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Role of platelet-activating factor (PAF) in platelet accumulation in rabbit skin: effect of the novel long-acting PAF antagonist, UK-74,505

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1 The contribution of platelet-activating factor (PAF) to platelet deposition and oedema formation induced by exogenous soluble mediators, zymosan particles and associated with a reversed passive Arthus (RPA) reaction in rabbit skin was investigated by use of a novel long-acting PAF receptor antagonist, UK-74,505.

2 Oedema formation and platelet accumulation were simultaneously measured by i.v. injection of [^{125}I]-albumin and ^{111}In-labelled rabbit platelets. UK-74,505 was either administered i.v. or used to pretreat radiolabelled platelets *in vitro* before their injection into recipient animals. Platelets pretreated with UK-74,505 were also labelled with the fluorescent calcium indicator, Fura-2, to assess their *ex vivo* reactivity to PAF at the end of the *in vivo* experiment.

3 UK-74,505 (0.5 mg kg^{-1}), administered i.v., inhibited PAF-induced oedema formation, but did not affect oedema induced by zymosan particles, bradykinin (BK), histamine, formyl-methionyl-leucyl-phenylalanine (FMLP), zymosan-activated plasma (ZAP, as a source of C5a des Arg), leukotriene B_4 (LTB_4) or interleukin-8 (IL-8).

4 UK-74,505, administered i.v. also suppressed the small platelet accumulation induced by exogenous PAF, but had no effect on accumulation induced by IL-8 or ZAP. Although oedema induced by zymosan was not affected by i.v. UK-74,505, zymosan-induced platelet accumulation was significantly attenuated by the antagonist.

5 The RPA reaction in rabbit skin was associated with marked oedema formation and platelet accumulation which were both inhibited by i.v. UK-74,505.

6 *In vitro*, UK-74,505 inhibited aggregation and the increase in intracellular calcium concentration induced by PAF in rabbit washed platelets in a concentration-dependent manner ($IC_{50} = 1.6 \times 10^{-8}$ M and 1.1×10^{-8} M, respectively). Platelets pretreated with 10^{-6} M UK-74,505, and maintained at 37°C, were unresponsive to PAF, whilst responding normally to thrombin, for up to 4 h.

7 In a second series of *in vivo* experiments, platelets were labelled with ^{111}In and loaded with Fura-2. The platelets were then pretreated with 10^{-6} M UK-74,505, washed, and injected into recipient rabbits. These platelets, prepared from blood samples taken at the end of the *in vivo* experiments, exhibited an 80% reduction in their response to PAF as measured *ex vivo* with Fura-2. However, in contrast to the effects of i.v. UK-74,505, platelets pretreated with the antagonist did accumulate effectively in the RPA reaction, a significant reduction only being observed in responses at the lowest antibody dose. In addition, pretreatment of platelets had no effect on the small platelet accumulation induced by PAF.

8 These results suggest that PAF is an important mediator of oedema formation and platelet accumulation in the RPA reaction in rabbit skin. However, they question the role of PAF receptors on platelets in this model. The results also indicate that PAF may be involved in platelet accumulation induced by zymosan in rabbit skin.

Keywords: Reversed passive Arthus (RPA) reaction; oedema formation; platelet accumulation; platelet-activating factor (PAF); PAF antagonist; inflammation

Introduction

Platelet accumulation has been observed in different animal models of acute inflammation including carrageenin-induced paw oedema (Vincent *et al.*, 1978), the dermal reversed passive Arthus (RPA) reaction (Henson & Cochrane, 1969) and the local Shwartzman reaction (Movat & Burrowes, 1985). Platelet accumulation has also been associated with certain pathological conditions in man such as the Adult Respiratory Distress Syndrome (Heffner *et al.*, 1987). Platelets can potentially contribute to the development of inflammation as they contain a wide variety of proinflammatory substances, including vasoactive amines and peptides, proteases, chemotactic factors for leucocytes and components of the coagulation pathway. Several studies have suggested that platelets are involved in the early stages of inflammation, often prior to the development of tissue damage, haemorrhage and thrombosis (Issekutz *et al.*, 1983), but the sequence of events leading to platelet accumulation and its relationship to other components of the inflammatory process are unknown.

Together with oedema formation, neutrophil infiltration and haemorrhage, platelet accumulation is a characteristic feature of the Arthus reaction (Stetson, 1951; Humphrey, 1955a; Cochrane & Janoff, 1974; Kravis & Henson, 1977). This model of vascular injury is initiated by the local deposition of immune complexes within the vessel wall and previous reports have demonstrated the importance of neutrophils (Humphrey, 1955a; Cochrane & Janoff, 1974; Warren *et al.*, 1989) and complement fragments such as C5a (Cochrane *et al.*, 1970; Cochrane & Janoff, 1974; Lewis & Turk, 1975; Jose *et al.*, 1983; Yeh *et al.*, 1991; Rossi *et al.*, 1992). The role of platelets and their contribution to the development of the reaction remain unclear. Some studies reported an inhibition of the Arthus reaction in platelet-depleted animals (Henson & Cochrane, 1969), whereas other

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investigators did not observe any modification of the reaction in the absence of platelets (Humphrey, 1955b; Cochrane & Janoff, 1974).

The phospholipid platelet-activating factor (PAF) appears to play an important role in the development of the Arthus reaction as PAF antagonists, administered locally or i.v., have been shown to reduce oedema formation associated with RPA reactions in rabbit and rat skin (Deacon *et al.*, 1986; Hellewell & Williams, 1986; Issekutz & Szejda, 1986; Williams *et al.*, 1986; Warren *et al.*, 1989; Hellewell, 1990; Rossi *et al.*, 1992). Neutrophil depletion has been shown to suppress oedema formation in RPA reactions in rat skin, an inhibition that is reported to be partially reversed by local injection of neutrophils (Warren *et al.*, 1989). Preincubation of the neutrophils with a PAF antagonist restored this inhibition (Warren *et al.*, 1989). Furthermore, it has been shown that i.v. injection of PAF antagonists partially suppresses platelet accumulation (Issekutz & Szejda, 1986). Kravis & Henson (1977) have suggested that PAF released from basophils or mast cells by an IgE-dependent mechanism is responsible for the early phase of platelet accumulation observed in the Arthus reaction. However, endothelial cells, neutrophils undergoing phagocytosis, and platelets themselves are also possible sources of PAF (Chignard *et al.*, 1980; Camussi *et al.*, 1983; Hellewell & Williams, 1986). It is not known whether PAF mediates platelet accumulation by acting directly on the platelet *in vivo*, or by an indirect mechanism that involves PAF interacting with receptors on other inflammatory cells such as the neutrophil.

The objectives of this study were to investigate the accumulation of platelets and its relationship to oedema formation in defined inflammatory reactions. For this purpose we monitored the accumulation of ^{111}In-labelled platelets and [^{125}I]-albumin in rabbit skin in response to soluble inflammatory mediators and particulate stimuli. Two types of the latter were employed: zymosan, and immune complexes deposited in an Arthus reaction. We used a novel PAF antagonist, UK-74,505 (Cooper *et al.*, 1990; Parry *et al.*, 1990; Alabaster *et al.*, 1991), to investigate the contribution of PAF in platelet deposition and oedema formation *in vivo*. UK-74,505 has the advantage of potency and a long duration of action which enabled measurements to be made *in vivo* over either short (30 min) or long (4 h) periods required for development of responses to exogenous mediators or particulate stimuli respectively. Dissociation of the compound from PAF receptors is very slow (Cooper *et al.*, 1990; Parry *et al.*, 1990; Alabaster *et al.*, 1991), which permitted PAF receptors to be blocked on ^{111}In-platelets by pretreatment *in vitro* before i.v. injection. This enabled us to investigate the role of platelet PAF receptors in platelet deposition *in vivo*.

Methods

Animals

New Zealand White rabbits (2.5–3.5 kg) were purchased from Froxfield Farm, Hampshire.

Generation of antiserum for the Arthus reaction

Arthus antiserum, anti-bovine-γ-globulin (anti-BGG) antibody, was raised in rabbits as previously described (Hellewell & Williams, 1986). Briefly, 4 × 0.25 ml of BGG (2 mg ml^{-1} in saline) emulsified with an equal volume of Freund's complete adjuvant was administered subcutaneously. This was followed 14 days later by booster s.c. injections (4 × 0.25 ml) of the same concentration of BGG in Freund's incomplete adjuvant. At day 28, a s.c. injection of alum-precipitated BGG (300 μg/rabbit) was given. Blood was collected by carotid cannulation at day 38. The serum from five rabbits was pooled, heat-inactivated at 56°C for 30 min and stored in aliquots at − 20°C. Heat-inactivated rabbit serum from normal rabbits was used as the control.

Preparation of zymosan-activated plasma (ZAP)

ZAP as a source of rabbit C5a des Arg was prepared by incubating heparinized (10 u ml^{-1}) rabbit plasma with zymosan (5 mg ml^{-1}) for 30 min at 37°C. Zymosan was removed by centrifugation (2 × 10 min, 2500 *g*) and ZAP was stored in aliquots at − 20°C. The C5a des Arg content of ZAP was approximately 5×10^{-7} M as measured by radioimmunoassay (Jose *et al.*, 1983).

Isolation and preparation of rabbit washed platelets

Washed platelets were prepared from rabbit blood as previously described (Radomski & Moncada, 1983). Briefly, blood was collected from a carotid artery or marginal ear vein into tri-sodium citrate (0.38%, w/v) and centrifuged at 250 *g* for 20 min to obtain platelet-rich plasma (PRP). The isolated PRP was then used for aggregation assays or further centrifuged at 750 *g* for 10 min in the presence of prostacyclin (PGI_2, 300 ng ml^{-1}) for the preparation of washed platelets. The resultant platelet pellet was resuspended in 10 ml Tyrode solution containing 300 ng ml^{-1} PGI_2 (PGI_2/Tyrode). The washed platelets were then centrifuged at 720 *g* for 10 min and finally resuspended in PGI_2-free Tyrode. The platelet concentration in PRP or Tyrode solution was determined with a Coulter counter (model ZM, Coulter Electronics Ltd, Luton, Bedfordshire).

Measurement of platelet aggregation

Aggregation of rabbit platelets either in suspension in plasma (PRP) or after washing with Tyrode solution was assessed photometrically in a dual channel aggregometer (Chronolog 440 VS) linked to a dual pen recorder (Chronolog 707). Aliquots of platelet suspension (250 μl) were incubated for 5 min at 37°C with continuous stirring at 700 r.p.m. and then stimulated with PAF or thrombin. Responses were allowed to develop for 3 min and expressed as a percentage of maximum light transmission. Platelet-poor-plasma and Tyrode solution were used as references (100% light transmission) for PRP and washed platelet suspension respectively. Platelet concentrations ranged from 190,000 to 560,000 platelets per μl in PRP and 200,000 to 250,000 platelets per μl in washed platelet suspensions. In some experiments, the effect of UK-74,505 on PAF or thrombin-induced washed platelet aggregation was investigated either by adding the antagonist to the platelet suspension 2 min before the aggregation assay or by pretreating the platelets during their preparation (see below).

Measurement of platelet intracellular calcium concentration ($[Ca^{2+}]_i$) with Fura-2

Platelet $[Ca^{2+}]_i$ was measured by monitoring the fluorescence of platelets loaded with the calcium indicator Fura-2 (Grynkiewicz *et al.*, 1985). Briefly, Fura-2 acetoxymethyl ester was added to PRP at a final concentration of 2 μM and the cell suspension was incubated for 45 min at 37°C in the presence of PGI_2 (300 ng ml^{-1}). The PRP was then centrifuged at 750 *g* for 10 min and washed platelets were prepared as described above. For the assay, aliquots of platelet suspension (200,000 to 300,000 platelets per μl in 200 μl) were dispensed into quartz cuvettes and the external $[Ca^{2+}]$ adjusted to 1 mM with $CaCl_2$. Changes in fluorescence induced by PAF or thrombin were monitored by a Perkin Elmer LS50 fluorescence spectrometer fitted with a thermostatically controlled cell holder incorporating an electronic stirrer and maintained at 37°C by a water circulator. Fluorescence readings were taken at excitation wavelengths 340 nm and 380 nm and emission wavelength 510 nm. The $[Ca^{2+}]_i$ was calculated from the ratio of the two fluorescence

readings using a K_d value of 224 nM. Responses were allowed to develop for 3 min and data were expressed as maximal increase in $[Ca^{2+}]_i$ over the basal levels.

^{111}In-labelling of washed rabbit platelets

Rabbit platelets ($8 \times 10^9 - 2 \times 10^{10}$), in some experiments loaded with Fura-2 as described above, were resuspended in PGI_2/Tyrode and incubated with $^{111}InCl_3$ (100 to 200 μCi in 10 to 20 μl) chelated with 2-mercaptopyridine-*N*-oxide (40 μg in 0.1 ml 50 mM phosphate buffered saline (PBS), pH 7.4). After a 10 min incubation at room temperature, the suspension was diluted to 10 ml with PGI_2/Tyrode and centrifuged for 10 min at 720 *g*. The platelets were then washed twice by repeating the same procedures of centrifugation and resuspension in PGI_2/Tyrode. In some experiments, platelets were treated with UK-74,505 before their last wash, as described below. The cells were finally resuspended in PGI_2-free Tyrode before injection into the animals. Supernatant aliquots were collected during washes and after the final suspension in order to assess labelling efficiency. In these experiments, labelling efficiency values varied between 80 and 95% and were not statistically different between control and UK-74,505-treated platelets.

Pretreatment of rabbit platelets with UK-74,505

Labelled or unlabelled platelets were pretreated with UK-74,505 before their last wash. At this time, the platelet pellet was resuspended in 2 ml of PGI_2/Tyrode and divided into 2 aliquots of 1 ml. UK-74,505 was added to one aliquot at a final concentration of 10^{-6} M and the other aliquot was used as control. After a 15 min incubation at room temperature, both suspensions were diluted to 10 ml with PGI_2/Tyrode, centrifuged and finally resuspended in PGI_2-free Tyrode.

Measurement of platelet accumulation and plasma protein leakage in rabbit skin

Platelet accumulation and oedema formation in rabbit dorsal skin were simultaneously measured by i.v. injection of ^{111}In-labelled platelets, in some experiments also loaded with Fura-2, and ^{125}I-labelled human serum albumin ([^{125}I]-HSA). Rabbits were anaesthetized with i.v. Sagatal and their dorsal skin shaved. [^{125}I]-HSA (5 μCi kg^{-1}) mixed with Evans blue dye (10 mg kg^{-1}) in saline was injected i.v., followed 5 min later by platelets in 3 ml volumes. Platelets were labelled with ^{111}In only for experiments investigating the effects of i.v. UK-74,505, and double labelled with Fura-2 and ^{111}In in experiments where platelets were pretreated with UK-74,505. The number of platelets injected into pairs of animals varied between 3.5 and 8×10^9 but was the same within each pair of animals. When necessary, UK-74,505 or its vehicle, was injected i.v. 10 min after the platelets. After a further 10 min, the agents under investigation, all prepared in saline containing 0.1% bovine serum albumin (BSA) (saline/BSA) were injected i.d. in 0.1 ml volumes, each agent having six replicates per animal according to a balanced site plan. Where indicated, agonists were co-injected with prostaglandin E_2 (PGE_2) (3×10^{-10} mol/site) as a potentiating agent of plasma protein leakage. The RPA reaction was elicited by injecting anti-BGG antiserum i.d. undiluted (100%), diluted with saline/BSA 1/2 (50%) and 1/4 (25%) at the same time as the other agents tested, followed 5 min later by an i.v. injection of BGG (5 mg kg^{-1}). In this model, no significant reaction was observed when saline was given i.v. instead of BGG. In experiments where the platelets were double labelled with ^{111}In and Fura-2, the animals were bled after 4 h via the carotid artery and platelets isolated for measurement of $[Ca^{2+}]_i$, as described above. In other experiments, at the end of the *in vivo* test period, 30 min or 4 h, a 10 ml blood sample was collected into heparin by cardiac puncture. The animals were then killed by an overdose of anaesthetic, the dorsal skin removed and the skin sites excised with a 17-mm diameter punch. Radioactivity in skin sites and plasma samples was counted in a gamma counter with automatic spill-over and cross talk correction (Packard Cobra, Meridien, CT, U.S.A.). Oedema formation was expressed as μl of plasma by dividing skin sample ^{125}I-counts by ^{125}I-counts in 1 μl of plasma. The ^{111}In-count per platelet was determined and used to express platelet accumulation in terms of number of platelets per site per 10^9 cells injected. Blood samples were also used to determine the percentage of circulating labelled platelets and the level of free ^{111}In. In all experiments, the level of free ^{111}In was less than 3% and the percentage of circulating labelled platelets varied between 75 and 100% of those injected. These values were the same in all groups of animals.

Materials

BSA (low endotoxin and fatty acid free), BGG, bradykinin (BK), histamine, zymosan, formyl-methionyl-leucyl-phenylalanine (FMLP), PGE_2, and PGI_2 were purchased from Sigma Chemical Co, Poole, Dorset. [^{125}I]-HSA (20 mg ml^{-1} albumin in sterile isotonic saline, 50 μCi ml^{-1}) and $^{111}InCl_3$ (2 mCi in 0.2 ml sterile pyrogen free 0.04 N HCl) were from Amersham International, Amersham, Buckinghamshire. Sagatal (pentobarbitone sodium, 60 mg ml^{-1}) was from May and Baker, Dagenham, Essex. Fura-2 acetoxymethyl ester was from Cambridge Bioscience, Cambridge. Freund's complete and incomplete adjuvant were from Difco Laboratories, West Molesey, Surrey. Evans blue was from British Drug House, Poole, Dorset. Viaflex (sterile, pyrogen-free isotonic saline solution) was from Baxter Healthcare Ltd., Thetford, Norfolk. PAF was from Bachem, Saffron Walden, Essex. Leukotriene B_4 (LTB_4) was from Cascade Biochem Ltd., Reading, Berkshire. Recombinant human interleukin-8 (IL-8) was from British Bio-technology Ltd., Cowley, Oxford. Sterile Tyrode solution (composition, mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4,H_2O 0.36, $NaHCO_3$ 12, glucose 5.6) was from Gibco BRL, Paisley. UK-74,505 [4-(2-chlorophenyl)-1,4-dihydro-3-ethoxycarbonyl-6-methyl-2-[4-(2-methylimidazo[4,5-c]pyrid-1-yl)phenyl]-5-[N-(2-pyridyl)carbamoyl)pyridine], initially dissolved in 0.1 N HCl and diluted in saline, was a gift from Dr M.J. Parry, Pfizer Central Research, Sandwich, Kent.

Statistical analysis

All data are presented as the mean ± s.e.mean of the indicated number of experiments. Statistical significance was assessed by use of two way analysis of variance for *in vivo* experiments and paired and unpaired Student's *t* tests for *in vitro* and *ex vivo* experiments, respectively. *P* values less than 0.05 were considered to be significant.

Results

Effect of systemic UK-74,505 on agonist-induced oedema formation over 30 min in rabbit skin

Initially, we determined the effective dose of UK-74,505 required to inhibit the PAF-induced inflammatory response in rabbit skin. Figure 1 (a) shows that PAF ($10^{-11} - 10^{-9}$ mol/site) administered i.d. in the presence of the vasodilator PGE_2 (3×10^{-10} mol/site) induced a dose-dependent oedema formation over a 30 min test period. In this system, as previously reported (Wedmore & Williams, 1981), PAF and PGE_2 act synergistically to induce oedema formation. Only small responses were produced by PAF alone at the top dose employed. In animals pretreated i.v. with 0.1 mg kg^{-1} UK-74,505, the oedema responses evoked by the different doses of PAF were inhibited by greater than 80%. Increasing the

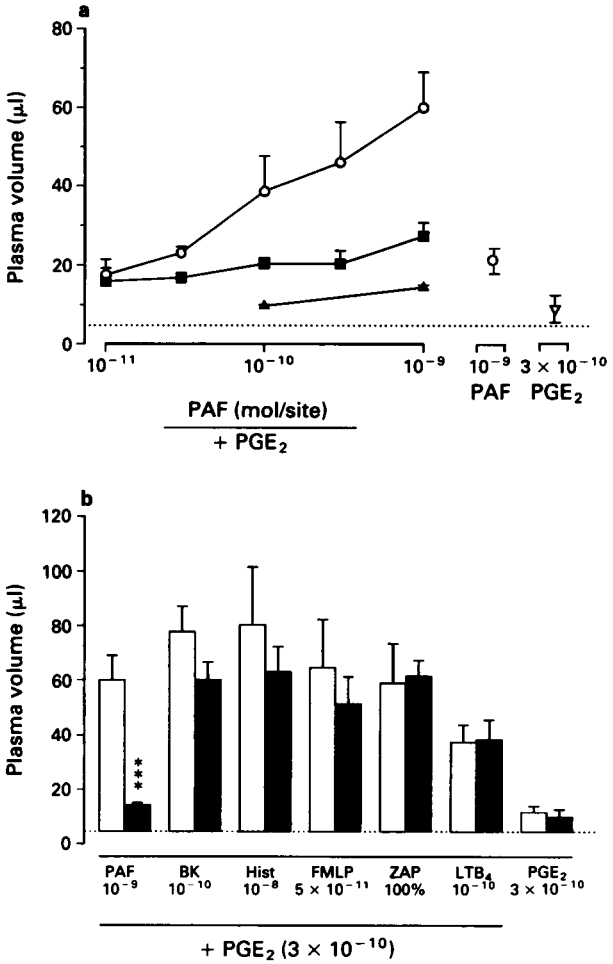

Figure 1 Effect of UK-74,505 on oedema formation induced by PAF (a) and by different soluble inflammatory stimuli (b) in rabbit skin. (a) Rabbits received an i.v. injection of either vehicle (open symbols) or UK-74,505 (0.1 mg kg^{-1}, ■ or 0.5 mg kg^{-1}, ▲) followed 10 min later by i.d. injections of increasing doses (mol/site) of PAF in the presence of prostaglandin E_2 (PGE_2, 3×10^{-10} mol/site). (b) Rabbits received an i.v. injection of either vehicle (open columns) or 0.5 mg kg^{-1} UK-74,505 (solid columns) followed 10 min later by i.d. injections of the indicated doses (mol/site) of PAF, bradykinin (BK), histamine (Hist), formyl-methionyl-leucyl-phenylalanine (FMLP), zymosan-activated plasma (ZAP) and leukotriene B_4 (LTB_4), all tested in the presence of PGE_2 (3×10^{-10} mol/site). After 30 min, the animals were killed and plasma protein exudation was measured as indicated in the Methods section. Results are expressed as μl plasma and presented as mean ± s.e.mean of $n = 3-6$ experiments. The dotted line represents control responses obtained after i.d. injection of saline/BSA. In (a) the open circle and triangle represent the responses obtained in PAF (10^{-9} mol/site) and PGE_2 (3×10^{-10} mol/site) injected sites respectively. Significant differences from control are indicated by $***P<0.001$.

dose of UK-74,505 to 0.5 mg kg^{-1} resulted in almost total suppression of PAF-induced oedema formation.

The specificity of UK-74,505 was then evaluated by examining its effect on plasma exudation induced by other inflammatory mediators over a 30 min test period. Figure 1 (b) shows that the optimum dose of 0.5 mg kg^{-1} UK-74,505, virtually abolished the response induced by PAF (10^{-9} mol/site), but had no effect on plasma leakage induced by BK, histamine, FMLP, ZAP (as a source of C5a des Arg) or LTB_4, all tested in the presence of PGE_2 (3×10^{-10} mol/site).

Effect of systemic UK-74,505 on oedema formation and platelet accumulation induced by inflammatory stimuli over 4 h in rabbit skin

Having determined an effective and specific dose for the i.v. injection of UK-74,505 (i.e. 0.5 mg kg^{-1}), we investigated the effect of this PAF antagonist on oedema formation (Figure 2a) and platelet accumulation (Figure 2b) induced by exogenous soluble inflammatory mediators and zymosan particles measured over a 4 h period. As found with the 30 min experimental protocol, oedema formation induced by PAF + PGE_2 over 4 h was effectively inhibited in rabbits pretreated with 0.5 mg kg^{-1} i.v. UK-74,505 (Figure 2a). In control animals, zymosan (which induces endogenous prostaglandin production (Williams & Jose, 1981)), IL-8 + PGE_2 and ZAP + PGE_2 all induced marked oedema formation. These responses were unchanged in animals pretreated with i.v. UK-74,505 (Figure 2a).

In these experiments, PAF, PAF + PGE_2, IL-8, IL-8 + PGE_2, or ZAP induced a small, but measurable, accumulation of ^{111}In-labelled platelets (5,000–15,000 platelets/site) in the skin, whereas platelet deposition evoked by zymosan or ZAP + PGE_2 was much more pronounced (25,000–30,000

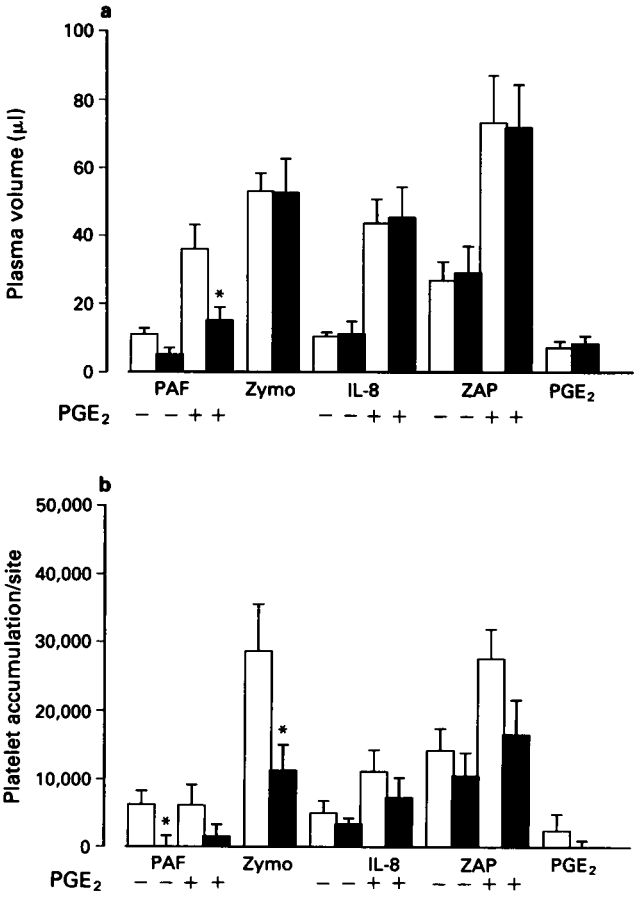

Figure 2 Effect of UK-74,505 on oedema formation (a) and platelet accumulation (b) induced by different soluble inflammatory stimuli in rabbit skin. Rabbits received an i.v. injection of either vehicle (open columns) or 0.5 mg kg^{-1} UK-74,505 (solid columns) followed 10 min later by i.d. injections of PAF (10^{-9} mol/site), zymosan (Zymo, 300 μg/site), interleukin-8 (IL-8, 3×10^{-11} mol/site) and undiluted zymosan activated plasma (ZAP), in the presence or the absence of prostaglandin E_2 (PGE_2, 3×10^{-10} mol/site). After 4 h, the animals were killed and plasma protein exudation and platelet accumulation were measured as indicated in the Methods section. Results are expressed as μl plasma (oedema formation) and number of platelets per site per 10^9 platelets injected (platelet accumulation) and presented as mean ± s.e.mean of responses obtained after subtraction of saline/BSA values in $n = 5-6$ rabbits. In control animals, oedema formation and platelet accumulation in response to i.d. injection of bradykinin (BK) (10^{-10} mol/site) + PGE_2 (3×10^{-10} mol/site) were 62.5 ± 6.2 μl ($n = 7$) and 6074 ± 527 platelets/site ($n = 7$) respectively. Oedema formation and platelet accumulation in saline/BSA injected sites were 8.9 ± 1.7 μl and 8866 ± 1773 platelets/site in control animals and 9.7 ± 1.8 μl and 8684 ± 1373 platelets/site in animals pretreated with UK-74,505. Significant differences from control are indicated by $*P<0.05$.

platelets/site) (Figure 2b). PGE_2 had a marked potentiating effect on platelet accumulation when in combination with IL-8 and ZAP. BK + PGE_2 induced large oedema responses, but only small numbers of platelets accumulated in this case (see Figure 2 legend). The platelet deposition induced by PAF + PGE_2, IL-8, IL-8 + PGE_2, ZAP or ZAP + PGE_2 was not significantly affected in animals given 0.5 mg kg^{-1} i.v. UK-74,505, whereas platelet accumulation provoked by PAF alone or zymosan was significantly suppressed, the latter being inhibited by greater than 50% (Figure 2b). Interestingly, the inhibition of zymosan-induced platelet accumulation occurred without any effect on the plasma leakage response (Figure 2).

Effect of UK-74,505 on rabbit platelet aggregation and changes in $[Ca^{2+}]_i$ induced by PAF in vitro

The potency of UK-74,505 in inhibiting PAF-induced responses of rabbit washed platelets was assessed *in vitro*. Figure 3a shows the concentration-dependent aggregation of washed platelets evoked by PAF (10^{-11} to 3×10^{-8} M). Maximal aggregation of $48.3 \pm 4.2\%$ ($n = 4$ preparations) was observed at 10^{-8} M PAF and the calculated ED_{50} value for the autacoid was $3.86 \pm 0.41 \times 10^{-10}$ M ($n = 4$ preparations). PAF was one hundred times less potent in inducing aggregation of platelets in PRP ($ED_{50} = 5.03 \pm 0.80 \times 10^{-8}$ M, $n = 3$ preparations) and its maximal effect on this preparation ($32.0 \pm 3.3\%$) was smaller than on washed platelets (Figure 3a). The effect of UK-74,505 was investigated against washed platelet aggregation induced by the submaximal dose of 3×10^{-9} M PAF (Figure 3b). The antagonist incubated for 2 min prior to platelet stimulation inhibited the response induced by PAF in a concentration-dependent manner (Figure 3b), with a calculated IC_{50} value of $1.63 \pm 0.13 \times 10^{-8}$ M ($n = 3$ preparations).

As found for aggregation, PAF induced a concentration-dependent increase in platelet $[Ca^{2+}]_i$ (Figure 3c). The range of PAF concentrations used was similar to those necessary to trigger an aggregatory response and the calculated ED_{50} value was $3.24 \pm 0.85 \times 10^{-10}$ M ($n = 5$ preparations). UK-74,505 incubated for 2 min prior to stimulation inhibited the increase in $[Ca^{2+}]_i$ evoked by 3×10^{-9} M PAF in a concentration-dependent manner (Figure 3d), with a calculated IC_{50} value of $1.13 \pm 0.28 \times 10^{-8}$ M ($n = 4$ preparations). These results demonstrate that UK-74,505 exhibits similar inhibitory activity towards rabbit platelet aggregation and increases in $[Ca^{2+}]_i$ induced by PAF. With both *in vitro* test systems maximal inhibition of the effects of PAF was achieved with 10^{-6} M of the antagonist.

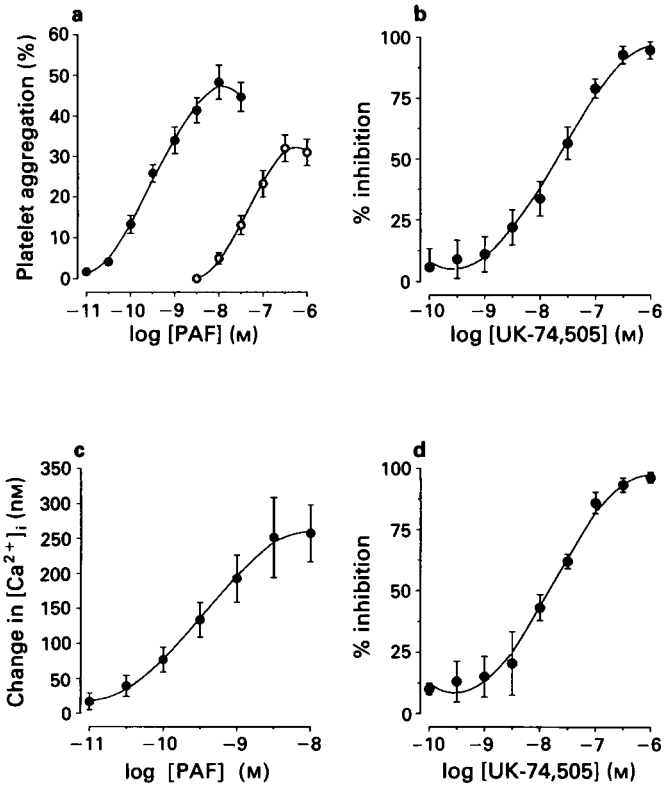

Figure 3 Effect of UK-74,505 on platelet aggregation and changes in $[Ca^{2+}]_i$ induced by PAF in rabbit washed platelets. (a) Concentration-dependent PAF-induced aggregation of platelets in PRP (○) or in Tyrode solution (●). Results are expressed as a percentage of light transmission and presented as mean ± s.e.mean of $n = 3$ preparations for PRP and $n = 4$ preparations for washed platelets. (b) Inhibition by UK-74,505 of washed platelet aggregation induced by 3×10^{-9} M PAF. UK-74,505 was added to the platelet suspension 2 min prior to challenge. Results are expressed as percentage inhibition of aggregation induced by 3×10^{-9} M PAF and presented as mean ± s.e.mean of $n = 3$ preparations. (c) Concentration-dependent PAF-induced changes in $[Ca^{2+}]_i$ in washed platelets. Results are expressed as $[Ca^{2+}]_i$ after subtraction of basal values and presented as mean ± s.e.mean of $n = 5$ preparations. Basal $[Ca^{2+}]_i$ was 191 ± 36 nM ($n = 5$). (d) Inhibition by UK-74,505 of changes in $[Ca^{2+}]_i$ induced by 3×10^{-9} M PAF in washed platelets. UK-74,505 was added to the platelet suspension 2 min prior to challenge. Results are expressed as percentage inhibition of the increase in $[Ca^{2+}]_i$ induced by PAF and presented as mean ± s.e.mean of $n = 4$ preparations. Basal $[Ca^{2+}]_i$ was not significantly different before and after addition of UK-74,505.

Pretreatment of rabbit platelets with UK-74,505

Based on the above experiments, a concentration of 10^{-6} M UK-74,505 was used to pretreat labelled rabbit platelets. However, before initiating the *in vivo* experiments, the time-course of the inhibitory activity of this dose was assessed *in vitro* on PAF-induced platelet aggregation. These experiments were carried out in order to determine whether pretreatment with UK-74,505 antagonized platelet PAF receptors for the 4 h *in vivo* period necessary to develop an RPA reaction in rabbit skin. Washed platelets were pretreated with UK-74,505, washed again to remove unbound compound and maintained for 4 h at 37°C. At the end of the incubation period, aggregation in response to 3×10^{-9} M PAF and, for comparison, 3.2 u ml^{-1} thrombin was assessed. As indicated in Figure 4, untreated washed platelets maintained for 4 h at 37°C retained their responsiveness to both PAF and thrombin throughout this incubation period. In contrast, platelets

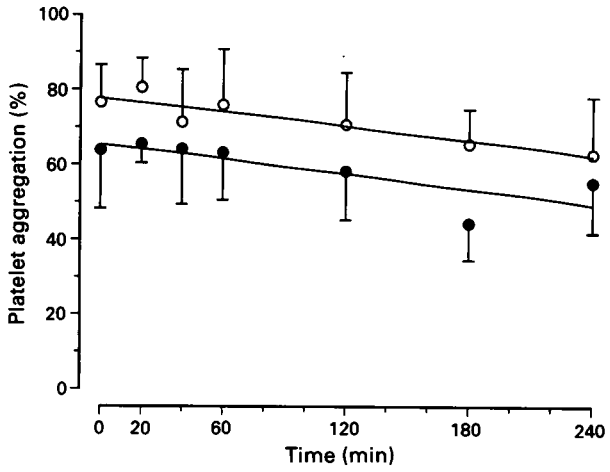

Figure 4 Time-course of rabbit washed platelet aggregation induced by 3×10^{-9} M PAF (●) or 3.2 u ml^{-1} of thrombin (○). After their final suspension, washed platelets were kept at 37°C in a water bath. Platelet aliquots were taken at different times during the 4 h incubation that followed and their aggregation to PAF or thrombin was assessed. Results are expressed as a percentage of maximum light transmission and presented as mean ± s.e.mean of $n = 4$ preparations.

pretreated with UK-74,505 exhibited a significantly inhibited response when tested with PAF after the 4 h period (Figure 5a). This inhibition was specific since the responses to thrombin were unaffected by pretreatment with UK-74,505 (Figure 5a). UK-74,505 remained bound to platelet PAF receptors during the 4 h incubation at 37°C, since pretreated platelets centrifuged for 10 min and resuspended in fresh Tyrode solution at the end of the 4 h period still exhibited an inhibited aggregatory response to PAF (Figure 5a). Furthermore, the

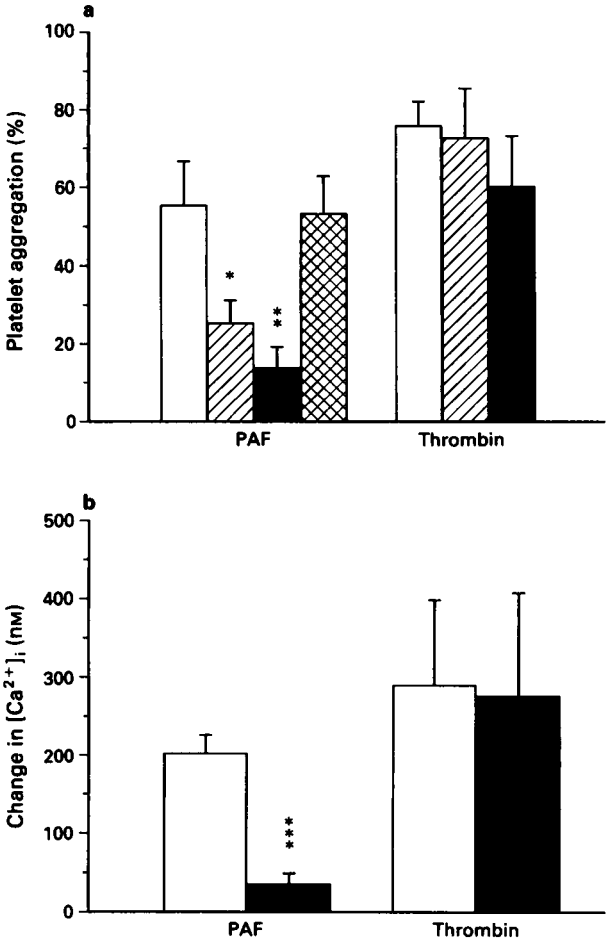

Figure 5 *In vitro* (a) and *ex vivo* (b) responsiveness to PAF and thrombin of control and UK-74,505 pretreated platelets after a 4 h period. (a) PAF- or thrombin-induced aggregation of control and UK-74,505 pretreated washed rabbit platelets incubated at 37°C for 4 h. Washed platelets were prepared and pretreated with UK-74,505 (10^{-6} M for 15 min) as indicated in the Methods section. The platelet preparations were then kept at 37°C for 4 h and at the end of the incubation period their reactivity to PAF (3×10^{-9} M) or thrombin (3.2 u ml^{-1}) was assessed. Results are expressed as a percentage of maximum light transmission and presented as mean ± s.e.mean of $n = 4$ preparations (open columns, control platelets; hatched columns, UK-74,505-pretreated platelets; solid columns, UK-74,505-pretreated platelets centrifuged and resuspended in fresh Tyrode solution; cross-hatched columns, control platelets incubated for 2 min with the supernatant of UK-74,505-pretreated platelets at a final dilution of 1/10). $*P<0.05$; $**P<0.01$, paired Student's t test. (b) *Ex vivo* measurement of changes in $[Ca^{2+}]_i$ induced by PAF (10^{-7} M) or thrombin (1 u ml^{-1}) in labelled platelets isolated from recipient rabbits. Washed platelets were loaded with Fura-2 and labelled with ^{111}In and pretreated (solid columns) or not (open columns) with UK-74,505, as indicated in the Methods section. Platelets were then injected into different recipient rabbits in order to measure their accumulation in response to various stimuli. After 4 h, rabbits were bled and their platelets isolated. Results are expressed as $[Ca^{2+}]_i$ after subtraction of basal values and presented as mean ± s.e.mean of $n = 6$ preparations. Basal values of $[Ca^{2+}]_i$ were 120 ± 19 nM and 123 ± 8 nM for control and UK-74,505-pretreated platelets, respectively ($n = 6$ preparations). Significant differences from control are indicated by $***P<0.001$.

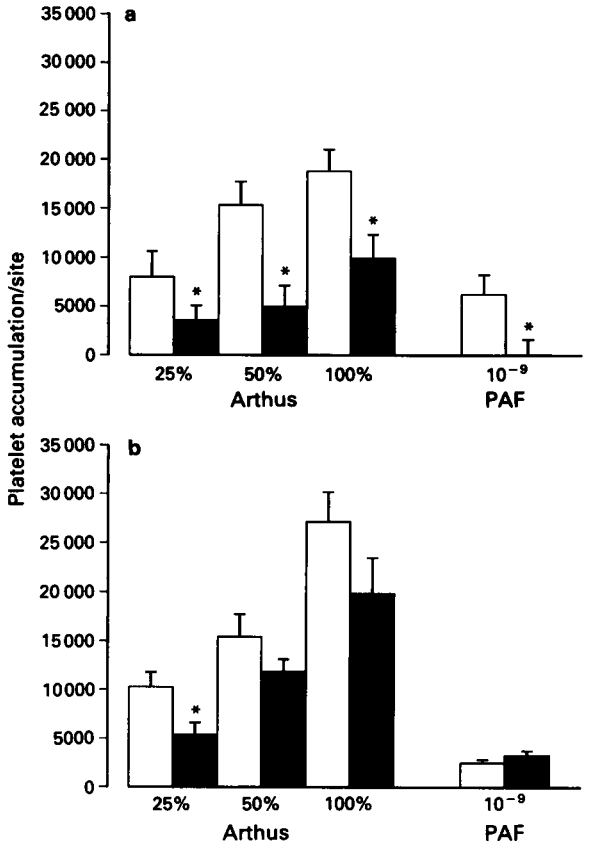

Figure 6 Platelet accumulation induced by PAF (10^{-9} mol/site) and increasing concentrations of Arthus antiserum in rabbit skin: effect of i.v. injection of 0.5 mg kg^{-1} UK-74,505 (a, solid columns) or pretreatment of ^{111}In-labelled platelets with UK-74,505 (b, solid columns). Platelet accumulation was expressed as number of platelets per site per 10^9 platelets injected and presented as mean ± s.e.mean of the responses obtained after subtraction of normal rabbit serum or saline/BSA values in $n = 6-7$ rabbits. Significant differences from control are indicated by $*P<0.05$. Oedema formation in response to 50% Arthus antiserum was significantly reduced in animals pretreated with i.v. UK-74,505 compared to control animals (14.9 ± 4.2 µl ($n = 6$) versus 30.5 ± 3.9 µl ($n = 6$), $P<0.05$). Platelet accumulation and oedema formation in response to normal rabbit serum were not significantly different from saline/BSA controls.

4 h supernatant of UK-74,505-pretreated platelets obtained after centrifugation, did not inhibit aggregation of control platelets in response to PAF (Figure 5a). However, centrifugation of UK-74,505-pretreated platelets and resuspension in fresh Tyrode solution at the end of the 4 h incubation period did not affect their normal responsiveness to thrombin (Figure 5a).

In order to determine if PAF receptors on platelets pretreated with the antagonist remained blocked after 4 h *in vivo*, platelets were ^{111}In-labelled and loaded with Fura-2. The platelets were then treated with UK-74,505 and washed. Treated and untreated platelets for comparison were then injected i.v. into rabbits for the experiments described in the next section. At the end of a 4 h period, blood samples were taken and platelets prepared. Responses to PAF were then determined in terms of changes in $[Ca^{2+}]_i$ measured with Fura-2. Platelets pretreated with the antagonist clearly remained unresponsive to PAF after the 4 h period *in vivo* whereas responses to thrombin were unaffected (Figure 5b).

Effect of systemic UK-74,505 or in vitro *pretreatment of rabbit platelets with UK-74,505 on oedema formation and platelet accumulation in an RPA reaction in rabbit skin*

Intradermal administration of increasing amounts of Arthus antiserum (25%, 50% and 100%) in rabbits, followed by i.v.

injection of antigen, provoked a significant and dose-dependent platelet accumulation over the 4 h test period (Figure 6, open columns). A similar dose-response curve for oedema formation was obtained (data not shown). Platelet deposition triggered by the different dilutions of Arthus antiserum was significantly inhibited when rabbits were pretreated with 0.5 mg kg^{-1} i.v. UK-74,505 (Figure 6a). The small platelet deposition induced by i.d. PAF was also inhibited in these animals (Figure 6a). Intravenous injection of the PAF antagonist also suppressed oedema responses in the RPA reaction (see legend to Figure 6), as shown previously with other antagonists (Hellewell & Williams, 1986; Issekutz & Szejda, 1986).

Thus, i.v. UK-74,505 effectively inhibited platelet accumulation in RPA reactions; however, radiolabelled platelets with their PAF receptors blocked by pre-incubation with the antagonist still accumulated in high numbers in RPA sites (Figure 6b). A significant reduction in accumulation was only seen in sites receiving 25% antiserum. Further, the small platelet accumulation induced by i.d. PAF was also unaffected by preincubating platelets with the antagonist (Figure 6b). In these experiments, oedema responses in Arthus sites were not significantly different in the two groups of rabbits (data not shown).

Discussion

In this study a novel PAF antagonist, UK-74,505, was employed to investigate the possible contribution of endogenous PAF to oedema formation and platelet accumulation induced in rabbit skin in response to soluble mediators and particulate stimuli. A unique property of the compound is avid binding to PAF receptors which enabled the effect of selectively blocking platelet receptors to be studied.

Intravenous UK-74,505 blocked oedema formation induced by intradermally-injected PAF, but had no effect on oedema formation induced by BK and histamine, or on neutrophil-dependent oedema formation induced by ZAP (as a source of C5a des Arg), FMLP, LTB_4 or IL-8 (Figures 1 and 2). Mediators were tested in the presence of the vasodilator, PGE_2, in order to induce substantial oedema responses by synergism. These experiments demonstrated the potency and selectivity of the antagonist. This selectivity is not seen with certain other PAF antagonists such as CV-3988 (Hellewell & Williams, 1989) which makes interpretation of some previous results difficult (Issekutz & Szejda, 1986). Oedema induced by i.d. injection of zymosan particles, which stimulates the generation of C5a and a vasodilator prostaglandin locally, was also unaffected by i.v. UK-74,505.

PAF, ZAP, IL-8 and zymosan were tested i.d. for their ability to induce the accumulation of ^{111}In-labelled platelets in rabbit skin (Figure 2). Interestingly, PAF alone, or PAF + PGE_2 which induced substantial oedema responses, induced only small, but significant, platelet accumulation. Some platelet accumulation was induced by IL-8 and this was significantly potentiated by PGE_2. ZAP-induced platelet accumulation and large accumulation was seen with the addition of PGE_2 (to our knowledge this is the first demonstration of potentiation of platelet accumulation by a prostaglandin). Zymosan particles also induced substantial platelet accumulation. Only the platelet accumulation induced by PAF and zymosan was significantly suppressed by i.v. UK-74,505. A striking observation was the effective inhibition of zymosan-induced platelet accumulation by the antagonist, with no effect on the oedema response. These results suggest that PAF is generated locally in response to zymosan and that it is important in platelet accumulation. These results are in agreement with data published by Dewar *et al.* (1984), showing that PAF causes platelet accumulation in guinea-pig skin. However, in our model, endogenous PAF does not appear to be important for zymosan-induced oedema formation, perhaps because this is masked by the powerful oedema-inducing effect of endogenous C5a by a neutrophil-dependent process. The observation that neutrophil chemoattractants induced platelet accumulation that is not significantly inhibited by i.v. UK-74,505 may suggest that this platelet accumulation is secondary to neutrophil adherence to the endothelium, e.g. platelet adherence to stimulated neutrophils (Issekutz *et al.*, 1983) or, alternatively, release of platelet stimulatory activity from neutrophils (Chignard *et al.*, 1986). ZAP appeared to be very active in this respect, which may reflect its more protracted action in the skin. Exogenous PAF induced substantial oedema when in combination with PGE_2, but small platelet accumulation. PAF is unstable in tissue fluid and these results may indicate that a sustained production of PAF is important for platelet accumulation, whereas an acute effect on endothelial cells can induce plasma protein leakage. These points require further investigation, but it is clear that platelet accumulation is not merely a consequence of increased microvascular permeability as BK + PGE_2 induced large oedema responses with very little platelet accumulation (Figure 2 legend).

These observations and the special binding properties of the antagonist led us to address the importance of PAF receptors on platelets in the process of platelet accumulation *in vivo*. PAF induced a concentration-related aggregation of rabbit platelets *in vitro* correlating with changes in $[Ca^{2+}]_i$ (Figure 3). Both these responses were inhibited by UK-74,505 (Figure 3). Platelets retained their responsiveness to PAF and thrombin when tested for aggregation after a 4 h incubation period at 37°C (Figure 4). Responses to PAF remained selectively blocked when platelets were incubated with UK-74,505, washed and then aggregation responses were tested after the 4 h incubation period (Figure 5a).

^{111}In-labelled platelets, loaded with Fura-2 and with their PAF receptors blocked by pre-incubation with UK-74,505, were then administered i.v. to rabbits. RPA reactions were induced in the skin, and platelet accumulation and plasma protein leakage measured after 4 h. Platelets prepared from blood samples taken at 4 h remained selectively unresponsive to PAF, as evidenced by changes in $[Ca^{2+}]_i$ (Figure 5b). However, these blocked platelets were still able to accumulate in Arthus sites (Figure 6b), whereas i.v. UK-74,505 (with access to PAF receptors on other cells as well as platelets) was far more effective in suppressing platelet accumulation (Figure 6a). Similarly, platelet accumulation induced by exogenous PAF was inhibited by i.v. antagonist and not modified in animals injected with UK-74,505-pretreated platelets. Intravenous UK-74,505 also suppressed oedema formation in the Arthus sites, (Figure 6 legend), as shown previously with other selective antagonists (Hellewell & Williams, 1986; Issekutz & Szejda, 1986).

These results suggest that endogenous PAF is not an important secondary mediator for oedema formation and platelet accumulation induced by the soluble mediators tested in this study. However, endogenous PAF is implicated in oedema formation in the RPA reaction, although not in response to zymosan. This difference may be explained by the different sites of PAF generation in these two responses. Both immune complexes and zymosan can activate complement locally and generate C5a that will cause neutrophil accumulation and neutrophil-dependent oedema formation. In the RPA reaction, PAF is likely to be generated by phagocytosing neutrophils very close to the microvascular endothelium and can increase permeability by a direct effect on endothelial cells. When zymosan is injected i.d. the particles will be relatively remote from vessels and PAF liberated during phagocytosis may be metabolized before reaching an effective concentration near the endothelium.

The situation with respect to platelet accumulation appears to be different. In this case i.v. PAF antagonist inhibited the response induced by both zymosan (Figure 2b) and immune complexes (Figure 6a). To account for this, we propose that released PAF acts over a short range and stimulates the secondary release of an unidentified, relatively-stable med-

iator that induces platelet accumulation. The source of endogenous PAF may be the neutrophil and it is possible that this PAF may have an autocrine function in acting on neutrophil PAF receptors to release the secondary mediator. This hypothesis is supported by the results of the final experiments (Figure 6b) demonstrating that platelets in which PAF receptors are blocked by preincubation with the antagonist still accumulate effectively in Arthus sites. The results with i.d. PAF also support this idea although platelet accumulation was small presumably because of the instability of PAF *in vivo*. Platelet accumulation induced by intradermally-injected PAF was clearly blocked by i.v. antagonist, which we propose would prevent PAF stimulating release of the secondary mediator. However, preincubation of platelets with the antagonist had no effect on platelet accumulation induced by i.d. PAF. An alternative explanation for the results obtained in Figure 6 (b) is that PAF, added exogenously or generated by neutrophils during the RPA reaction, activates the recipient animal's own platelets which then in turn cause recruitment of the antagonist-treated radiolabelled platelets by releasing a mediator other than PAF. However, our results in Figure 5 (b) render this latter hypothesis unlikely since platelets isolated from recipients receiving UK-74,505-treated platelets (this platelet preparation containing both Fura-2 loaded PAF-receptor blocked platelets and the recipient animal's own untreated platelets) exhibited only a minimal increase in $[Ca^{2+}]_i$ in response to PAF.

In conclusion, we have demonstrated a role for PAF in platelet accumulation induced by particulate, but not soluble inflammatory stimuli. Interestingly, the results suggest that platelet PAF receptors may not play a significant role in mediating platelet accumulation and the activation of PAF receptors on neutrophils rather than on platelets may be more important. In this respect, UK-74,505 is a valuable tool for assessing the role of PAF receptors on leucocytes in inflammatory reactions and such experiments are currently in progress. With long-acting, selective and potent PAF antagonists such as UK-74,505 a clearer understanding of the mechanisms involved in inflammatory reactions such as the RPA reaction may be forthcoming.

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References

ALABASTER, V.A., KEIR, R.F., PARRY, M.J. & DE SOUZA, R.N. (1991). UK-74,505, a novel and selective PAF antagonist, exhibits potent and long lasting activity in vivo. In *New Drugs for Asthma Therapy*. ed. Anderson, G.P., Chapman, I.P. & Morley, J. pp. 221–227. Basle: Birkhauser Verlag.

CAMUSSI, G., AGLIETTA, M., MALAVASI, F., TETTA, C., PIACIBELLO, W., SANOVIO, F. & BUSSOLINO, F. (1983). The release of platelet-activating factor from human endothelial cells in culture. *J. Immunol.*, **131**, 2397–2403.

CHIGNARD, M., LE CONEDIC, J.P., VARGAFTIG, B.B. & BENVENISTE, J. (1980). Platelet-activating factor (PAF-acether) secretion from platelets: effect of aggregating agents. *Br. J. Haematol.*, **46**, 455–464.

CHIGNARD, M., SELAK, M.A. & SMITH, J.B. (1986). Direct evidence for the existence of a neutrophil-derived platelet activator (neutrophilin). *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8609–8613.

COCHRANE, G.C. & JANOFF, A. (1974). The Arthus reaction: a model of neutrophil and complement-mediated injury. In *The Inflammatory Process*. ed. Zweifach, B.W., Grant, L. & McCluskey, R.T. pp. 85–162. New York: Academic Press.

COCHRANE, C.G., MULLER-EBERHARD, H.J. & AIKIN, B.S. (1970). Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. *J. Immunol.*, **105**, 55–69.

COOPER, K., FRAY, M.J., RICHARDSON, K. & STEELE, J. (1990). UK-74,505, a novel and highly potent dihydropyridine PAF antagonist: synthesis and structure-activity relationships. *J. Lipid Mediators*, **2**, 212.

DEACON, R.W., MELDEN, M.K., SAUNDERS, R.N. & HANDLEY, D.A. (1986). PAF involvement in dermal extravasation in the reverse passive Arthus reaction. *Fedn. Proc.*, **45**, 995.

DEWAR, A., ARCHER, C.B., PAUL, W., PAGE, C.P. MACDONALD, D.M. & MORLEY, J. (1984). Cutaneous and pulmonary histopathological responses to platelet activating factor (paf-acether) in the guinea-pig. *J. Pathol.*, **144**, 25–34.

GRYNKIEWICZ, G., POENIE, M. & TSIEN, R.Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.*, **260**, 3440–3450.

HEFFNER, J.E., SAHN, S.A. & REPINE, J.E. (1987). The role of platelets in the adult respiratory distress syndrome. Culprits or bystanders? *Am. Rev. Respir. Dis.*, **135**, 482–492.

HELLEWELL, P.G. (1990). The contribution of platelet-activating factor to immune complex-mediated inflammation. In *Platelet-Activating Factor in Endotoxin and Immune Diseases*. ed. Handley, D.A., Saunders, R.N., Houlihan, W.J. & Tomesch, J.C. pp. 367–386. New York: Marcel Dekker Inc.

HELLEWELL, P.G. & WILLIAMS, T.J. (1986). A specific antagonist of platelet-activating factor suppresses oedema formation in an Arthus reaction but not oedema induced by leukocyte chemoattractants in rabbit skin. *J. Immunol.*, **137**, 302–307.

HELLEWELL, P.G. & WILLIAMS, T.J. (1989). Antagonism of PAF-induced oedema formation in rabbit skin: a comparison of different antagonists. *Br. J. Pharmacol.*, **97**, 171–180.

HENSON, P.M. & COCHRANE, C.G. (1969). Immunological induction of increased vascular permeability. I. A rabbit passive cutaneous anaphylactic reaction requiring complement, platelets, and neutrophils. *J. Exp. Med.*, **129**, 153–165.

HUMPHREY, J.H. (1955a). The mechanism of Arthus reactions. I. The role of polymorphonuclear leucocytes and other factors in reversed passive Arthus reactions in rabbits. *Br. J. Exp. Pathol.*, **36**, 268–282.

HUMPHREY, J.H. (1955b). The mechanism of Arthus reactions. II. The role of polymorphonuclear leucocytes and platelets in reversed passive reactions in the guinea-pig. *Br. J. Exp. Pathol.*, **36**, 283–285.

ISSEKUTZ, A.C., RIPLEY, M. & JACKSON, J.R. (1983). Role of neutrophils in the deposition of platelets during acute inflammation. *Lab. Invest.*, **49**, 716–724.

ISSEKUTZ, A.C. & SZEJDA, M. (1986). Evidence that platelet activating factor may mediate some acute inflammatory responses. Studies with the platelet activating factor antagonist, CV3988. *Lab. Invest.*, **54**, 275–281.

JOSE, P.J., FORREST, M.J. & WILLIAMS, T.J. (1983). Detection of the complement fragment C5a in inflammatory exudates from the rabbit peritoneal cavity using radioimmunoassay. *J. Exp. Med.*, **158**, 2177–2182.

KRAVIS, T.C. & HENSON, P.M. (1977). Accumulation of platelets at sites of antigen-antibody-mediated injury: a possible role for IgE antibody and mast cells. *J. Immunol.*, **118**, 1569–1573.

LEWIS, E. & TURK, J.L. (1975). Comparison of the effect of various antisera and cobra venom factor on inflammatory reactions in guinea-pig skin. II. The Arthus reaction and the local Shwartzman reaction. *J. Pathol.*, **115**, 111–125.

MOVAT, H.Z. & BURROWES, C.E. (1985). The local Shwartzman reaction: endotoxin-mediated inflammatory and thrombo-hemorrhagic lesions. In *Handbook of Endotoxin*, Vol 3: *Cellular Biology of Endotoxin*. ed. Berry, L.J. pp. 260–302. Amsterdam: Elsevier Science Publishers B.V.

PARRY, M.J., ALABASTER, V.A., CHEESEMAN, H.E., DE SOUZA, R.N. & KEIR, R.F. (1990). In vitro and in vivo profile of UK-74,505: a novel, highly potent and selective dihydropyridine PAF antagonist. *J. Lipid Mediators*, **2**, 181.

RADOMSKI, M. & MONCADA, S. (1983). An improved method for washing of human platelets with prostaglandin. *Thromb. Res.*, **30**, 383–389.

ROSSI, A.G., NORMAN, K.E., DONIGI-GALE, D., SHOUPE, T.S., EDWARDS, R. & WILLIAMS, T.J. (1992). The role of complement, platelet-activating factor and leukotriene B_4 in the reversed passive Arthus reaction. *Br. J. Pharmacol.*, **107**, 44–49.

STETSON, C.A. (1951). Similarities in the mechanisms determining the Arthus and Schwartzman phenomena. *J. Exp. Med.*, **94**, 347–358.

VINCENT, J.E., BONTA, I.L. & ZIJLSTRA, F.J. (1978). Accumulation of blood platelets in carrageenin rat paw oedema. Possible role in the inflammatory process. *Agents Actions*, **8**, 291–295.

WARREN, J.S., MANDEL, D.M., JOHNSON, K.J. & WARD, P.A. (1989). Evidence for the role of platelet-activating factor in immune complex vasculitis in the rat. *J. Clin. Invest.*, **83**, 669–678.

WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289**, 646–650.

WILLIAMS, T.J., HELLEWELL, P.G. & JOSE, P.J. (1986). Inflammatory mechanisms in the Arthus reaction. *Agents Actions*, **19**, 66–72.

WILLIAMS, T.J. & JOSE, P.J. (1981). Mediation of increased vascular permeability after complement activation: histamine-independent action of rabbit C5a. *J. Exp. Med.*, **153**, 136–153.

YEH, C.G., MARSH, H.C., CARSON, G.R., BERMAN, L., CONCINO, M.F., SCESNEY, S.M., KUESTNER, R.E., SKIBBENS, R., DONAHUE, K.A. & IP, S.H. (1991). Recombinant soluble human complement receptor type 1 inhibits inflammation in the reversed passive Arthus reaction in rats. *J. Immunol.*, **146**, 250–256.

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