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Nitric oxide induces vascular permeability changes in the guinea pig conjunctiva

Frans Meijer [a,*], Jan M. Ruijter [b], Jan L. Van Delft [c], Nico J. Van Haeringen [a]

[a] *Biochemical Laboratory, The Netherlands Ophthalmic Research Institute, Amsterdam, Netherlands*
[b] *Department of Visual System Analysis, The Netherlands Ophthalmic Research Institute, Amsterdam, Netherlands*
[c] *Department of Ophthalmology, Academic Hospital Leiden, Leiden, Netherlands*

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Abstract

The role of nitric oxide (NO) as an inflammatory mediator in the mechanism of increased microvascular permeability was examined in a guinea pig model of allergic conjunctivitis. Topical challenge with antigen, compound 48/80, histamine or platelet activating factor (PAF) resulted in a marked increase of the conjunctival vascular permeability. Vascular permeability was determined by measuring the albumin content in the lavage fluid of the challenged eyes after 30 min. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) eyedrops caused a significant inhibition of the clinical score and the vascular permeability after challenge with either antigen, histamine or PAF. Aminoguanidine prophylaxis also resulted in a significant inhibition of both the clinical score and vascular permeability in response to all the used provocative agents except PAF. Our observations indicate that NO is an important factor in the induction of the vascular permeability provoked by histamine but seems to play no role in the mechanism by which PAF exerts increased vascular permeability in the conjunctiva.

Keywords: Nitric oxide (NO); Allergic conjunctivitis; Vascular permeability; N^G-Nitro-L-arginine methyl ester; Aminoguanidine; Histamine; PAF (platelet-activating factor)

1. Introduction

Increased vascular permeability and edema are prominent clinical findings in the immediate hypersensitivity response of allergic conjunctivitis. The pathogenesis of allergic conjunctivitis is complex but is characterized by the interaction of antigen, immunoglobulin E antibody and the mast cell resulting in the degranulation of the latter. Mediators such as histamine, prostaglandins and possibly platelet-activating factor (PAF) are released or produced by the mast cell (Abelson and Schaefer, 1993). These mediators have been shown to produce the clinical signs and symptoms of allergic conjunctivitis including increased vascular permeability and edema when applied topically.

Nitric oxide (NO) has been described as an important mediator in several types of inflammation including allergy (Lin et al., 1993), acute carrageenin induced inflammation (Ialenti et al., 1992), lipopolysaccharide induced shock (Kilbourn et al., 1990) and neurogenic inflammation (Lippe et al., 1993). The highly reactive and instable nitric oxide molecule is produced by NO synthase from L-arginine. Two subtypes of NO synthase are described, the constitutive, calcium dependent enzyme and the inducible, calcium independent enzyme. In the inflammatory process the inducible NO synthase starts to synthesize large amounts of NO when activated by cytokines.

NO exerts its activity by binding to iron containing enzymes thereby activating or inactivating the enzyme.

* Corresponding author. Biochemical Laboratory, The Netherlands Ophthalmic Research Institute, P.O. Box 12141, 1100 AC Amsterdam, Netherlands. Tel. (+31) 20-5664528, fax (+31) 20-6916521.

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In this way it activates guanylate cyclase which subsequently produces cyclic guanosine monophosphate (cGMP) (Moncada et al., 1991). By relaxing the vascular smooth muscle cells via cGMP, NO dilates bloodvessels and this may account for the increase in local blood flow in inflamed tissue.

N^G-Nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) which are competitive inhibitors of both sub-types of NO synthase, showed a significant inhibition of the vascular permeability (Ialenti et al., 1992; Hughes et al., 1990). Aminoguanidine, a selective inhibitor of inducible NO synthase, has also been described in several studies in relation to microvascular permeability changes (Tilton et al., 1994; Corbett et al., 1992).

The inflammatory response associated with allergy involves several mediators interacting with each other by stimulation or inhibition. Synergistic effects are described for NO and mediators such as bradykinin, histamine and PAF in guinea pig skin (Teixeira et al., 1993)

In this study we have investigated the role of NO as a mediator of increased vascular permeability and its effects in relation to other mediators in a guinea pig model of allergic conjunctivitis using N^G-nitro-L-arginine methyl ester (L-NAME) and aminoguanidine.

2. Materials and methods

2.1. Experimental protocol

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 saline (0.5 ml). Two to three weeks later ovalbumin solution, compound 48/80, histamine or PAF were applied topically in 20 μl eyedrops. Ovalbumin eye drops were prepared as a 0.1% solution in phosphate buffered saline. Just prior to the performance of the experiment histamine, compound 48/80 and PAF were also prepared in phosphate buffered saline. PAF (C-18) was dissolved in H_2O and stored at 4°C according the instructions of the manufacturer and used as a stock solution. Both eyes were challenged with the provocative agents mentioned above.

L-NAME and aminoguanidine were dissolved in a mixture (1 : 2) of phosphate buffered saline and hypromellose 0.3% (hydroxyproylmethylcellulose) and used as a pretreatment 30 and 60 min before provocation. Ketorolac was applied topically 1 h before challenge with antigen. Sodium nitroprusside and L-arginine were dissolved in phosphate buffered saline and administered 15 min before histamine challenge. All solutions were applied as 20 μl eyedrops. The contralateral eye served as a control receiving only the solvent.

Clinical score was determined and lavage fluid was collected 30 min after challenge. Lavage fluid was collected by washing the eye with phosphate buffered saline (20 μl) using a plastic micropipette, avoiding to touch the eye. After three forced blinks the lavage fluid was collected and stored at −20°C until use. Animals were not anesthetized during the performance of the experiments.

Animals were housed and cared for in accordance with the guidelines of the Association for Research in Vision and Ophthalmology for the use of animals in ophthalmic and vision research.

2.2. Reagents

Aminoguanidine, N^G-nitro-L-arginine methyl ester (L-NAME), ovalbumin, L-arginine, sodium nitroprusside, histamine and compound 48/80 were obtained from Sigma (St. Louis, MO, USA). PAF (C-18) was purchased from Cayman (Ann Arbor, MI, USA), anti-guinea pig albumin antiserum and guinea pig albumin were obtained from Nordic (Tilburg, Netherlands). Ketorolac (Acular), a non-steroidal inflammatory drug for ophthalmic use was a kind gift of Allergan (Nieuwegein, Netherlands).

2.3. Clinical score

The inflammatory signs were estimated by two independent observers weighing the total impression of hyperemia, edema and swelling. The clinical score was expressed by visual analogic scales 0–100%.

2.4. Measurement of the vascular permeability

Total albumin levels in lavage fluid were determined using radial immunodiffusion. Samples were tested in an appropriate dilution. Agar (1.5%) plates containing a 1/400 dilution of anti-guinea pig albumin antiserum were used for this purpose. Various concentrations of guinea pig albumin were used as a standard.

Table 1
Effect of L-NAME (200 μg/eye) on the clinical score (%) determined 30 min after drug provocation as compared to the control eye

Drugs	Dose/eye	Control	L-NAME	n
Ovalbumin	20 μg	79±6	43±5 [a]	10
Histamine	20 μg	91±6	37±6 [a]	10
PAF	10 μg	82±4	51±4 [a]	10

[a] Significantly different from control by Student's paired t-test, $P < 0.05$.

Table 2
Effect of L-NAME (200 μg/eye) on the albumin recovery in lavage fluid (μg/ml) determined 30 min after drug provocation as compared to the control eye

Drugs	Dose/eye	Control	L-NAME	n
Ovalbumin	20 μg	336.6±51.4	108 ±32.1 [a]	10
Histamine	20 μg	202.5±14.8	42.2±11.0 [a]	10
PAF	10 μg	171.6±15.6	55.4±15.4 [a]	10

[a] Significantly different from control by Student's paired t-test, $P < 0.05$.

2.5. Statistics

All results are expressed as means ± S.E.M. The results were analyzed by Student's paired t-test or by a repeated measures analysis of variance (ANOVA). A two-way ANOVA was used to compare experiments on different days between different groups of animals; this will be specified when appropriate. A P value < 0.05 was considered as significant.

3. Results

3.1. Effects of L-NAME and aminoguanidine on the clinical score

The clinical signs and symptoms (redness, edema, tearing and itching) appeared within 15 min after topical provocation with the used inflammatory agents. The maximal clinical score was observed after 30 min. After 24 h the clinical reaction had completely resolved. After challenge with the provocative agents, the clinical score showed some variation between the animals probably due to the state of sensitization and because of individual variation. After challenge with the provocative agent to both eyes we did not observe any differences in effect between the eyes within one animal. Pretreatment with L-NAME resulted in a significant inhibition of the clinical score after ovalbumin, histamine or PAF challenge (Table 1). Aminoguanidine treatment showed a significant inhibition of the clinical score after provocation with antigen, compound 48/80 and histamine (Table 3). No effect was observed on the clinical score after topical provocation with PAF.

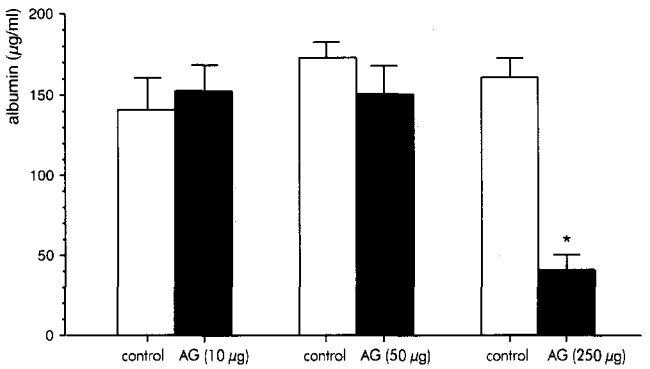

Fig. 1. Effect of aminoguanidine on albumin recovery (μg/ml) in lavage fluid after 30 min induced by topical provocation with histamine (20 μg) per eye, control (open columns) vs. aminoguanidine (filled columns) ($n = 4$). * Significant difference from control eyes by Student's paired t-test, $P < 0.05$.

3.2. Effect of L-NAME and aminoguanidine on vascular permeability

Pretreatment with L-NAME resulted in a significant inhibition of the vascular permeability in response to antigen, histamine or PAF provocation (Table 2). Aminoguanidine treatment showed a dose dependent inhibition of the vascular permeability caused by histamine (Fig. 1). A dose up to 250 μg aminoguanidine per eye had no effect on the vascular permeability. Aminoguanidine treatment showed a significant decrease of the vascular permeability after topical challenge with ovalbumin, compound 48/80 and histamine (Table 4). Maximum inhibitory effect of aminoguanidine was observed after histamine provocation. Aminoguanidine showed no effect on the increased vascular permeability when eyes were topically challenged with PAF. Also no effect was observed in experiments using aminoguanidine vs. the solvent as a pretreatment before PAF provocation in different groups of animals.

Table 3
Effect of aminoguanidine (200 μg/eye) on the clinical score (%) determined 30 min after drug provocation as compared to the control eye

Drugs	Dose/eye	Control	Aminoguanidine	n
Ovalbumin	20 μg	70±6	58±6 [a]	22
Compound 48/80	100 μg	43±6	27±4 [a]	12
Histamine	20 μg	86±4	49±5 [a]	46
PAF	10 μg	74±4	68±4	40

[a] Significantly different from control by a repeated measures ANOVA, $P < 0.05$.

Table 4
Effect of aminoguanidine (200 μg/eye) on the albumin recovery in lavage fluid (μg/ml) determined 30 min after drug provocation as compared to the control eye

Drugs	Dose/eye	Control	Aminoguanidine	n
Ovalbumin	20 μg	169.6±18.7	99.8±24.3 [a]	21
Compound 48/80	100 μg	81.8±13.9	38.8±11.4 [a]	10
Histamine	20 μg	134.0±21.5	29.6±10.4 [a]	40
PAF	10 μg	95.4±27.5	105.9±24.7	36

[a] Significantly different from control by a repeated measures ANOVA, $P < 0.05$.

3.3. Effect of L-arginine and sodium nitroprusside

Topical administration of sodium nitroprusside clearly showed vasodilatation of the limbal blood vessels of the conjunctiva. When eyes were challenged with histamine, sodium nitroprusside augmented the clinical score and the vascular permeability in comparison to control eyes. L-Arginine treatment also enhanced the clinical score and the vascular permeability of the eyes in response to histamine challenge (Fig. 2A and B).

3.4. Effect of ketorolac

Ketorolac pretreatment showed a significant effect on the clinical score after histamine provocation as compared to control eyes but did not have any effect towards the increase in vascular permeability. Addition of aminoguanidine to the ketorolac solution showed a marked effect on the clinical score and significantly inhibited the vascular permeability after histamine challenge (Fig. 3A and B).

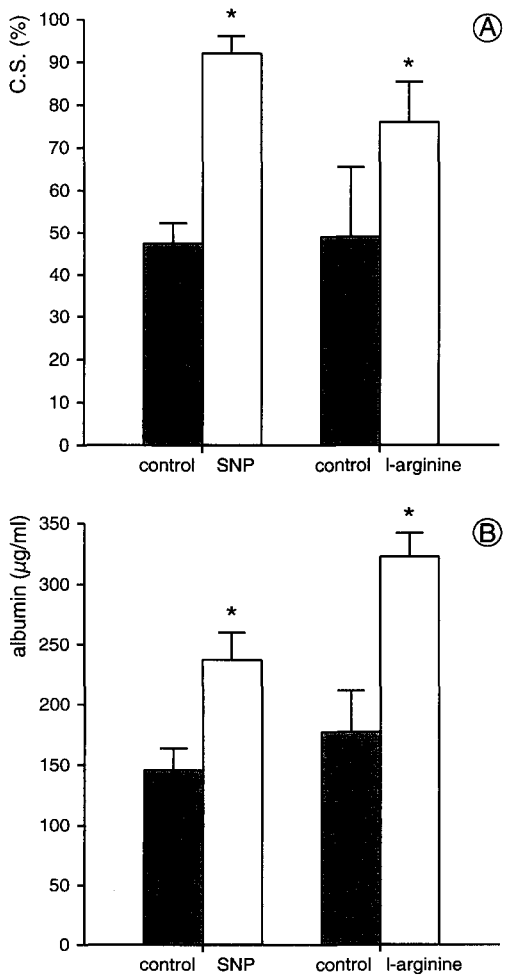

Fig. 2. Effect of pretreatment with sodium nitroprusside (200 μg/eye) or L-arginine (600 μg/eye) in response to histamine challenge (10 μg) on clinical score (A) and albumin recovery (μg/ml) in lavage fluid (B) after 30 min ($n = 12$). * Significant difference from control by Student's paired t-test, $P < 0.05$.

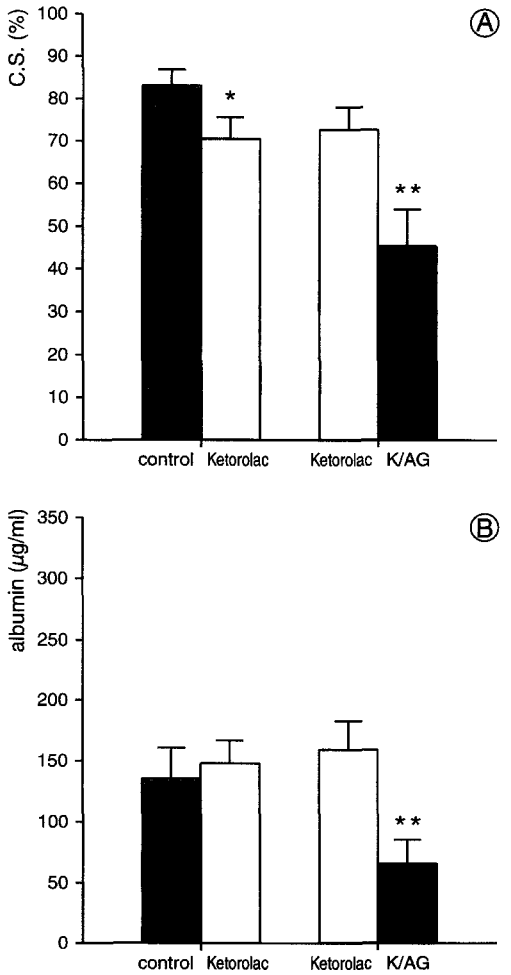

Fig. 3. Effect of pretreatment with ketorolac (100 μg/eye) and a mixture of aminoguanidine (200 μg/eye) and ketorolac (100 μg/eye) (K/AG) in response to histamine challenge (10 μg) on clinical score (A) and albumin recovery (μg/ml) in lavage fluid (B) after 30 min ($n = 12$). * Significant difference from control by a repeated measures ANOVA, $P < 0.05$. ** Significant difference from ketorolac by a repeated measures ANOVA, $P < 0.05$.

4. Discussion

In this study we conclude that NO induces increased conjunctival blood flow and vascular permeability and acts as a mediator in provoking vasodilatation and increased vascular permeability in a guinea pig model of allergic conjunctivitis.

Administration of antigen, compound 48/80 or histamine to the eyes of sensitized guinea pigs induces albumin leakage from the conjunctival blood vessels in

our study. After topical provocation with these agents other studies showed a similar effect towards the vascular permeability of the conjunctiva (Woodward et al., 1986; Gary et al., 1988). Our results resemble the observations of others, using different techniques in order to express increased vascular permeability of the conjunctiva after provocation with antigen and other mediators (Gary et al., 1988; Kamei et al., 1991). Because albumin is not produced by the lacrimal gland of the guinea pig (Thörig et al., 1985), the concentration of albumin on the ocular surface of the eye is a result of the flow of tissue fluids and other serum proteins across the epithelium to the ocular surface in response to several mediators. The lavage fluid collected from the surface of the eye at this stage represents a mixture of 'leaking' serum and reflex tearing from the lacrimal gland resulting in some variation between animals but not between the eyes of one animal. Stock et al. (1990) observed that in the conjunctival system the clinical evaluation was as good as an experimental estimate of the mediator effect measured by the extravasation of Evans blue. Because in our model the clinal 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 lavage fluid.

Treatment with L-NAME or aminoguanidine showed a significant inhibition of both the clinical score and the vascular permeability after provocation with antigen, compound 48/80 or histamine. Furthermore addition of sodium nitroprusside, a nitrovasodilatator known to release NO as its active moiety, increased the vascular permeability even further in comparison to control eyes after provocation with histamine. Topical administration of L-arginine also showed a significant potentiation on the effect of histamine on vascular permeability. This indicates that NO is an important factor in the process of increasing the vascular permeability after provocation with different inflammatory agents.

NO acts by diffusion into target cells where it can bind to iron containing enzymes, resulting in their activation or inhibition (Lowenstein et al., 1994). It has been reported that NO can directly activate cycloxygenase by forming a complex with the heme center of the enzyme leading to the production of prostaglandins (Salvamini et al., 1993, 1994). Prostaglandins most likely act as mediators in allergy. Increased levels of prostaglandins were found in lavage fluids of patients with asthma (Ferreri et al., 1988) and allergic rhinitis (Ramis et al., 1991). Furthermore, increased levels of PGD_2 were found in tears of allergic patients when topically challenged with antigen (Helleboid et al., 1991). Recently ketorolac eyedrops were used by patients suffering from acute allergic conjunctivitis. These eyedrops appeared to be significantly more effective than placebo in relieving erythema, itching and edema (Tinkelman et al., 1993). In our model ketorolac showed a significant inhibition of the clinical score after provocation with antigen, whereas no effect on the vascular permeability could be observed. This implies that in our model prostaglandins are not involved or play a minor role in the process of increasing vascular permeability of the conjunctiva. However, when aminoguanidine was co-administered as a pretreatment with ketorolac before challenging the eyes with histamine, a significant effect on the vascular permeability was observed. This indicates that NO is involved in microvascular permeability changes but that these changes are not mediated via secondary production of prostaglandins.

Histamine, released from the granules of the mast cell, seems to be the major mediator in allergic conjunctivitis (Allansmith and Ross, 1986) and increased levels of histamine have been detected in the tears of patients with allergic conjunctivitis (Proud et al., 1990). The increase in vascular permeability of the conjunctiva after antigen or compound 48/80 provocation seems to be a histamine dominated process as can be concluded from the superior effect of antihistaminics after provocation with these agents (Woodward et al., 1989; Abelson et al., 1994). In a recent study it was suggested that histamine increases vascular permeability via a NO dependent mechanism (Yuan et al., 1993). At the cellular level it was shown that histamine caused increased vascular permeability by the formation of venular leaky sites (Mayhan, 1994). In this model L-NMMA significantly inhibited the vascular permeability after histamine provocation, indicating that NO is involved in this process. It was hypothesized that histamine induced the endothelial cytoskeleton changes via the production of NO and subsequent synthesis of cGMP by guanylate cyclase. The effects of L-NAME and aminoguanidine on the vascular permeability of the conjunctiva after provocation or release of histamine could be the result of a decrease of venular leaky sites. On the other hand increased vascular permeability of the conjunctiva could be the result of relaxation of vascular smooth muscle, resulting in an increase of conjunctival blood flow.

Because PAF mimics the effects of antigen provocation including increased vascular permeability, it may play a role in the immediate hypersensitivity response of allergic conjunctivitis (Abelson and Schaefer, 1993; Stock et al., 1990). The mechanism of action of PAF is still not completely understood. It has been suggested that PAF acts via the secondary synthesis of lipoxygenase products while other studies claim a direct effect of PAF on target cells (Spencer, 1992). Interaction between NO formation and PAF synthesis has been described (Filep and Földes-Filep, 1993; Teixeira et al., 1993). Our experiments showed no inhibitory effect of aminoguanidine on the increase of vascular

permeability after provocation with PAF. This could imply that PAF does not increase vascular permeability via NO in the guinea pig conjunctiva. The inhibitory effect of aminoguanidine in response to antigen, compound 48/80 and histamine challenge could possibly reflect the involvement of the inducible NO synthase in the generation of NO in the acute phase of allergic conjunctivitis. Because we used sensitized animals in our study, the presence of inflammatory cells in the conjunctiva such as macrophages, leucocytes and mast cells could be the source of inducible NO synthase. A hypothesis about the involvement of the inducible NO synthase in asthmatic patients in relation to allergen challenge has recently been reviewed (Barnes and Liew, 1995). Another explanation for the effect of aminoguanidine in our model could be that NO is generated by the constitutive NO synthase of conjunctival vascular endothelial cells in response to antigen, compound 48/80 or histamine. Histamine, by stimulation of the constitutive enzyme, has been shown able to release NO from endothelial cells in vitro (Palmer et al., 1988). Because aminoguanide is not very selective towards the inducible NO synthase but also employs an effect towards the constitutive enzyme (Hasan et al., 1993; Misko et al., 1993) and regarding the 1% concentration we needed to observe an effect of aminoguanidine on the vascular permeability, aminoguanidine may inhibit both the constitutive and the inducible isoform of NO synthase. The inhibitory effect of L-NAME towards PAF provocation seems to reflect a stronger inhibition of the NO production in comparison to aminoguanidine. This seems to result in a decrease of the local conjunctival blood flow, which is comparable to the effect of topical applied vasoconstrictive agents to the conjunctiva (Abelson et al., 1990) thereby inhibiting the vascular permeability.

In conclusion, we suggest that NO plays an important role in vascular permeability changes in the guinea pig conjunctiva in response to different provocative agents. Therefore L-NAME or aminoguanidine may have a therapeutic potential for the treatment of allergic conjunctivitis.

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