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A study of PAF-induced ocular inflammation in the rat and its inhibition by the PAF antagonist, L-652,731

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A significant inflammatory reaction in the rat conjunctiva followed the subconjunctival injection of synthetic platelet activating factor (PAF) in doses which ranged from 10 ng to 1 μg, an inflammatory response being evaluated as the increase in both tissue weight and extravasation of Evans blue dye in the conjunctival tissue. Inflammation was still present 6 h after the injection of 0·1 μg of PAF. Orally administered indomethacin or BW 755C failed to alter the response to 0·1 μg of PAF. In contrast, the PAF-induced inflammation was blocked by the oral administration of the PAF receptor antagonist, L-652,731, a dose as low as 5 mg kg^{-1} eliciting a significant inhibition. The topical administration of L-652,731, (two doses of 100 μg as a 1% suspension), elicited a slight, but significant blockade of 23%. Its antagonistic action was more striking when it was co-injected subconjunctivally with 0·1 μg of PAF, a dose as low as 3 μg evoking a significant blockade. The topical administration of 0·1 μg of PAF did not elicit a significant inflammatory reaction and this contrasts with the results obtained after its subconjunctival injection.

Platelet activating factor (PAF), in addition to being released through an IgE-dependent mechanism from rabbit basophils (Benveniste et al 1972), is generated by a wide variety of cells implicated in inflammation (Vargaftig et al 1981). Besides its platelet aggregating effect, PAF has been shown in-vitro to be a potent mediator of neutrophil stimulation (O'Flaherty et al 1981; Shaw et al 1981) and smooth muscle contraction (Findlay et al 1981). Systemically administered PAF elicits many effects including hypotension (Blank et al 1979), inflammation (Bonnet et al 1981) and asthma (Page et al 1982) and the injection of PAF into the skin of animals (Stimler et al 1981; Humphrey et al 1982) and man (Basran et al 1983) causes an inflammatory response reminiscent of allergic skin reactions.

Allergen-induced conjunctivitis is a common affliction and experiments were undertaken to determine if locally administered PAF inflamed the conjunctiva of the rat.

Methods

Solutions of synthetic PAF (1-*O*-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine; Bachem, Switzerland) in sterile saline (0·9% NaCl) were injected subconjunctivally (25 μL) into both eyes of ether anaesthetized, male Wistar rats, ca 240 g. Immediately thereafter, a solution of Evans blue in saline (12·5 mg kg^{-1}) was injected via the tail vein. Rats were killed at predetermined times by cervical dislocation. The upper conjunctiva was dissected, weighed and stored frozen until dye extraction. This was done using a 0·5% aqueous solution of sodium sulphate in acetone (30% : 70%; v/v) and by sonication for 30 min. The extracted Evans blue was assayed spectrophotometrically at 620 nm and expressed as μg/conjunctiva.

Conjunctival inflammation was evaluated as an increase in both tissue weight and Evans blue content. A concurrently run, vehicle-treated group of rats received Evans blue and the animals were killed at the same time as those in the treated group and results were corrected for the tissue weight and Evans blue content of these controls. Differences between treated groups were analysed statistically using a two-tailed Student's *t*-test.

Results

The subconjunctival injection of PAF in doses ranging from 1 ng to 1 μg elicited a dramatic dose-dependent inflammatory response at 30 min (Table 1); the inflammation after 0·1 μg was still present at 6 h, the last recorded time (Table 2). The oral administration of 5 mg kg^{-1} of L-652,731 (*trans*-2,5-bis-(3,4,5,-trimethoxyphenyl)tetrahydrofuran, supplied by Merck Sharp & Dohme Research Laboratories, Rahway, NJ) at 90 min before PAF injection, 0·1 μg, significantly reduced the inflammatory response (Table 3). A more marked inhibitory effect was observed at 10 and 20 mg kg^{-1}. In contrast, the oral administration of 5 and 10 mg kg^{-1} of the cyclooxygenase inhibitor indomethacin 60 min before PAF, 0·1 μg, had little effect, the respective inhibitions being 9 and 13%. The corresponding reductions were 8 and 13% after a similar

Table 1. Inflammatory response of the rat conjunctiva to locally injected PAF (25 μL) into the upper conjunctiva of ether-anaesthetized rats which were then injected with Evans blue (12·5 mg kg^{-1} i.v.) and killed 30 min after PAF injection. Results are expressed as the increase in tissue weight and Evans blue content from vehicle-treated control values and are the mean ± s.e.m. of 10 determinations.

PAF (ng)	Tissue wt (mg)	Evans blue (μg)
1	2·0 ± 1·6	0·4 ± 0·2
10	15·8 ± 3·1	3·0 ± 0·9
100	78·2 ± 7·2	20·6 ± 2·7
1000	199·9 ± 10·7	38·4 ± 3·6

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Table 2. Duration of PAF-induced inflammation of the rat conjunctiva. PAF (0·1 μg) was injected into the upper conjunctiva and rats were killed at times indicated. For remainder of experimental details, see Table 1. Results are the mean ± s.e.m. of 10 determinations.

Time after injection (h)	Tissue wt (mg)	Evans blue (μg)
0·5	50·1 ± 7·4	14·4 ± 1·7
1	45·8 ± 4·6	9·3 ± 0·8
2	40·5 ± 4·0	10·0 ± 0·9
4	31·1 ± 5·4	6·4 ± 0·8
6	22·1 ± 4·6	5·2 ± 0·7

pretreatment with 5 and 10 mg kg^{-1} of the dual cyclooxygenase-lipoxygenase inhibitor BW 755C (3-amino-1-[(*m*-trifluoromethyl)phenyl]-2-pyrazoline). The topical administration of two doses (100 μg as 10 μL of a 1% suspension) of L-652,731 at 30 and 15 min before PAF, 0·1 μg, elicited a significant reduction (23%) in inflammation, the Evans blue values for the PAF and L-652,731 + PAF groups (n = 20) being 19·2 ± 1·5 and 14·7 ± 1·0 μg, respectively. The effect of L-652,731 was more striking when administered subconjunctivally with PAF, 3, 10 and 30 μg of L-652,731 significantly reducing PAF-induced inflammation, as reflected in Evans blue levels, by 26, 57 and 71%, respectively (Table 4).

Discussion

These experiments clearly demonstrate, for the first time, that conjunctival inflammation in the rat can be elicited by PAF. To elicit an inflammatory response, PAF must be injected into the conjunctiva because it was found in preliminary experiments that the topical instillation of 0·1 μg of PAF in 10 μL failed to elicit a conjunctival inflammation. The lowest injected dose of PAF causing a significant inflammatory response was 10 ng. This is equivalent to 20 pmol and is comparable with doses which, on intradermal injection, increase cutaneous vascular permeability in the rat (Humphrey et al 1982; Goldenberg & Meurer 1984; Hwang et al 1985b).

L-652,731 is a recently described specific PAF receptor antagonist (Hwang et al 1985a) which, on oral administration, attenuates PAF-induced hypotension, extravasation, vascular lysosomal hydrolase release and neutropenia in the rat (Doebber et al 1986; Wu et al 1986). A pretreatment time of 90 min was used in those studies and, using the same pretreatment time, we have demonstrated that oral doses of L-652,731 as low as 5 mg kg^{-1} can block PAF-induced conjunctival inflammation in the rat. L-652,731 was effective in reducing the response to PAF when both were co-injected into the rat conjunctiva. A significant reduction of PAF-induced inflammation was achieved by a dose of L-652,731 as low as 3 μg. The compounds were co-administered to avoid damaging the tissue. The topical administration of two doses L-652,731 (100 μg as a 1% suspension) had a slight, but significant inhibitory effect but this dose is much higher than the doses which were active on injection into the cunjunctiva. L-652,731 is not soluble at 1% and it is feasible that a more effective inhibition on topical administration could be achieved by a PAF receptor antagonist more soluble than L-652,731.

In contrast to L-652,731, oral pretreatment with indomethacin or BW 755C failed to attenuate PAF-induced conjunctival inflammation. Others have previously reported that systemic administration of both indomethacin and BW 755C failed to reduce PAF-induced cutaneous vascular permeability in the rat

Table 3. Effect of 90 min pretreatment with L-652,731 (oral) on PAF (0·1 μg)-induced inflammation of the rat conjunctiva. For other experimental details, see Table 1. Results are the mean ± s.e.m. of 10–20 determinations.

L-652,731 (mg kg^{-1})	Tissue wt (mg)			Evans blue (μg)		
	Control	Treated	% Inhib.	Control	Treated	% Inhib.
5	32·4 ± 2·4	16·7 ± 2·1*	48·4	8·3 ± 0·5	6·1 ± 0·5*	26·5
10	47·7 ± 6·1	9·6 ± 3·2*	79·9	16·2 ± 2·2	7·7 ± 3·4*	52·5
20	52·1 ± 4·2	14·4 ± 1·7*	72·4	14·7 ± 0·5	6·1 ± 0·5*	58·5

* Differs from control, $P < 0·05$.

Table 4. Effect of the administration subconjunctivally of L-652,731 with PAF (0·1 μg) on PAF-induced inflammation of the rat conjunctiva. For other experimental details, see Table 1. Results are the mean ± s.e.m. of 20 determinations.

L-652,731 (μg)	Tissue wt (mg)			Evans blue (μg)		
	PAF	PAF + L-652,731	% Inhib.	PAF	PAF + L-652,731	% Inhib.
3	52·1 ± 3·8	38·5 ± 2·6*	26·1	19·4 ± 1·6	14·3 ± 0·9*	26·3
10	52·1 ± 3·8	14·9 ± 2·3*	71·4	18·9 ± 1·2	8·1 ± 0·8*	57·1
30	54·9 ± 4·9	17·3 ± 2·1*	68·5	19·4 ± 1·8	5·6 ± 0·6*	71·1

* Differs from PAF, $P < 0·05$.

(Goldenberg & Meurer 1984; Hwang et al 1985b; Swingle & Reiter 1986). This suggests that the biosynthesis of prostaglandins or leukotrienes is not involved in PAF-induced conjuctival inflammation in the rat.

In summary, the local injection of low doses of PAF into the rat conjunctiva elicits a marked inflammatory response. This phenomenon is not dependent upon eicosanoids, as reflected in the ineffectiveness of orally administered indomethacin and BW 755C. In contrast, the PAF receptor antagonist L-652,731 significantly decreased the oedematous response to PAF after oral, topical and subconjunctival administration. The usefulness of PAF receptor antagonists on other forms of conjunctival inflammation remains to be determined.

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REFERENCES

Basran, G. S., Page, C. P., Paul, W., Morley, J. (1983) Eur. J. Pharmacol. 86: 143–144

Benveniste, J., Henson, P. M., Cochrane, C. G. (1972) J. Exp. Med. 136: 1356–1377

Blank, M. L., Snyder, F., Byers, L. W., Brooks, B., Muirhead, E. E. (1979) Biochem. Biophys. Res. Commun. 90: 1194–1200

Bonnet, J., Loiseau, A. M., Orvoen, M., Bessin, P. (1981) Agents Actions 11: 559–562

Doebber, T. W., Wu, M. S., Biftu, T. (1986) J. Immunol. 136: 4659–4668

Findlay, S. R., Lichtenstein, L. M., Hanahan, D. J., Pinckard, R. N. (1981) Am. J. Physiol. 241: C 130–C 133

Goldenberg, M. M., Meurer, R. D. (1984) Prostaglandins 28: 271–278

Humphrey, D. M., McManus, L. M., Satouchi, K., Hanahan, D. J., Pinckard, R. N. (1982) Lab. Invest. 46: 422–427

Hwang, S.-B., Lam, M.-H., Biftu, T., Beattie, T. R., Shen, T. Y. (1985a) J. Biol. Chem. 260: 15639–15645

Hwang, S.-B., Li, C.-L., Lam, M.-H., Shen, T. Y. (1985b) Lab. Invest. 52: 617–630

O'Flaherty, J. T., Wykle, R. L., Miller, C.H., Lewis, J. C., Waite, M., Bass, D. A., McCall, C. E., DeChatelet, L. R. (1981) Am. J. Pathol. 103: 70–79

Page, C. P., Paul, W., Basran, G. S., Morley, J. (1982) New Engl. J. Med. 307: 251–252

Shaw, J. O., Pinckard, R. N., Ferrigni, K. S., McManus, L. M., Hanahan, D. L. (1981) J. Immunol. 127: 1250–1255

Stimler, N. P., Bloor, C. M., Hugli, T. E., Wykle, R. L., McCall, C. E., O'Flaherty, J. T. (1981) Am. J. Pathol. 105: 64–69

Swingle, K. F., Reiter, M. J. (1986) Agents Actions 18: 359–365

Vargaftig, B. B., Chignard, M., Benveniste, J., Lefort, J., Wal, F. (1981) Ann. N.Y. Acad. Sci. 370: 119–137

Wu, M. S., Biftu, T., Doebber, T. W. (1986) J. Pharmacol. Exp. Ther. 239: 841–845

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Distribution and urinary excretion of the desethylmetabolites of chloroquine in the rat

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The tissue distribution of desethylchloroquine and bisdesethylchloroquine has been studied in rats after single intraperitoneal administration of the drugs at a dose of 10 mg kg^{-1}. Concentrations of the chloroquine metabolites in the liver, heart, lungs, kidney and spleen were 34 to 250 times higher than their plasma concentrations 24 h after the drugs had been injected. Urinary excretion of the drugs was studied in rats after single intravenous administration of 2·5, 5 or 10 mg kg^{-1} doses. The total estimated urinary excretion of desethylchloroquine and bisdesethylchloroquine was 25 and 64% respectively of the administered dose, with the maximum urinary excretion occurring on the first day. The results show that the desethylmetabolites of chloroquine are concentrated in the tissues in the same manner as the parent compound.

The major metabolites of chloroquine are desethylchloroquine which forms about 25% of total plasma quinolines and bisdesethylchloroquine (BDCQ) which forms about 6% (McChesney et al 1967). Both desethylchloroquine (Aderounmu & Fleckenstein 1983) and bisdesethylchloroquine (Ajayi et al 1987) have antimalarial activity which is at least as great as that of the parent compound against chloroquine-sensitive *Plasmodium falciparum*. Desethylchloroquine is concentrated in red blood cells to at least the same extent as chloroquine itself (Gustafsson et al 1983). The pharmacodynamics and pharmacokinetics of the metabolites might therefore be important in the overall antimalarial activity of chloroquine and possibly also in the development of resistance to it and also in the adverse reaction and toxicity profile seen during treatment. We have therefore examined the tissue distribution and urinary excretion of desethyl- and bisdesethylchloroquine using the rat as a model.

Materials and methods

Male and female Swiss albino rats, 210–240 g, were used. Rats were divided into 4 groups of three, one

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