1986). In addition, dexamethasone inhibits the induction of NO synthase *in vivo* (Knowles *et al.*, 1990), which may contribute to its anti-inflammatory activity in these models.

The present study suggests that neither prostaglandins nor leukotrienes have a role in the later phase of carrageenin paw oedema or in the dermal RPA in the rat skin. The anti-inflammatory activity of SOD-PEG and desferrioxamine suggests that the increases in vascular permeability in these models are mediated by oxygen radicals. The results presented illustrate qualitative differences in activity between the combination of selective CO and 5-LO inhibitors and BW755C, a so-called dual inhibitor of CO and 5-LO. Indeed, the activity of BW755C in the present study is best explained as being due to CO inhibition, for the initial suppression of carrageenin paw oedema, while activity as a scavenger of oxygen radicals may account for inhibition of the subsequent phases, as well as its activity in dermal RPA. Previous studies on the prevention of gastric mucosal injury in the rat by

BW755C have also indicated that this action is independent of CO and 5-LO inhibition (Boughton-Smith & Whittle, 1988). These observations illustrate clearly that the use of BW755C to evaluate the role of leukotrienes in models of inflammation is potentially misleading, and therefore should be discontinued. These findings also suggest that the rat dermal RPA and the later phases of the carrageenin paw oedema may be suitable models for the *in vivo* assessment of the anti-inflammatory actions of novel anti-oxidants and inhibitors of oxygen radical generation.

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