Antagonism of Paf-induced oedema formation in rabbit skin: a comparison of different antagonists

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1 Eight platelet activating factor (Paf) antagonists were evaluated as inhibitors of oedema formation in rabbit skin induced by intradermal injection of Paf plus prostaglandin E₂ (PGE₂). Antagonists were tested by both intradermal (i.d.) and intravenous (i.v.) routes.

2 Intradermal injection of two antagonists structurally-related to Paf (SRI 63-675 and CV-3988) resulted in a partial inhibition of Paf-induced oedema formation but at high doses of antagonist, marked agonist activities were detected. CV-3988 administered i.v. inhibited Paf-induced plasma leakage by 73–80%; however, oedema responses to a range of other inflammatory mediators were also reduced, albeit to a lesser extent (40–60%). SRI 63-675 administered i.v. did not significantly inhibit Paf-induced oedema.

3 The antagonist 48740 RP administered either i.d. or i.v. showed partial, but selective, inhibition of Paf-induced oedema formation, although the doses required were high when compared with other antagonists.

4 BN 52021 was a weak Paf antagonist when injected i.d., but following i.v. administration the responses to Paf were inhibited by 63–71%. Responses to all other mediators tested were unaffected.

5 Kadsurenone and its synthetic derivatives, L-652,731 and L-659,989 all blocked responses to Paf in the skin. L-659,989 was the most potent, achieving almost total inhibition when injected i.d. and i.v.; moreover, it was selective for Paf. L-652,731 was more potent than kadsurenone.

6 WEB 2086 given i.d. and i.v. showed similar activity to L-659,989 and it was also selective for Paf-induced oedema formation.

7 These results illustrate that in rabbit skin not all Paf antagonists are selective for Paf, some showing agonist-like activity which can mask antagonist properties. It is suggested that before ascribing a role for endogenous Paf in an inflammatory reaction based on results with antagonists, the activity of the antagonists in the model under investigation should be rigorously established.

Introduction

Intradermal injection of platelet activating factor (Paf) in the rabbit and other species induces an increase in local microvascular permeability and synergism with vasodilators, such as prostaglandin E₂ (PGE₂), to produce marked oedema formation has been observed (Wedmore & Williams, 1981a; Humphrey *et al.*, 1982; Morley *et al.*, 1983; McGivern & Basran, 1984; Pirotzky *et al.*, 1984; Hellewell & Williams, 1986). Paf appears to act directly to induce

permeability changes since its actions are not reduced in animals depleted of circulating neutrophils (Wedmore & Williams, 1981a) or platelets (Pirotzky *et al.*, 1984).

Since the initial description of CV-3988 as a Paf antagonist (Terashita *et al.*, 1983), a large number of structurally unrelated compounds have been reported as selective Paf antagonists (for reviews, see Braquet & Godfroid, 1986; Braquet *et al.*, 1987; Saunders & Handley, 1987). These have been characterized primarily on the basis of inhibition of Paf-induced platelet aggregation and may be grouped as follows: antagonists structurally-related to Paf; natural products and synthetic analogues; novel synthetic compounds; and products belonging to well-known pharmacological classes (Braquet &

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Godfroid, 1986). Based on the activities of Paf and the actions of these antagonists, Paf has been implicated as a contributory mediator in a wide range of models of inflammation (Braquet *et al.*, 1987). The mechanisms underlying inflammation are complex and it is likely that many mediators interact to produce the final tissue response. Therefore, it is important to establish the selectivity and efficacy of the antagonist in the model under investigation before ascribing a role to Paf in an inflammatory reaction. For example, we have previously reported that the Paf antagonist L-652,731 (Hwang *et al.*, 1985) reduced oedema formation in an Arthus reaction in rabbit skin (Hellewell & Williams, 1986), a reaction that is dependent on generation of the complement peptide C5a (Cochrane & Janoff, 1974; Jose *et al.*, 1983). However, to exclude the possibility that the antagonist was working via a mechanism other than Paf antagonism, we established that L-652,731 was selective for Paf in rabbit skin and did not antagonize C5a or modify blood flow.

In the present study, we have investigated the activities of eight structurally diverse Paf antagonists on oedema formation in rabbit skin induced by the intradermal injection (i.d.) of Paf. Examples of Paf-related compounds tested were CV-3988 (Terashita *et al.*, 1983) and SRI 63-675 (Handley *et al.*, 1987); of natural products were BN-52021 (Braquet *et al.*, 1985) and kadsurenone (Shen *et al.*, 1985), and synthetic derivatives L-652,731 (Hwang *et al.*, 1985) and L-659,989 (Ponpipom *et al.*, 1988); of synthetic compounds was 48740 RP (Sedivy *et al.*, 1985); and of pharmacological agents was WEB 2086 (Casals-Stenzel *et al.*, 1987). The antagonists were tested by both the i.d. and intravenous (i.v.) routes.

Methods

Animals

Male New Zealand White specific pathogen-free rabbits (2.5–3.5 kg) were purchased from Hacking and Churchill, Huntingdon, Cambridgeshire.

Solubilization of antagonists

For i.d. and i.v. injection, CV-3988 was dissolved in saline (62 mg ml⁻¹ and 10 mg ml⁻¹ respectively) and diluted appropriately. SRI 63-675 was dissolved in saline for both i.d. and i.v. injection (6.91 mg ml⁻¹ and 10 mg ml⁻¹ respectively). Similarly, 48740 RP was dissolved in saline at 28 mg ml⁻¹ and 50 mg ml⁻¹ for i.d. and i.v. administration, respectively. For i.d. injection, BN 52021 was dissolved at 84.88 mg ml⁻¹ in dimethylsulphoxide (DMSO) and further diluted in saline; for i.v. administration,

10 mg of BN 52021 was solubilized in 1 ml 0.1 N NaOH and adjusted to pH 7.4 with 1 N HCl (approximately 0.1 ml). Kadsurenone, L-652,731 and L-659,989 were dissolved in DMSO at concentrations of 34 mg ml⁻¹, 40 mg ml⁻¹ and 48 mg ml⁻¹ respectively for i.d. injection; further dilutions were made in warmed (50°C) saline. Of these latter compounds, only L-659,989 was tested i.v. and it was dissolved in dimethylformamide at 50 mg ml⁻¹. Finally, WEB 2086 was dissolved in saline (4.56 mg ml⁻¹) for i.d. injection; for i.v. administration, 10 mg was solubilized in 0.3 ml 0.1 N HCl, neutralized with 0.23 ml 0.1 N NaOH and made up to 1 ml with saline.

For i.d. injection, the local concentration of DMSO did not exceed 0.5%. Above this concentration, non-specific effects of the vehicle were detected.

Measurement of local oedema formation in rabbit skin

Oedema formation was measured as the local accumulation of i.v. injected ¹²⁵I-human serum albumin into skin sites. Rabbits were anaesthetized with sodium pentobarbitone (30 mg kg⁻¹), the fur on the dorsal skin shaved and 1.5 µCi kg⁻¹ of ¹²⁵I-albumin mixed with a solution of Evans blue dye in sterile saline (10 mg dye kg⁻¹) was injected i.v. via a marginal ear vein. Agents under test were freshly prepared in sterile saline from stock solutions and 0.1 ml injected i.d. in six replicates per treatment according to a balanced site injection plan. After 30 min, a 10 ml cardiac blood sample was taken into heparin and plasma prepared. The animal was then killed with sodium pentobarbitone and the dorsal skin removed. Injection sites were punched out with a 17 mm diameter punch and counted together with three 1 ml plasma samples in a 12 head gamma counter (LKB, Wallac 1260 Multigamma). The amount of plasma protein that had accumulated in each skin site was calculated in terms of µl plasma by dividing the ¹²⁵I-count rate in each skin site by the ¹²⁵I-count rate in 1 µl plasma.

When Paf antagonists were tested i.d., they were mixed before injection at the appropriate concentrations with combinations of inflammatory agents under test. For testing of antagonists via the i.v. route, experiments were performed with pairs of animals, one rabbit receiving the antagonist, the other receiving an appropriate volume of vehicle. After 5 min pretreatment, each rabbit was injected i.d. with the same combination of inflammatory mediators and killed 30 min later and oedema formation assessed as described above.

Statistical analysis

Data are presented as the mean ± s.e.mean and have been analysed with Student's paired *t* test after i.d.

Table 1 Effect of local administration of eight Paf antagonists on Paf-induced oedema formation in rabbit skin

Antagonist	Dose (mol per site)	% inhibition	P
SRI 63-675	10^{-8}	45 ± 5 (5)	<0.001
CV-3988	10^{-7}	36 ± 4 (4)	<0.05
48740 RP	10^{-6}	57 ± 7 (4)	<0.01
BN 52021	10^{-7}	37 ± 6 (9)	<0.001
Kadsurenone	5×10^{-8}	51 ± 6 (5)	<0.02
L-652,731	5×10^{-8}	65 ± 6 (16)	<0.001
L-659,989	5×10^{-8}	90 ± 2 (5)	<0.0001
WEB 2086	10^{-7}	96 ± 2 (6)	<0.0001

Plasma exudation was induced by i.d. injection of a fixed dose of Paf (10^{-9} mol per site) mixed with prostaglandin E_2 (3×10^{-10} mol per site) and the antagonists at the doses shown. Number of rabbits given in parentheses.

administration of antagonists, and with Student's *t* test after i.v. administration of antagonists. A *P* value of <0.05 was considered statistically significant. The background response to PGE_2 alone has been subtracted from all data before calculating the percentage inhibition.

Drugs and materials

Prostaglandin E_2 (PGE_2), bradykinin, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) and zymosan were purchased from Sigma Chemical Co., Poole, Dorset; histamine and Evans blue dye were from BDH Chemicals, Poole, Dorset; 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphorylcholine (Paf) was from Bachem, Saffron Walden, Essex; sodium pentobarbitone was from May and Baker, Dagenham, Essex; sterile 0.9% saline (Steriflex) was from the Boots Company, Nottingham, Nottinghamshire; ^{125}I -human serum albumin ($50 \mu Ci ml^{-1}$, 20 mg albumin ml^{-1}) was from Amersham International plc, Amersham, Buckinghamshire. Leukotriene B_4 was a gift from Dr S.J. Foster, I.C.I., Pharmaceuticals Division, Macclesfield, Cheshire.

Zymosan-activated plasma (ZAP) was used as a source of $C5a$ des Arg. Briefly, rabbit heparinized ($10 u ml^{-1}$) plasma was incubated with zymosan ($5 mg ml^{-1}$) for 30 min at $37^\circ C$ followed by centrifugation, twice for 10 min at 3000 *g*, to remove the zymosan. Aliquots of ZAP were stored at $-25^\circ C$.

The following Paf antagonists were obtained as gifts: CV-3988 (RS-2-methyl-3-(octadecyl-carbamoyloxy)-propyl-2-(3-thiazolo)ethyl phosphate) from Dr K. Nishikawa, Takeda Chemical Industries Ltd, Osaka, Japan; SRI 63-675 (*cis*-($\pm$)-1-[2-[hydroxy[[tetrahydro-2,5-dimethyl-5-[(ocycylaminocarbonyl)-oxy]methyl]furan-2-yl]methoxy-phosphinyloxy]ethyl]-quinolinium hydroxide, inner salt) from Dr D.A. Handley, Sandoz Research Institute, East Hanover, New Jersey, U.S.A.; 48740 RP (3-(3-pyridyl)-1H,3H-pyrrolo[1,2-c]-

thiazole-7-carboxamide) from Dr P. Sedivy, Rhône-Poulenc Santé, Vitry sur Seine, France; BN 52021 (3-*t*-butyl-hexahydro-4,7b,11-trihydroxy-8-methyl-9H-1,7a-epoxymethano-1H,6aH-cyclopenta-[c]furo[2,3-b]furo[3',2':3,4]cyclopenta[1,2-d]furan-5,9,12(4H)trione) from Dr P. Braquet, Institut Henri Beaufour, Le Plessis Robinson, France; kadsurenone (from Piper futokadsura), L-652,731 (*trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran) and L-659,989 (*trans*-2-(3-methoxy-5-methylsulphonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran) from Drs J.C. Chabala and T.Y. Shen, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, U.S.A.; WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno(3,2f)(1,2,4)-triazolo-(4,3-a)(1,4)-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) from Dr H. Heuer, Boehringer Ingelheim KG, Ingelheim am Rhein, FRG.

Results

The effects of local administration of eight Paf antagonists on oedema formation induced by intradermal injection of Paf + PGE_2 in rabbit skin are summarized in Table 1. The doses of antagonists quoted were either the maximal doses that could be achieved because of limited solubility, or were the doses that gave maximal inhibition for each compound. Aspects of these data are presented in detail below.

SRI 63-675 and CV-3988

Figure 1 demonstrates the synergism between Paf and PGE_2 in producing oedema formation in rabbit skin (Wedmore & Williams, 1981a; Hellewell & Williams, 1986). Responses to either agent injected alone were only slightly above the background value obtained after injection of saline vehicle alone. However, when injected together there was marked oedema formation.

Figure 1 Effect of i.d. injection of SRI 63-675 on oedema formation in rabbit skin induced by i.d. injection of a fixed dose of Paf (10^{-9} mol per site) + prostaglandin E_2 (PGE_2) (3×10^{-10} mol per site). SRI 63-675 was injected i.d. at the doses shown. The symbols represent: oedema responses to Paf (□), PGE_2 (◇), Paf + PGE_2 (○); responses to Paf + PGE_2 + SRI 63-675 (●); responses to SRI 63-675 + PGE_2 (▲); responses to SRI 63-675 alone (■). The dashed line shows the control value obtained after i.d. injection of saline. Data represent 6 replicate injections.

Local injection of SRI 63-675 produced a dose-related inhibition of Paf-induced oedema formation up to 10^{-8} mol per site (Figure 1). At 10^{-7} mol per site SRI 63-675 however, no inhibition was observed. As the data illustrate in Figure 1, this reversal of inhibition can be explained by an intrinsic permeability-increasing activity of SRI 63-675. Thus, although responses to SRI 63-675 injected alone (10^{-10} – 10^{-8} mol per site) were small, synergism with PGE_2 to produce marked oedema formation was observed. Since PGE_2 did not produce oedema formation, the implication was that SRI 63-675 possessed permeability-increasing activity which masked the full antagonist properties of the molecule. At 10^{-7} mol per site of SRI 63-675, addition of PGE_2 did not potentiate the oedema responses. This effect of a high dose of SRI 63-675 may be a detergent-like effect on skin cells and another Paf antagonist which virtually abolishes the actions of Paf (WEB 2086, 10^{-7} mol per site; see below), was unable to block the agonist-like activity of SRI 63-675 (data not shown). In view of the non-specific effects of SRI 63-675 when given i.d., which would limit its usefulness in investigating models of inflammation, its selectivity for Paf by this route was not investigated.

When tested i.v. up to 10 mg kg^{-1} , SRI 63-675 showed no significant inhibition of Paf-induced oedema formation. Similar observations have been made in the guinea-pig with this antagonist (D.A. Handley, personal communication) and related com-

pounds (Deacon *et al.*, 1986). The reason for this lack of activity is unclear, but may relate to rapid inactivation.

When injected i.d., CV-3988 showed similar agonist-like activity to SRI 63-675 (data not shown); thus the percentage inhibition at 10^{-7} mol per site was the maximal inhibition obtained with this compound (Table 1). At higher doses, substantial plasma leakage was found with the antagonist alone and in combination with PGE_2 . Therefore, we did not investigate the selectivity of CV-3988 given by this route. When injected i.v. at 10 mg kg^{-1} , CV-3988 produced an effective inhibition of oedema formation induced by intradermal injection of Paf + PGE_2 (Figure 2). In animals treated with CV-3988, responses to Paf at 10^{-9} and 10^{-10} mol per site were inhibited by $73 \pm 6\%$ ($P < 0.001$) and $80 \pm 6\%$ ($P < 0.005$) respectively ($n = 6$). However, CV-3988 inhibited responses to all other mediators tested by 40–60%. For example, the response to bradykinin (10^{-10} mol per site) + PGE_2 was reduced by

Figure 2 Effect of i.v. administration of CV-3988 on oedema responses in rabbit skin. Responses were obtained to the agents indicated, mixed with prostaglandin E_2 (PGE_2), in control rabbits (○) (saline, 1 ml kg^{-1}) and in rabbits treated with CV-3988 (10 mg kg^{-1}) (●). Doses of agents are given in mol per site except for zymosan-activated plasma (ZAP) which is given as a dilution. PGE_2 was injected at 3×10^{-10} mol per site. Bk = bradykinin; Hist = histamine. The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data represent 6 rabbits per group. * $P < 0.05$, ** $P < 0.005$ compared to control.

$47 \pm 7\%$ ($P < 0.01$). In addition, the oedema response to i.d. zymosan ($300 \mu\text{g}$ per site; Wedmore & Williams, 1981b) was significantly ($P < 0.01$) reduced from $42 \pm 7 \mu\text{l}$ to $16 \pm 2 \mu\text{l}$ ($n = 6$).

48740 RP

We have previously observed that i.d. administration of 48740 RP antagonizes Paf-induced oedema formation in rabbit skin (Hellewell & Williams, 1985; 1987) and summary data from 4 experiments is shown in Table 1. Figure 3 shows the effect of i.v. injection of 48740 RP (50 mg kg^{-1}) on Paf-induced oedema formation. The response to 10^{-9} mol per site Paf was inhibited by $54 \pm 7\%$ ($n = 5$, $P < 0.02$). Even at the high dose of 48740 RP required to produce the partial suppression of Paf-induced oedema formation, responses to all other mediators were not significantly affected (Figure 3).

BN 52021

As shown in Table 1, local administration of BN 52021 antagonized Paf-induced oedema formation, yet it was weak, showing similar activity to CV-3988. The compound possessed no agonist-like activity (data not shown). When tested i.v. at 10 mg kg^{-1} , BN

Figure 3 Effect of i.v. administration of 48740 RP on oedema responses in rabbit skin. Responses were obtained in control rabbits (○) (saline, 1 ml kg^{-1}) and in 48740 RP-treated rabbits (●) (50 mg kg^{-1}). See Figure 2 for details of doses of inflammatory agents. The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data are from 4 rabbits per group. * $P < 0.02$.

Figure 4 Effect of i.v. administration of BN 52021 on oedema responses in rabbit skin. Responses were obtained in control rabbits (○) (injected with 0.1 N NaOH neutralized with HCl , 1 ml kg^{-1}) and in BN 52021-treated rabbits (●) (10 mg kg^{-1}). See Figure 2 for details of doses of inflammatory agents. The dashed line shows the control value obtained after i.d. injection of saline (Sal). Results from 5 rabbits per group. * $P < 0.01$, ** $P < 0.002$.

52021 showed selective inhibition of plasma leakage induced by Paf + PGE_2 (Figure 4). The degree of inhibition was incomplete, but substantial; responses to 10^{-9} mol and 10^{-10} mol Paf were reduced by $63 \pm 9\%$ ($P < 0.01$) and $71 \pm 7\%$ ($P < 0.002$) respectively ($n = 5$). In view of the unusual structure of BN 52021 (Braquet *et al.*, 1985) and its effectiveness when administered i.v., we wondered whether access of the compound to the venular endothelium (the site of plasma leakage) was restricted when given i.d. and this may have explained its weak activity. We therefore pretreated skin sites with BN 52021 (10^{-7} mol per site) before injecting Paf (10^{-9} mol per site) + PGE_2 . In 3 rabbits, co-administration of BN 52021 with Paf inhibited the oedema response by $44 \pm 8\%$, compared to $43 \pm 12\%$ after 5 min pretreatment.

Kadsurenone, L-652,731 and L-659,989

All 3 of these antagonists inhibited Paf-induced oedema formation (Table 1). L-652,731 was more potent than kadsurenone (approximately 10 times from dose-response curves, data not shown), and in turn, L-659,989 was 30–100 times more active than L-652,731, almost abolishing the effect of Paf (Figure 5). From the data in Figure 5, 50% inhibition of the response to Paf + PGE_2 was obtained with approximately 10^{-8} mol per site L-652,731 (similar to our previous observations; Hellewell & Williams, 1986) and with approximately 2×10^{-10} mol per site

Figure 5 Comparison of the ability of L-652,731 and L-659,989 administered i.d. to inhibit oedema formation in rabbit skin induced by a fixed dose of Paf (10^{-9} mol per site) + prostaglandin E_2 (PGE_2) (3×10^{-10} mol per site). The symbols represent: oedema response to Paf + PGE_2 alone (○); responses to Paf + PGE_2 + L-652,731 (●); responses to Paf + PGE_2 + L-659,989 (▲); response to L-652,731 (5×10^{-8} mol per site) + PGE_2 (▼); response to L-659,989 (5×10^{-8} mol per site) + PGE_2 (■); response to PGE_2 (◆). The dashed line shows the control value obtained after i.d. injection of saline. Data from 6 replicate injections.

L-659,989. At the doses shown in Table 1, none of these antagonists displayed agonist activity; data for L-652,731 and L-659,989 are shown in Figure 5. Kadsurenone did not affect oedema responses induced by bradykinin (10^{-10} mol per site) + PGE_2 (data not shown), and we have published evidence

Figure 6 L-659,989 administered i.d. is selective for Paf-induced oedema formation in rabbit skin. Open columns represent responses to the agents indicated mixed with prostaglandin E_2 (PGE_2) (3×10^{-10} mol per site) and the stippled columns show the effect of adding L-659,989 (5×10^{-8} mol per site). Agents were used at the doses shown, except for zymosan-activated plasma (ZAP) which was used undiluted (0.1 ml per site). The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data from 6 replicate injections.

Figure 7 Effect of i.v. administration of L-659,989 on oedema responses in rabbit skin. Responses were obtained in control rabbits (○) (dimethylformamide vehicle, 0.1 ml kg^{-1}) and in L-659,989-treated rabbits (●) (5 mg kg^{-1}). See Figure 2 for details of doses of inflammatory agents. The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data from 5 rabbits per group. * $P < 0.0001$, ** $P < 0.001$.

elsewhere that L-652,731 is a selective Paf antagonist (Hellewell & Williams, 1986). Figures 6 and 7 demonstrate the selectivity of L-659,989 when given i.d. and i.v. respectively. In Figure 6, the oedema response to Paf + PGE_2 was inhibited by 96% (i.e. reduced almost to the leakage level seen with PGE_2 alone), while responses to all other mediators tested were unaffected. In Figure 7, the leakage responses to 10^{-9} and 10^{-10} mol Paf were inhibited by $83 \pm 2\%$ ($P < 0.0001$) and $82 \pm 6\%$ ($P < 0.001$)

Figure 8 Effect of i.d. administration of WEB 2086 on oedema formation in rabbit skin induced by i.d. injection of a fixed dose of Paf (10^{-9} mol per site) + prostaglandin E_2 (PGE_2) (3×10^{-10} mol per site). The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data from 4 rabbits.

respectively by L-659,989 (5 mg kg^{-1} , i.v., $n = 5$). When tested at a lower dose (2 mg kg^{-1} , i.v.), L-659,989 inhibited the oedema response to Paf (10^{-9} mol per site) + PGE_2 by approximately 70% (2 rabbits) compared to PGE_2 alone.

WEB 2086

Local administration of WEB 2086 produced a dose-related inhibition of Paf-induced oedema formation (Figure 8), with 50% inhibition of the response obtained with approximately 10^{-9} mol per site. In addition, the compound displayed no agonist activity at 10^{-7} mol per site (Figure 8). Local and i.v. administered WEB 2086 displayed selectivity for Paf (Figures 9 and 10); at 10^{-7} mol per site WEB 2086, only the leakage response induced by Paf was inhibited (Figure 9), and similarly, at 10 mg kg^{-1} i.v. (Figure 10). At 10^{-9} and 10^{-10} mol per site Paf, responses were reduced by $91 \pm 3\%$ ($P < 0.002$) and $86 \pm 7\%$ ($P < 0.005$) respectively ($n = 5$).

Discussion

In this study we have tested eight reported Paf antagonists in the same *in vivo* model of Paf-induced oedema formation in rabbit skin. Each of the compounds displayed activity as an inhibitor of Paf-induced plasma leakage, but there was considerable variability in activity and selectivity. The compounds most problematic in terms of non-specific effects

Figure 9 WEB 2086 administered i.d. is selective for Paf-induced oedema formation in rabbit skin. Open columns represent responses to the agents indicated mixed with prostaglandin E_2 (PGE_2 , 3×10^{-10} mol per site) and the stippled columns show the effect of adding WEB 2086 (10^{-7} mol per site). Agents were used at the doses shown, except for zymosan-activated plasma (ZAP) which was used undiluted (0.1 ml per site). The dashed line shows the control value obtained after i.d. injection of saline (Sal). Data from 6 replicate injections.

Figure 10 Effect of i.v. administration of WEB 2086 on oedema responses in rabbit skin. Responses were obtained in control rabbits ($\circ$) (vehicle, 1 ml kg^{-1}) and in WEB 2086 (10 mg kg^{-1}) treated rabbits ($\bullet$). See Figure 2 for details of doses of inflammatory agents. The dashed line shows the control value obtained after i.d. injection of saline. Data from 5 rabbits per group. * $P < 0.002$, ** $P < 0.005$.

were the lipid-like Paf-related antagonists, CV-3988 and SRI 63-675. When administered i.d., both compounds displayed antagonist properties but these were masked at higher doses by agonist-like activities. These agonist effects may result from non-specific lipid interactions with cells in the skin as indicated by the failure of WEB 2086 to inhibit the response (our unpublished data). Intravenous SRI 63-675 failed to antagonize the effects of i.d. Paf, but this may be related to rapid inactivation of the compound in the circulation. In guinea-pigs and primates, the half-life of SRI 63-675 after i.v. injection is approximately 9–12 min (Handley *et al.*, 1987) but the half-life in rabbits has not been reported. We allowed 5 min to elapse between i.v. injection of antagonist (10 mg kg^{-1}) and i.d. injection of Paf and this may have been sufficient time for inactivation to occur. CV-3988 injected i.v. produced effective inhibition of Paf-induced oedema formation, although this action was not selective for Paf; responses to all other agents tested also being inhibited, albeit to a lesser extent. One possible explanation for this non-selectivity is that this Paf-related compound lowers systemic blood pressure. When administered i.v. in rabbits Paf is known to cause rapid hypotension (Pinckard *et al.*, 1982) and a decrease in arterial pressure has been shown to be associated with decreased

oedema formation in skin in response to a variety of agents (Rampart & Williams, 1986). However, following an i.v. bolus injection of CV-3988 (10 mg kg^{-1}) we found a transient ($< 3 \text{ min}$) decrease in systemic blood pressure of approx. 30% in anaesthetized rabbits, and it is thus unlikely that this would explain the observations of non-selectivity. Agonist activity of CV-3988 (10^{-4} M) has been reported following its infusion into the pulmonary circulation of the rat (Voelkel *et al.*, 1986). CV-3988 has been used by other investigators to implicate Paf as a mediator of various types of inflammation in animal models, including the rabbit (Issekutz & Szpejda, 1986). We question the use of the compound for this purpose because of its non-selective action.

The antagonist 48740 RP displayed antagonism of Paf-induced oedema formation. Although 50 mg kg^{-1} i.v. produced only a 54% inhibition of the response to Paf, the antagonist appeared to be selective at this dose. We did not try higher doses of 48740 RP in the rabbit and do not know if total inhibition of the Paf response could be obtained.

Of interest was the observation that BN 52021 was a moderately effective and selective inhibitor when injected i.v., despite producing weak inhibition of Paf-induced oedema when injected i.d. The reason for this may relate to restricted access of the antagonist to endothelial cell Paf receptors when injected i.d., although our pretreatment studies do not support this concept. BN 52021 has been tested in man for its ability to inhibit skin and platelet responses to Paf. At the maximal dose tested (120 mg orally), partial suppression of Paf-induced weal and flare and *ex vivo* platelet aggregation were observed (Chung *et al.*, 1987).

The *in vivo* potencies of kadsurenone, L-652,731 and L-659,989 compare favourably with potencies *in vitro* on inhibition of Paf binding to human and rabbit platelet membranes (Ponpipom *et al.*, 1988). We have previously observed that L-652,731 inhibited oedema formation in the Arthus reaction (Hellewell & Williams, 1986; 1987), but not oedema associated with a passive cutaneous anaphylactic reaction (Hellewell & Williams, 1987; Hellewell *et al.*, 1987). The former observation has also been shown by others using the same antagonist in the rat (Mandel *et al.*, 1988). We have also tested L-659,989 on both oedema formation and neutrophil accumulation in the Arthus reaction. Local administration of L-659,989 ($5 \times 10^{-8} \text{ mol per site}$) reduced oedema formation by approximately 60% and neutrophil accumulation was also inhibited, but to a lesser extent (Hellewell, 1989; Hellewell *et al.*, unpublished observations). We found similar results with i.v. administered L-659,989 (5 mg kg^{-1}). In these earlier experiments, and those described in the present

study, responses to C5a, which has been presumed to be a major mediator in the Arthus reaction, were unaffected by the antagonist. In addition, L-659,989 does not interfere with binding of C5a to its receptor on the neutrophil (Ponpipom *et al.*, 1988).

Finally, WEB 2086 also proved to be a potent Paf antagonist *in vivo* in the rabbit, showing similar activity to L-659,989. At $10^{-9} \text{ mol per site}$, Paf-induced oedema formation was inhibited by 50%, which is similar to observations made in rat skin (Casals-Stenzel *et al.*, 1987). This is 5 times the dose of L-659,989 required to cause a similar inhibition, and comparing Figures 5 and 8, it appears that at the lower doses of antagonist, L-659,989 was more potent than WEB 2086. WEB 2086 produced a slightly greater inhibition of Paf-induced oedema at the top doses of antagonist tested, but we were able to test WEB 2086 at a higher concentration than L-659,989. When injected i.v., WEB 2086 was more effective than L-659,989 but, again for reasons of limited solubility of L-659,989, we were able to test WEB 2086 at a higher dose. The dose of WEB 2086 required i.v. to inhibit Paf-induced responses in the rabbit skin is high when compared with that needed to block responses to i.v. Paf in the guinea-pig. For example, $10 \mu\text{g kg}^{-1}$ i.v. was required to achieve total inhibition of plasma leakage in the airways induced by i.v. Paf in the guinea-pig (Evans *et al.*, 1988), although in the same species 1 mg kg^{-1} i.v. was required to block the decrease in respiratory flow and hypotension observed following intratracheal instillation of Paf (Casals-Stenzel *et al.*, 1987). In addition, $20 \text{ mg kg}^{-1} \text{ h}^{-1}$ i.v. of WEB 2086 (equivalent to 10 mg kg^{-1} over 30 min as carried out in our experiments) was needed to attenuate the pulmonary changes induced by i.v. Paf and endotoxin in sheep (Purvis *et al.*, 1988). One possibility is that in the rabbit and sheep, WEB 2086 is inactivated more rapidly than in the guinea-pig and also, the antagonist may be more effective when Paf is administered by the same route.

The results of this study indicate that not all reported Paf antagonists behave as selective Paf antagonists *in vivo* in rabbit skin, and the same compound can show different potencies depending on the route of administration. Taken together, these observations suggest that before ascribing a role for endogenous Paf in mediating a particular inflammatory reaction *in vivo* based on the inhibitory properties of a given antagonist, it is important to establish that the antagonist is selective in that model.

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