EXTENDED REPORT

Clinical and biological efficacy of preservative-free NAAGA eye-drops versus levocabastine eye-drops in vernal keratoconjunctivitis patients

A Leonardi, D Bremond-Gignac, M Bortolotti, D Violato, P Pouliquen, L Delval, J M Grouin, I A Fregona

Br J Ophthalmol 2007;**91**:1662–1666. doi: 10.1136/bjo.2007.117515

See end of article for authors' affiliations

Correspondence to: A Leonardi, Clinica Oculistica, Department of Neuroscience, Ophthalmology Unit, Via Giustiniani 2, 35128 Padua, Italy; andrea.leonardi@unipd.it

Accepted 1 June 2007
Published Online First 21 June 2007

Aims: This comparative and randomised pilot study assessed the clinical and biological efficacy of Naaxia Sine® eye-drops versus levocabastine eye-drops in the treatment of vernal keratoconjunctivitis (VKC).
Methods: Twenty-three VKC patients were randomised and treated bilaterally for 28 days with *N*-acetyl-aspartyl-glutamate (NAAGA) or levocabastine (LEVO) eye-drops. The primary efficacy variable, overall evolution of eosinophil cationic protein (ECP) tear concentrations, was assessed in a masked fashion on D0, D7 and D28. Clinical symptoms and signs were reported at the same time points. Biological parameters were analysed with a non-parametric rank-based approach. Global tolerance was assessed by the investigator and patient.
Results: At all time points, ECP tear levels were significantly reduced in the NAAGA compared with the LEVO group ($p = 0.023$). Reduction of eosinophil leucocytes and tear lymphocytes was higher not significant in the NAAGA group. The same trend was observed for the evolution of total ocular symptom score. There were no significant differences between treatment groups in the occurrence of adverse effects, except for burning which was more frequent in the LEVO group ($p = 0.002$).
Conclusion: The anti-eosinophilic actions of NAAGA were shown by a significant reduction of ECP tear concentrations. A decreased lymphocyte count and an overall improvement of the symptomatology were also noted. Moreover, the tolerability of NAAGA appeared to be better.

Vernal keratoconjunctivitis (VKC) is a severe chronic ocular allergic disease, occurring throughout the year but increasing in intensity during spring and summer.[1 2] It is observed in children and young adults, generally up to 25 years old. Typical clinical symptoms are itching, tearing, foreign-body sensation and severe photophobia. Major clinical signs are conjunctival hyperaemia, papillary hypertrophy and limbal gelatinous infiltration. Two clinical forms have been observed: a tarsal form characterised by the presence of giant papillae on the upper tarsal conjunctiva, and a bulbar form characterised by the presence of limbal giant papillae.[3] A mixed vernal can also be encountered, combining the palpebral and limbal lesions.[4 5] The cornea is often damaged with a superficial keratopathy or the presence of corneal shield ulcers and neovascularisation. Cataract and steroid-induced glaucoma are the major ocular complications.[3]

Recent studies have suggested that a Th2-driven mechanism is part of the etiopathology.[3] VKC is not exclusively IgE-mediated: in addition to mast cells and eosinophils, a complex network of interleukins and cell mediators are involved.[3] Biopsies have shown a significant increase in inflammatory cells, including mast cells, basophils and eosinophils.[2] One of the principal eosinophil granule proteins is eosinophil cationic protein (ECP). It plays a major role in the pathogenesis of VKC,[6–11] having a toxic effect on human corneal epithelium[12 13] and potentially leading to vernal corneal shield ulcer. ECP levels in biological fluids correlate with the severity of some allergic diseases, and so measuring its tear levels has been considered as a marker for eosinophil activation.[1]

Treatment of VKC is challenging: antihistamines and mast-cell stabilisers are effective, but sometimes require association with corticosteroid eye-drops. Clinical studies have shown the efficacy and safety of *N*-acetyl-aspartyl-glutamate (NAAGA) eye-drops in vernal conjunctivitis.[14–16] Experimental studies demonstrated that NAAGA is a mast-cell stabiliser that has anti-inflammatory actions: in addition to inhibition of mast-cell degranulation, the effects of complement activation,[17] the production of inflammatory mediators (leucotrienes)[18–21] and the expression of eosinophil surface-adhesion molecules[19 22 23] are decreased.

Naaxia Sine® is an ophthalmic formulation of NAAGA in a multidose preservative-free phial (ABAK system®) of particular interest for VKC treatment. The objective of this pilot study was to evaluate its clinical and biological efficacy in the treatment of VKC patients compared with the activity of levocabastine, a reference H1 antihistamine, with particular focus on ECP tear concentrations.

METHODS

Patients

Patients at least 4 years of age had to present mild or moderate VKC with severity ranging from 3 to 4 (clinical score). A 5-day washout period was required for topical or systemic anti-allergic or other ophthalmic treatment, and a 10-day washout period for non-steroidal anti-inflammatory drugs and corticosteroids. Exclusion criteria were as follows: patients having received long-acting corticosteroids within the past 4 weeks, ocular laser treatment within the past 3 months, ocular surgery within the last year, ocular trauma or infection within the past 3 months, best corrected visual acuity <1/10, abnormality or clinically significant ocular disorder, relevant medical or surgical history. Signed written informed consent was obtained from each patient or legal tutor. The study was approved by an independent ethics committee and was conducted in compliance with ethical principles of the Declaration of Helsinki.

Abbreviations: ECP, eosinophil cationic protein; LEVO, levocabastine; NAAGA, *N*-acetyl-aspartyl-glutamate; VKV, vernal keratoconjunctivitis

Study design

This study was a randomised, pilot study with an open recording of the clinical signs, as treatment packaging was different (multidose ABAK phial vs classic phials). A masked fashion was ensured for ECP assays and thus for the assessment of the primary efficacy variable. At inclusion (D0), eligible patients underwent an ocular examination, including ocular symptoms, best corrected visual acuity and slit lamp examination with fluorescein test. All eligible patients were then randomly allocated to 1 of the 2 study medication groups: LEVO (Janssen) eye-drops 4 times/day or NAAGA (Laboratoires Théa, Clermont-Ferrand, France) 6 times per day. Patients were to be treated bilaterally for 28 days. For ethical reasons, fluorometholone eye-drops (rescue medication) could be concomitantly administered to patients requiring further relief of symptoms. Patients attended two follow-up visits: D7 and D28. At all time points, tears were collected for the measurement of eosinophil cationic protein (ECP; μg/l) and for tear cytology (number of cells/μL).

Tear sampling and analysis

Tears samples were collected using a capillary tube from the external canthus of each eye; samples of both eyes were pooled in a phial. After a 10-min centrifuge, supernatant was collected and frozen at −20°C. ECP tear levels were measured using RIA (Pharmacia Upjohn, Uppsala, Sweden); results are expressed in μg/l.

Tear cytology (number of eosinophils, neutrophils, lymphocytes and basophils) was performed on 1 μL using precoloured slides (Testsiplets, Boeringher), counting cells present in a 0.25 mm^2 area of the central field.

Efficacy

The primary efficacy variable was ECP tear concentration. Secondary efficacy variables were the number of eosinophils, neutrophils, lymphocytes and basophils in tears and change in global clinical score (ie, the sum of scores (0–38) for signs and symptoms). Grading for signs was as follows: redness/chemosis/discharge: 0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe; papillae: 1 = mild hyperaemic scattered papillae, 2 = moderate diffuse hyperaemic swollen papillae, 3 = as before but more severe, 4 = hyperaemic swollen giant papillae covering the superior tarsal plate; limbal infiltrates: 1 = mild limbus hyperaemia and swelling, 2 = moderate limbus hyperaemia and swelling, 3 = as before but more severe, 4 = 360° limbus hyperaemia and swelling; corneal epithelial disease: 1 = fine superficial epithelial defects involving less than half the cornea, 2 = diffuse fine superficial epithelial defects involving more than half the cornea, 3 = confluent epithelial defects of mucous plaque formation, 4 = oval corneal ulcers. Grading for the symptoms itching/tearing/photophobia/foreign body sensation was as follows: 0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe; and the use of rescue medication (fluorometholone eye-drops).

Safety

Safety and tolerance were assessed using symptoms (irritation, stinging, burning, itching, others), signs (others than those reported as efficacy variable), global local tolerance by the investigator and patient, best-corrected visual acuity, ocular and systemic adverse events.

Statistical analysis

Analyses were performed as an intention-to-treat set. For each patient, data for the right eye were taken into account. Missing data were not estimated and replaced.

Biological parameters

As the residuals obtained from the fitted ANCOVA random mixed models for analysing biological parameters over the 2 visits (day 7 and day 28) did not follow a normal distribution, a non-parametric rank-based approach was used. Distribution of biological parameters within each treatment group was summarised at each visit by their medians and interquartile ranges (1st quartile; 3rd quartile). Differences in changes from baseline to each visit between treatment groups were tested by the exact Wilcoxon test and estimated according to the Hodges–Lehmann semiparametric method. The overall change was analysed, considering the average of changes from baseline to the two visits and applying the same non-parametric approach.

Clinical parameters

A standard normal ANCOVA mixed model adjusting for patient random effect, baseline score as a covariate, visit and treatment effects and the interaction between treatment and visit effects was fitted to test the overall treatment effect and the treatment effect at each visit separately. The distributions of the total clinical and symptom scores were summarised by their means and standard deviations.

RESULTS

One patient per group discontinued the study after D7 for lack of efficacy, and 3 did not have data for ECP tear concentrations at D0 (1 in the NAAGA group and 2 in the LEVO group) due to technical reasons. No major protocol deviations were reported. Thus, the intention-to-treat population with tear sampling was identical to the per protocol population: 10 patients per group.

Demographic data

The median age was 9.0 years, and the proportion of males (70%) was higher than females (30%). At baseline, there were no differences in signs and symptoms between treatment groups, except for the total mean score of signs, which was significantly higher in the NAAGA group: 7.3 versus 6.3 ($p = 0.034$).

Efficacy variables

Baseline data

Clinical results confirmed that VKC involved both eyes equally, so it was considered valid to choose the right eye for analysis of clinical outcome.

Medians and quartiles for ECP, eosinophil leucocytes and lymphocytes are displayed in tables 1–3. The baseline distribution ranges of the two groups overlapped substantially, and so no statistical baseline difference was shown.

Evolution of biological variables

Compared with the LEVO group, the ECP tear levels were significantly reduced in the NAAGA group between D0 and D7 ($p = 0.05$), between D0 and D28 ($p = 0.046$), and over the entire study period ($p = 0.023$) (table 1). This finding was supported by a non-statistically significant trend in reduction of eosinophils in tears from D0 and D28 ($p = 0.064$) in the NAAGA group (table 2). A significant reduction of lymphocytes was also noted between D0 and D7 ($p = 0.048$) in the NAAGA compared with the LEVO group (table 3).

Regarding the change in total symptom score, a trend in favour of the NAAGA group was noted (table 4). This was confirmed by differences in the use of rescue medication. Although not statistically significant, less fluorometholone was concomitantly administered in the NAAGA group during the 3rd week of treatment: 10% of patients in the NAAGA group versus 36% of patients in the LEVO group.

Table 1 Change in tear eosinophil cationic protein (ECP) (μg/l)

ECP tear concentrations (μg/l)				
	NAAGA (n = 10*)		LEVO (n = 10*)	
Visits	Mean (SD)	Median (quartiles)	Mean (SD)	Median (quartiles)
D0	1152.6 (654.0)	1175.0 (700.0; 1600.0)	1120.8 (1868.1)	47.6 (8.6; 2310.0)
D7	692.6 (954.2)	383.6 (170.0; 765.0)	1381.1 (1864.5)	161.0 (35.8; 2465.0)
D28	329.9 (324.5)	230.0 (62.0; 590.0)	1468.8 (2171.3)	116.0 (20.6; 2655.0)
		Treatment effect† estimate; 95% CI		p value†
D7–D0		−806.5 (−1302.1; 4.4)		0.050
D28–D0		−750.5 (−1270.0; −14.3)		0.046
Both visits–D0		−911.7 (−1426.0; −269.9)		0.023

*Naaga group: day 0 n = 10, day 7 n = 9 and day 28 n = 8. Levo group: day 0 n = 10; day 7 n = 9 and day 28 n = 9; †Hodges–Lehmann semiparametric estimate and Wilcoxon test p value.

Table 2 Change in eosinophil tear number (n/μl)

Eosinophil tear no. (n/μL)				
	NAAGA (n = 10*)		LEVO (n = 10*)	
Visits	Mean (SD	Median (quartiles)	Mean (SD)	Median (quartiles)
D0	23.5 (15.6)	21.0 (15.0; 27.0)	16.3 (12.1)	11.0 (7.0; 22.5)
D7	15.0 (12.5)	10.0 (5.0; 28.0)	12.3 (8.1)	10.0 (7.5; 18.0)
D28	5.8 (4.4)	4.0 (3.0; 10.0)	11.7 (8.7)	7.0 (5.0; 23.0)
		Treatment effect†; 95% CI		p value†
D7–D0		−2.0 (−11.0; 7.0)		0.593
D28–D0		−11.0 (−23.0; 1.0)		0.064
Both visits–D0		−6.3 (−14.5; 5.0)		0.186

*Naaga group: day 0 n = 10, day 7 n = 9 and day 28 n = 8. Levo group: day 0 n = 10; day 7 n = 9 and day 28 n = 9; †Hodges–Lehmann semiparametric estimate and Wilcoxon test p value.

Table 3 Change in lymphocyte tear number (n/μL)

Lymphocyte tear no. (n/μL)				
	NAAGA (n = 10*)		LEVO (n = 10*)	
Visits	Mean (SD)	Median (quartiles)	Mean (SD)	Median (quartiles)
D0	6.6 (2.8)	7.0 (4.0; 8.0)	6.4 (5.1)	5.0 (3.0; 8.5)
D7	4.4 (5.5)	2.0 (1.0; 5.0)	7.3 (5.7)	7.0 (3.5; 8.5)
D28	4.0 (4.4)	3.0 (2.0; 5.0)	6.6 (5.5)	5.0 (1.0; 10.0)
		Treatment effect† estimate; 95% CI		p value†
D7–D0		−4.0 (−7.0; 0.0)		0.048
D28–D0		−2.0 (−7.0; 1.0)		0.114
Both visits–D0		−3.5 (−6.5; 0.0)		0.077

*Naaga group: day 0 n = 10, day 7 n = 9 and day 28 n = 8. Levo group: day 0 n = 10; day 7 n = 9 and day 28 n = 9; †Hodges–Lehmann semiparametric estimate and Wilcoxon test p value.

Safety

For safety, any patient who received at least 1 dose of the study medication was included, so all 23 patients were included: 11 in the NAAGA group and 12 in the LEVO group. No ocular adverse events were reported. Only 1 patient in the LEVO group reported a systemic adverse event of gastritis, considered by the investigator unrelated to the study drug.

There were no significant differences between treatment groups in the occurrence of symptoms, except for burning: 50% of patients in the LEVO group presented burning sensations at day 7 versus none in the NAAGA group ($p = 0.002$).

Neither treatment had any impact on corrected visual acuity. A good safety profile was confirmed by the appraisal of patients and investigators. A total of 90.0% of patients in the NAAGA group rated the eye-drops as satisfactory or very satisfactory versus 72.7% in the LEVO group.

DISCUSSION

VKC is a chronic inflammatory disorder in which eosinophils play a relevant role in the pathogenesis of local inflammation and damage. During the active phases of the disease, eosinophils comprise 20–50% of inflammatory cells present in the tear fluid. They release toxic factors upon activation, such as major basic protein and ECP.[7 8] ECP tear levels range from 7.5 to 33 μg/l in normal subjects[6 7 12] whereas higher levels have been detected in patients with active VKC.[7] It was therefore relevant to measure ECP tear levels as the primary

Table 4 Change in total symptom score

Total symptom score				
	NAAGA (n = 11*)		LEVO (n = 12*)	
Visits	Mean (SD)	Median (quartiles)	Mean (SD)	Median (quartiles)
D0	5.8 (0.9)	6.0 (5.0; 6.0)	5.8 (1.3)	5.5 (5.0; 7.0)
D7	3.5 (2.0)	3.0 (2.0; 4.0)	4.1 (1.9)	4.5 (3.0; 6.0)
D28	2.7 (1.3)	2.0 (2.0; 4.0)	3.7 (2.2)	3.0 (2.0; 6.0)
		Treatment effect†; 95% CI		p value†
At D7		−0.6 (−2.2; 1.0)		0.445
At D28		−1.0 (−2.5; 0.4)		0.160
At both visits		−0.8 (−2.1; 0.5)		0.213

*Naaga group: day 0 n = 11, day 7 n = 11 and day 28 n = 10. Levo group: day 0 n = 12; day 7 n = 12 and day 28 n = 11; †difference in least-squares means and Student t test p value.

efficacy parameter to evaluate the anti-eosinophilic effect of NAAGA.

As previously reported in the literature,[1 6 7 12] wide variations in ECP levels were found in baseline tears of VKC patients. These variations might reflect different stages of eosinophil activation, as ECP levels are known to correlate with the severity of the disease.

Non-steroidal alternative treatments, such as mast-cell stabilisers or antihistamines, are used for the management of vernal keratoconjunctivitis. Levocabastine is an H1-antihistamine used for treatment of all forms of ocular allergy, whereas NAAGA is a mast-cell stabiliser with anti-inflammatory actions. NAAGA is known to reduce allergic and inflammatory processes by various inhibitory activities shown in vitro. NAAGA stabilises mast-cell membranes by inhibiting the release of preformed mediators, such as histamine, the main inflammatory mediator in allergic disease.[18 24] It also has inhibitory activity against the lipoxygenase involved in leucotriene B4 (LTB4) synthesis.[18 20 21] LTB4 is a highly chemotactic agent causing chemosis, vasodilatation, leucocyte accumulation, an increase in leucocyte adherence to the vascular endothelium and aggregation of polymorphonuclear cells.[25] Moreover, NAAGA inhibits activation of the complement system by both the classical and alternative pathways.[17 19 24 26–28] Activated complement is a powerful cytolytic agent able to provoke per se the degranulation of mast cells, eosinophils, and alteration of the adjacent tissues where the proteolytic phenomenon is triggered. Lastly, NAAGA inhibits the expression of adhesion molecules on endothelial cells and leucocytes induced by histamine or by the activated fraction of complement.[19 29–31] Expression of adhesion molecules is the physiological basis of the diapedesis that sustains chronic inflammatory processes.

Our results showed additional mechanisms of action of NAAGA: an anti-eosinophilic activity combined with a lymphocyte lowering effect. When compared with levocabastine, NAAGA was statistically more effective in lowering ECP tear levels on both days 7 and 28 ($p = 0.023$) and in lowering the number of eosinophils in tears, indicating an inhibitory effect of NAAGA on eosinophil activation. Both drugs showed a progressive reduction in clinical symptoms. Although not statistically significantly different, NAAGA resulted in better relief of symptoms, as well as a better corticosteroid-sparing effect: only 10% of NAAGA-treated patients versus 36% of levocabastine-treated patients used fluorometholone eye-drops. These data suggest a better anti-allergic effect of NAAGA for the treatment of mild VKC compared with the H1 antihistamine, levocabastine.

In addition, a benefit is expected from the unique NAAGA formulation (ABAK®). This multidose delivery system, in which non-preserved solutions are entirely protected from microbial contamination by a membrane filter with very low porosity (0,2 µ),[32 33] provides a preservative-free formulation crucial for treatment of diseases such as VKC in which preservatives may be responsible for allergy and/or for exacerbation of inflammation.

In conclusion, this trial in VKC patients showed that preservative-free NAAGA exerts an in vivo anti-eosinophilic action in addition to its mast cell stabilising properties and that it is effective in reducing the symptoms of VKC and its associated conjunctival inflammation.

ACKNOWLEDGEMENTS

We would like to thank the investigators' staff who performed the study, and A Richetin for manuscript preparation. This clinical trial was sponsored by Laboratoires Théa, Clermont-Ferrand, France.

Authors' affiliations

A Leonardi, M Bortolotti, D Violato, I A Fregona, Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy
D Bremond-Gignac, Service d'Ophtalmologie, Hôpital Robert Debré, 48, Boulevard Sérurier, 75935 Paris Cedex, France
P Pouliquen, L Delval, Laboratoires Théa, 12, rue Louis-Blériot, Z.I. du Brézet, 63017 Clermont-Ferrand, France
J M Grouin, University of Rouen, 1 rue Thomas Becket, 76821 MONT-SAINT-AIGNAN cedex France

Competing interests: AL, DB-G, MB, DV, JMG and IF have no financial interest in Laboratoires Théa and Naaxia Sine®. PP and LD are employees of Laboratoires Théa.

URL and trial registration number: http://www.clinicaltrials.gov/NCT00357019

REFERENCES

1 **Leonardi A**, Borghesan F, Avarello A, *et al.* Effect of lodoxamide and disodium cromoglycate on tear eosinophil cationic protein in vernal keratoconjunctivitis. *Br J Ophthalmol* 1997;**81**:23–6.
2 **Tabbara KF**, al Kharashi SA. Efficacy of nedocromil 2% versus fluorometholone 0.1%: a randomised, double masked trial comparing the effects on severe vernal keratoconjunctivitis. *Br J Ophthalmol* 1999;**83**:180–4.
3 **Bonini S**, Coassin M, Aronni S, *et al.* Vernal keratoconjunctivitis. *Eye* 2004;**18**:345–51.
4 **Lee Y**, Raizman MB. Vernal conjunctivitis. *Immunol Allergy Clin North Am* 1997;**17**:33–51.
5 **Leonardi A**, Smith LM, Secchi AG. Vernal keratoconjunctivitis. In: Abelson MB, eds. *Allergic diseases of the eye.* Philadelphia: Saunders, 2000:179–96.
6 **Montan PG**, Hage-Hamsten M. Eosinophil cationic protein in tears in allergic conjunctivitis. *Br J Ophthalmol* 1996;**80**:556–60.
7 **Leonardi A**, Borghesan F, Faggian D, *et al.* Eosinophil cationic protein in tears of normal subjects and patients affected by vernal keratoconjunctivitis. *Allergy* 1995;**50**:610–3.
8 **Leonardi A**. Vernal keratoconjunctivitis: pathogenesis and treatment. *Prog Retin Eye Res* 2002;**21**:319–39.
9 **Trocme SD**, Aldave AJ. The eye and the eosinophil. *Surv Ophthalmol* 1994;**39**:241–52.

10 **Trocme SD**, Kephart GM, Allansmith MR, *et al.* Conjunctival deposition of eosinophil granule major basic protein in vernal keratoconjunctivitis and contact lens-associated giant papillary conjunctivitis. *Am J Ophthalmol* 1989;**108**:57–63.
11 **Trocme SD**, Gleich GJ, Kephart GM, *et al.* Eosinophil granule major basic protein inhibition of corneal epithelial wound healing. *Invest Ophthalmol Vis Sci* 1994;**35**:3051–6.
12 **Leonardi A**, Borghesan F, Faggian D, *et al.* Tear and serum soluble leukocyte activation markers in conjunctival allergic diseases. *Am J Ophthalmol* 2000;**129**:151–8.
13 **Trocme SD**, Kephart GM, Bourne WM, *et al.* Eosinophil granule major basic protein deposition in corneal ulcers associated with vernal keratoconjunctivitis. *Am J Ophthalmol* 1993;**115**:640–43.
14 **Djennas M**, Benia ML, Brahimi O. Le traitement de la conjonctivite printanière: étude clinique de l'acide *N*-acetyl-aspartyl-glutamique* en collyre. *Objectif Médical* 1986;**2**:8–9.
15 **van Bijsterveld OP**, Aalders-Deenstra V. Klinische Erprobung von *N*-acetyl-aspartyl-Glutaminsaure Augentropfen bei subakuter und chronischer atopischer Konjunktivitis. *Klin Monatsbl Augenheilkd* 1986;**188**:625–7.
16 **Vingtain P**. Traitement des limbo-conjonctivites endémiques des tropiques (L.C.E.T.): Etude clinique de l'acide *N*-acétyl-aspartyl-glutamique en collyre. *Objectif Médical* 1991;**85**:1–3.
17 **Etievant M**, Leluc B, David B. In vitro inhibition of the classical and alternate pathways of activation of human complement by *N*-acetyl aspartyl glutamic acid (NAAGA). *Agents Actions* 1988;**24**:137–44.
18 **Goldschmidt P**, Bazan HEP, Hurst J, *et al.* Effects of *N*-acetyl aspartyl glutamic acid (NAAGA) in rabbit cornea anterior segment inflammation. ARVO. Sarasota, USA, May 2–7, 1993. *Invest Ophthalmol Vis Sci* 1993;**34**:3475–85.
19 **Goldschmidt P**, Blondin C, Haeffner-Cavaillon N. Anti-inflammatory activity of the dipeptide Naaga assessed with human circulating eosinophils. ARVO. Fort Lauderdale, USA, May 11–16, 1997. *Invest Ophthalmol Vis Sci* 1997;**38**:5027-B534.
20 **Goldschmidt PL**, Vulliez-Le Normand B, *et al.* Effects of *N*-acetyl-aspartyl glutamic acid and sodium cromoglycate on leukotriene B4 secretion by human leukocytes. *Allergy* 1990;**45**:363–9.
21 **Jambou D**, Lapalus P. Effect of *N*-acetyl-aspartyl-glutamate (Naaga) on in-vitro leukotriene synthesis by macrophage cell line P388D1. *Int J Tissue React* 1990;**12**:273–80.
22 **Goldschmidt P**, Blondin C, Boulhal H, *et al.* Cell-adhesion molecules (CD11b) and Rantes-expression are inhibited in human eosinophils by the dipeptide NAAGA. First Combined International Symposium on Ocular Immunology and Inflammation. Amsterdam, The Netherlands, June 27–July 1, 1998. *Ocul Immunol Inflamm* 1998;**6**:S16.
23 **Goldschmidt P**, Boulhal H, Cavaillon N. Induction on human eosinophils (Eo) of transmembrane cell adhesion molecules (VLA-4/CD49) by histamine (H) and its inhibition by *N*-acetyl-aspartyl glutamate (Naaga). ARVO. Fort Lauderdale, USA, May 9–14, 1999. *Invest Ophthalmol Vis Sci* 1999;**40**:3608-B466.
24 **Goldschmidt P**, Luyckx J. Effects of lodoxamide (LOD), disodium cromoglycate (DSCG) and *N*-acetyl-aspartyl-glutamate sodium salt (NAAGA) on ocular active anaphylaxis. *Allerg Immunol (Paris)* 1996;**28**:124–6.
25 **Abelson MB**, Schwartz E, Chapin MJ. Leukotrienes. In: Abelson MB, eds. *Allergic diseases of the eye.* Philadelphia: Saunders, 2000:93–8.
26 **Elena PP**, Goldschmidt P. Comparative evaluation of preserved and non preserved NAAGA eye-drops and nedocromil eye-drops on ocular active anaphylaxis. Jermov 97. Montpellier, France, October 15–19, 1997. *Ophthalmic Res* 2006;**29**:20.
27 **Feuillard J**, Maillet F, Goldschmidt P, *et al.* Comparative study of in vitro inhibition of activation of the classical and alternative pathways of human complement by the magnesium and sodium salts of the anti-inflammatory peptide *N*-acetyl-aspartyl-glutamic acid (NAAGA). *Agents Actions* 1991;**32**:343–6.
28 **Goldschmidt P**, Couturier C, Herman D, *et al.* Effects of three antiallergic drugs on the complement-induced degranulation C5a of human basophils obtained from patients suffering from allergy to pollen or acarians. ARVO. Fort Lauderdale, USA, May 14–19, 1995. *Invest Ophthalmol Vis Sci* 1995;**36**:658-B566.
29 **Blondin C**, Cholley B, Haeffner-Cavaillon N, *et al.* Effets modulateurs de collyres anti-allergiques sur l'activation du complément induite in vitro par des polluants particulaires. *J Fr Ophtalmol* 2003;**26**:328–36.
30 **Bouhlal H**, Blondin C, Haeffner-Cavaillon N, *et al.* Effets inhibiteurs du dipeptide *N*-acetyl-aspartyl-glutamate (NAAGA) sur l'adhérence des leucocytes humains aux cellules endothéliales activées et l'expression des molécules d'adhésion CD11b, CD49d, ICAM-1, ICAM-2, VCAM-1 et ELAM-1. *J Fr Ophtalmol* 2002;**25**:993–1000.
31 **Yanez B**, Martin R, Merayo-Lloves J, *et al.* Antiallergic efficacy of the antiallergic eye drop Lodoxamide 0.1% and Naaga 4.9% on ragweed-induced allergic conjunctivitis in mice. ARVO. Fort Lauderdale, USA, May 9–14, 1999. *Invest Ophthalmol Vis Sci* 1999;**40**:S686.
32 **Osumi M**, Yamada N, Toya M. Bacterial retention mechanisms of membrane filters. *PDA J Pharm Sci Technol* 1996;**50**:30–4.
33 **Rigal D**. Epithélium et thérapeutique. Epithélium et médicaments. In: Rigal D, Assouline M, Colin J, *et al*, eds. *L'épithélium cornéen.* Paris: Masson, 1993;**XXVIII**:391–419.