The role of platelet-activating factor (PAF) as a mediator of increased conjunctival vascular permeability was investigated in a guinea-pig model of immediate hypersensitivity. Vascular permeability of the conjunctiva was determined by measuring the albumin content in lavage fluid (LF) after topical challenge with either PAF or ovalbumin. PAF produced a dose-dependent increase of the vascular permeability within minutes. Topical pretreatment with levocabastine, a potent histamine H₁-antagonist demonstrated no effect towards the vascular permeability in response to PAF provocation. Pretreatment with eyedrops containing the specific PAF antagonist BN 52021 (1%) showed a significant inhibition of the vascular permeability (60.2%) and the clinical score (27.5%) after PAF challenge. In sensitized guinea-pigs, levocabastine showed a marked inhibition of both the vascular permeability (80.5%) and the clinical score (70%) after topical challenge with ovalbumin. BN 52021, although to a lesser extent, showed a similar effect towards the vascular permeability (26.8%) and the clinical score (28%) after antigen provocation. When BN 52021 and levocabastine were administered in combination, the vascular permeability was significantly decreased after antigen challenge in comparison with eyes pretreated with levocabastine alone. These results indicate that PAF plays a role in the acute phase of allergic conjunctivitis in the guinea-pig.

Key words: Allergic conjunctivitis, Guinea pig, H₁-antagonist, PAF antagonist, Platelet-activating factor (PAF), Vascular permeability

Platelet-activating factor: an inflammatory mediator in the acute phase of allergic conjunctivitis in a guinea-pig model

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Introduction

Allergic conjunctivitis is characterized by bilateral hyperaemia, itching, tearing and oedema. Increased vascular permeability has also been described as an important feature of allergic conjunctivitis.¹ It is an immediate hypersensitivity reaction (type I); after interaction of the antigen–IgE antibody complex with the mast cell, the clinical signs and symptoms appear within minutes. Histamine, released from the granules of the mast cell, seems to play a key role but other mediators including leukotrienes (LT), prostaglandins (PG) and platelet-activating factor (PAF) seem to take part in the inflammatory process.²

PAF has been shown to induce a wide variety of biological actions such as chemotaxis and activation of neutrophils³ and eosinophils;⁴ moreover PAF induces increased vascular permeability.⁵ On a molar basis, PAF appeared to be 1 000 to 10 000 times more potent in inducing vascular permeability changes in skin compared with histamine.⁶ Because of these effects, PAF has been implicated in the pathogenesis of allergic diseases including allergic conjunctivitis.

In a number of studies PAF receptor antagonists have been shown to be very effective in inhibiting various effects after PAF challenge in different models. In the eye, PAF antagonists have been shown to be capable of inhibiting corneal oedema formation and pupillary constriction after intracameral injection of PAF⁷ and they partially reduced the endotoxin-induced breakdown of the blood–aqueous barrier.⁸ Furthermore, the specific PAF antagonist BN 52021 showed a significant inhibition of oedema, leucocyte infiltration and vascularization of the cornea in a rabbit model of immunogenic keratitis⁹ after challenge with albumin. In view of these findings, we studied the inhibitory effect of BN 52021 towards microvascular permeability and clinical signs after PAF and antigen provocation in a guinea-pig model of immediate hypersensitivity.

Materials and Methods

Female Hartley strain guinea-pigs (weight range, 350–450 g) were sensitized to ovalbumin.
by i.p. injection of a mixture containing 10 µg ovalbumin and 0.1 g aluminium hydroxide suspended in 0.5 ml saline. Two to 3 weeks later, ovalbumin (0.5%) or PAF (10 µg) were applied topically in 25 µl eye drops. Ovalbumin eye drops were prepared as a 0.5% solution in phosphate buffered saline (PBS). Preliminary experiments (not reported here) had shown that this dose of ovalbumin produced a useful response in terms of clinical signs and microvascular permeability.

PAF (C18) was dissolved in water and stored at 4°C according to the manufacturer’s instructions. Just prior to the performance of the experiment, the stock solution was further diluted in PBS, using polypropylene tubes and pipette tips.

BN 52021 (1%) or levocabastine (0.05%) were dissolved in 0.3% hypromellose and applied at random to one eye 1 h before challenge with PAF or antigen. The contralateral eye served as a control and received the solvent.

Clinical score (CS) was determined and lavage fluid (LF) was collected at 0.5, 1, 2 and 4 h. LF was sampled by washing the eye with PBS (25 µl) using a polypropylene micropipette, avoiding touching the eye. After three forced blinks the LF was collected and stored at -20°C until used.

Ovalbumin was purchased from Sigma (St Louis, MO), PAF from Cayman (Ann Arbor, MI), guinea-pig albumin anti-serum and guinea-pig albumin from Nordic (Tilburg, the Netherlands). BN 52021 was donated by the institute Henri Beaufour (Le Plessis Robinson, France) and levocabastine hydrochloride was a gift from Janssen pharmaceutics (Beerse, Belgium). Animals were housed and cared in accordance with the guidelines of the ARVO statement for the use of animals in ophthalmic and vision research.

**Clinical score:** For estimation of the inflammatory signs, the total impression of hyperaemia, oedema and swelling was combined in a clinical score (CS) and expressed by visual analogic scales 0–100% by two independent observers.

**Albumin assay:** Total albumin concentration in LF was determined using radial immunodiffusion. Samples were tested in an appropriate dilution. Agar plates (1.5%) containing a 1/100 dilution of guinea-pig albumin anti-serum were used for this purpose. Various concentrations of guinea-pig albumin were used as a standard.

**Statistical evaluation:** Student’s t-test was used for statistical calculations. A p value < 0.01 was taken as significant.

**Results**

**Effects of PAF:** Topical application of PAF (10 µg) resulted, within 5 min, in an inflammatory reaction consisting of hyperaemia, conjunctival oedema and periorbital swelling which disappeared within 4 h. The maximum CS was observed after 30 min.

PAF provocation produced a dose related increase of the albumin concentration in LF (Fig. 1). The minimal dose to provoke a leakage of albumin from the conjunctiva vessels was 3 µg/ eye. Maximum levels of albumin in LF were found after 30 min, showing a decline to zero 4 h after challenge (Fig. 2). The increase of the vascular permeability with time showed a straight correlation with the development of clinical signs and symptoms.

Pretreatment with BN 52021 showed a significant effect on both the CS (Fig. 3) and the albumin concentration in LF (Fig. 4). The mean albumin content in LF after BN 52021 treatment was inhibited (by 60.2%) in comparison with control eyes.

Levocabastine showed no effect on the CS and failed to reduce the conjunctival microvascular permeability after PAF administration.

**Effects of ovalbumin:** Ovalbumin administration produced a dose-dependent increase in vascular permeability in sensitized animals (data not shown). As a useful concentration for our experiments, a 0.5% solution of ovalbumin was used (dose per eye 125 µg). The inflammatory response to ovalbumin showed a similar clinical reaction in comparison with PAF. After 24 h the
The role of PAF in allergic conjunctivitis

FIG. 2. Time course for albumin recovery in LF in response to PAF (10 μg/eye) and ovalbumin (250 μg/eye) after 30 min (n = 8). PAF, (○); ovalbumin, (●).

FIG. 3. Effect of levocabastine 0.05% (Levo) and BN 52021 1% (BN) on the clinical score in response to PAF provocation (10 μg/eye) after 30 min (n = 8).
*Significant difference from eyes receiving only the solvent (control) p < 0.01.

Levocabastine as a pretreatment significantly reduced the mean of the clinical score (Fig. 5) and showed a significant inhibition (80.5%) of the vascular permeability (Fig. 6). BN 52021 alone showed a significant effect towards the inflammatory response after ovalbumin challenge, reducing the clinical score and inhibiting the vascular permeability (26.8%). The combination of levocabastine (0.05%) and BN 52021 (1%) in one solution showed an even greater inhibitory effect towards the clinical score and vascular permeability (Figs. 5 and 6) in comparison with levocabastine alone. The mixture showed a significant inhibition of the mean clinical score (73%) and the vascular permeability (79%), as compared with the effect of levocabastine alone.

Discussion

This study shows that PAF plays a role in the acute phase of allergic conjunctivitis. Topical application of PAF to the eyes of guinea-pigs resulted in an inflammatory response including hyperaemia, oedema and swelling of the con-
F. Meijer, J. L. van Delft and N. J. van Haeringen

However, a substantial part of the inflammatory response remains present using histamine antagonists as a pretreatment, reflecting the possible involvement of other mediators. Our study shows that pretreatment with BN 52021 alone and in combination with levocabastine before antigen challenge resulted in a significant inhibition of the clinical score and vascular permeability (Figs. 5 and 6). This suggests that the non-histaminergic component of the acute phase of allergic conjunctivitis is at least partly mediated by PAF. Other reports also showed a significant effect of different PAF antagonists on the vascular permeability in various types of inflammation.4,17

The mechanism of action of PAF is still not completely understood. PAF receptors situated and a combination of both (Levo + BN) on the albumin recovery in LF in response to ovalbumin challenge (125 μg/eye) after 30 min (n = 5–8).

*Significant difference from eyes receiving only the solvent (control) p < 0.01.

**Significant difference from eyes receiving levocabastine (Levo) p < 0.01.

junction. Moreover, PAF provocation showed a dose dependent increase in conjunctival vascular permeability. Our results coincide with the observations of others who applied PAF to the eyes of rats10 and guinea-pigs.11,12 The dose of PAF needed to produce an inflammatory response of the conjunctiva seems very high when compared with the dose of PAF, less than 0.1 μg, used in other models.13 In a rabbit model of ocular hypertension, a dose of 250 μg per eye in the presence of 0.25% HSA was topically administered without any systemic side effects.14 This may be explained by the unique characteristics of the eye towards topical administration of PAF. (Possibly PAF is rapidly converted to lysophosphatidylcholine or PAF does not easily penetrate the outer surface of the eye).

Because albumin levels in LF and the CS both reached a maximum after 30 min, we selected this as a time point to test the effectiveness of the specific PAF antagonist BN 52021 and the H1-antagonist levocabastine after provocation with antigen or PAF.

Pretreatment with levocabastine reduced the inflammatory response after topical administration to antigen to sensitized guinea-pigs for the major part (Figs. 5 and 6). Woodward et al.15 observed a similar effect using a combination of H1 and H2-antagonists in guinea-pigs, and in a human study levocabastine appeared to be very effective in relieving the clinical signs and symptoms when used by patients with allergic conjunctivitis.16 These observations indicate the dominant role of histamine in the acute phase of allergic conjunctivitis. However, a substantial part of the inflammatory response remains present using histamine antagonists as a pretreatment, reflecting the possible involvement of other mediators. Our study shows that pretreatment with BN 52021 alone and in combination with levocabastine before antigen challenge resulted in a significant inhibition of the clinical score and vascular permeability (Figs. 5 and 6). This suggests that the non-histaminergic component of the acute phase of allergic conjunctivitis is at least partly mediated by PAF. Other reports also showed a significant effect of different PAF antagonists on the vascular permeability in various types of inflammation.4,17

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**Significant difference from eyes receiving levocabastine (Levo) p < 0.01.

Because albumin in the conjunctival system the
The role of PAF in allergic conjunctivitis

clinical evaluation was as good as an experimental estimate of the mediator effect measured by extravasation of Evans blue. Because, in our model, clinical score and albumin leakage showed a clear correlation, the observed differences in albumin leakage are due to pharmacological effects and not to the collection method of the LF.

Although the major response in the immediate hypersensitivity reaction seems to be histamine mediated, the effectiveness of BN 52021 in inhibiting the inflammatory response after ovalbumin challenge demonstrates the important role of PAF in the acute phase of allergic conjunctivitis.

References


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