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Effects of lodoxamide, disodium cromoglycate and fluorometholone on tear leukotriene levels in vernal keratoconjunctivitis

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Abstract

Purpose **We compared tear leukotriene B4 (LTB_4) and leukotriene C4 (LTC_4) levels of vernal keratoconjunctivitis (VKC) patients with those of age-matched controls and evaluated the effects of disodium cromoglycate (DCG) 2%, lodoxamide 0.1% and fluorometholone 0.1% on the tear LTB_4 and LTC_4 levels of the VKC patients.**

Methods **Thirty VKC patients were divided into three groups and their tear LTB_4 and LTC_4 levels measured with an enzyme-linked immunoassay technique before and after treatment with either lodoxamide 0.1%, DCG 2% or fluorometholone 0.1%. The results were compared with the tear LTB_4 and LTC_4 levels of 10 healthy control subjects. During this trial period, clinical scores for signs and symptoms of VKC were also evaluated.**

Results **In the VKC patients median tear LTB_4 and LTC_4 levels were 349.0 pg/ml (range 213.3–707.7 pg/ml) and 225.2 pg/ml (range 196.1–241.1 pg/ml) respectively – significantly higher than the control group ($p = 0.0065$ for LTB_4 and $p = 0.0003$ for LTC_4). After treatment, LTB_4 levels decreased significantly in all treatment groups when compared with baseline (for the lodoxamide group, $p = 0.01$; for the DCG group, $p = 0.008$; for the fluorometholone group, $p = 0.045$). LTC_4 levels were also significantly reduced after treatment in all three treatment groups (for the lodoxamide group, $p = 0.0209$; for the DCG group, $p = 0.0284$; for the fluorometholone group, $p = 0.0109$).**

Conclusions **Tear LTB_4 and LTC_4 levels are significantly higher in VKC patients than controls, which points to a possible role of lipoxygenase pathway products in the pathophysiology of ocular allergic disorders. Lodoxamide 0.1%, DCG 2% and fluorometholone 0.1% were all effective in reducing LTB_4 and LTC_4 levels in VKC.**

Key words Leukotriene B_4, Leukotriene C_4, Vernal keratoconjunctivitis, Lodoxamide, Disodium cromoglycate, Fluorometholone

Introduction

Vernal keratoconjunctivitis (VKC) is a chronic, recurrent ocular allergic disease that primarily affects children and adolescents.[1] It occurs more frequently in boys and is characterised by intermittent exacerbations that are often seasonal.[2,3]

Infiltration of the conjunctival epithelium and substantia propria by inflammatory cells, including mast cells, lymphocytes, eosinophils and basophils, is the main histological feature of VKC.[4] Although the cells associated with VKC have been identified by several studies,[5–7] much less is known about the mediators responsible for their recruitment.

Many mediators produced by the mast cells and neutrophils have been implicated in ocular allergic reactions. These mediators include histamine, eosinophil chemotactic factor, eosinophil granule major basic protein, platelet activating factor, neutral proteases, prostaglandins and leukotrienes.[8,9]

Leukotrienes are lipoxygenase pathway products of the arachidonic acid cascade. They are potent mediators of hypersensitivity and inflammatory reactions and are produced by mast cells, macrophages and polymorphonuclear leucocytes.[10] Their role in many allergic diseases is under investigation. Leukotriene B_4 (LTB_4) has a potent chemotactic and chemokinetic activity for eosinophils and polymorphonuclear leucocytes.[8] This activity may play an important role in the pathophysiology of VKC. Leukotriene C_4 (LTC_4) can induce smooth-muscle contraction and small vessel dilatation, and can increase secretion of glycoproteins from the epithelial glands.[11] Previous studies have shown release of LTC_4 into the tears after conjunctival antigen challenge.[12,13] Elevated tear LTC_4 levels were

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The authors do not have any financial interest in any of the drugs used in this study

also present in patients with contact-lens-induced giant papillary conjunctivitis.[14] As a result of the availability of commercial enzyme immunoassay (EIA) kits for the determination of leukotrienes in body fluids, it is now possible to determine the tear fluid leukotriene levels with a high degree of specificity and sensitivity.

Corticosteroids and mast cell inhibitors are commonly used drugs for the treatment of VKC. However, use of corticosteroids is complicated by many well-known undesirable effects including glaucoma, cataracts and inhibition of corneal wound healing.[8] On the other hand, disodium cromoglycate (DCG) and lodoxamide provide non-steroidal alternatives for the management of VKC. By inhibiting mast cell degranulation, these agents prevent the release of allergic mediators responsible for the immediate hypersensitivity response.[15]

The aims of this study were to compare tear LTB_4 and LTC_4 levels in VKC patients with those in age-matched controls and to evaluate the effects of two mast cell stabilisers (lodoxamide 0.1%; DCG 2%) and a corticosteroid (fluorometholone 0.1%) on tear LTB_4 and LTC_4 levels of VKC patients.

Materials and methods

Thirty patients with well-documented VKC (9 female, 21 male; mean age 14.07 years, SD 3.2 years) and 10 age-matched healthy control subjects (4 female, 6 male; mean age 15.90 years, SD 2.6 years) were included in the study.

At admission patients were assessed for eligibility on the basis of clinical signs, symptoms and a history of VKC. In addition, all the VKC patients had been free from medication for at least 4 weeks before trial entry and had moderately active disease without severe corneal ulcers at the time of recruitment. Patients with very severe corneal ulcers requiring combination treatment regimens were excluded. Other exclusion criteria included eye disorders other than VKC, especially other ocular allergic disorders such as allergic rhino-conjunctivitis and giant papillary conjunctivitis. Seasonal and perennial allergic rhino-conjunctivitis is differentiated from VKC by minimal corneal involvement, accompanying nasal symptoms, mild to moderate level of eye symptoms such as fewer, small papillae, and symptoms paralleling exposure to the allergens. In contrast VKC is characterised by extreme itching, pain, foreign body sensation, prominent mucus production, epithelial keratitis and corneal ulcers, and prominent (cobblestone) papillary reaction. Giant papillary conjunctivitis patients with a history of contact lens use, exposed sutures and ocular prostheses were also excluded.

Baseline tear fluid samples were collected from the selected VKC patients and control subjects. The VKC patients were divided in a double-masked, randomised fashion into three groups ($n = 10$ for each group). The first group was treated four times daily for 1 month with lodoxamide 0.1% ophthalmic solution (Alomide, Alcon), the second group was treated four times daily with DCG 2% ophthalmic solution (Opticrom 2%, Fisons) for 1 month and the third group was treated four times daily with fluorometholone 0.1% ophthalmic solution (FML, Allergan) for 1 month. On the last day of the treatment period a second tear fluid sample was collected.

A clinical score was determined on a 5-point scale (0-4, where 0 is 'absent', and 4 is 'very severe') at first visit and on the 10th, 20th and 30th days of the trial period for four major ocular signs (conjunctival erythema and chemosis, tearing and discharge, limbal hyperaemia and swelling, episcleral injection) and six major ocular symptoms of VKC (itching, tearing and discharge, photophobia, foreign body sensation, grittiness and swollen eyes). Composite scores for ocular signs and symptoms were obtained from the mean of scores for individual signs and symptoms.

Tear samples were collected from the lower lid marginal tears meniscus of left eyes using capillary tubes under slit lamp observation. Samples were kept at −20 °C until the time of EIA testing, as recommended by the manufacturer of the test kits.

An LTB_4 enzyme immunoassay kit (AMI code 6804 96) and an LTC_4 enzyme immunoassay kit (AMI catalogue no. 8-6805) from Advanced Magnetics (Cambridge, MA) were used to determine LTB_4 and LTC_4 levels in tear fluids samples. The sensitivity of the test was 8.9 pg/ml for LTB_4 and 7.62 pg/ml for LTC_4. Results are reported as picograms of leukotriene per millilitre of tear sample.

The Kruskal–Wallis test, a non-parametric analysis of variance, was used for simultaneous comparison between groups and, where significant, pairwise comparisons were performed by two-tailed Mann–Whitney *U*-tests. In addition, for comparison of the baseline tear leukotriene levels and tear leukotriene levels after treatment, Wilcoxon matched pairs signed-rank tests were used.

The study was carried out in accordance with the principles of the revised Declaration of Helsinki (Venice, 1993) and all patients or parents gave their written informed consent.

Table 1. *Baseline levels of leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) in tears of vernal keratoconjunctivitis (VKC) patients and the control group*

	VKC patients ($n = 30$)	Control group ($n = 10$)	p value[a]
LTB_4 (pg/ml)	349.0 (213.3–707.7)	229.9 (155.0–438.6)	$p = 0.0065$
LTC_4 (pg/ml)	225.2 (196.1–241.1)	198.7 (164.1–220.1)	$p = 0.0003$

Results are expressed as median (range).
[a]Statistical significance was $p < 0.05$.

Table 2. *Median tear leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) levels of vernal keratoconjunctivitis patients in the three treatment groups before and after treatment, and the median percentage change in LTB_4 and LTC_4 from baseline*

	LTB_4 (pg/ml)				LTC_4 (pg/ml)			
Treatment	Baseline	After treatment	Percentage change	p value[a]	Baseline	After treatment	Percentage change	p value[a]
Lodoxamide	342.4 (214.7–665.3)	218.3 (101.6–353.8)	−21	$p = 0.01$	224.9 (206.5–243.9)	217.3 (197.5–229.4)	−3	$p = 0.0209$
DCG	338.9 (213.3–569.9)	225.4 (173.3–271.2)	−29	$p = 0.008$	224.1 (196.1–240.2)	212.8 (187.5–239.7)	−3	$p = 0.0284$
Fluorometholone	397.4 (229.6–707.7)	247.9 (220.4–514.8)	−19	$p = 0.045$	225.7 (204.8–271.1)	217.6 (198.1–241.1)	−6	$p = 0.0109$

Results are expressed as median (range).
DCG, disodium cromoglycate.
[a]Statistical significance was $p < 0.05$.

Results

Data from 30 VKC patients and 10 controls were included in the statistical analysis.

The median baseline tear LTB_4 and LTC_4 levels measured in VKC patients and the control group are summarised in Table 1. In VKC patients the baseline levels of LTB_4 and LTC_4 were significantly higher than the control group ($p = 0.0065$ for LTB_4; $p = 0.0003$ for LTC_4).

In the second part of the study, Kruskal–Wallis tests were used to compare the baseline median tear LTB_4 and LTC_4 levels among the three treatment groups. The results were not significantly different ($p = 0.5263$ for LTB_4; $p = 0.3213$ for LTC_4).

The median tear LTB_4 levels after treatment are summarised in Table 2. When compared with the median baseline LTB_4 levels, median tear LTB_4 levels after treatment were significantly lower in all three treatment groups ($p = 0.01$ for the lodoxamide group; $p = 0.008$ for the DCG group; $p = 0.045$ for the fluorometholone group). The median tear LTB_4 level changes are compared in Fig. 1.

The median tear LTC_4 levels after treatment are presented in Table 2, and these levels were again significantly lower than the baseline LTC_4 levels in all three treatment groups ($p = 0.0209$ for the lodoxamide group; $p = 0.0284$ for the DCG group; $p = 0.0109$ for the fluorometholone group). The median tear LTC_4 level changes are presented in Fig. 2.

In all three treatment groups the median LTB_4 and LTC_4 levels after treatment were compared with those in the control group (Table 3). In the lodoxamide and DCG groups the median LTB_4 levels after treatment were lower than in the control group. In the fluorometholone group the LTB_4 levels after treatment were higher than in the control group but the difference was not statistically significant ($p = 0.073$). LTC_4 levels after treatment, although significantly lower than the baseline LTC_4 levels, were still significantly higher in all the treatment groups than in the control group ($p = 0.0089$ for the lodoxamide group; $p = 0.0499$ for the DCG group; $p = 0.0089$ for the fluorometholone group).

To compare the effectiveness of the three drugs, the median percentage reduction values presented in Table 2 were analysed using the Kruskal–Wallis test. There was no significant difference among the three treatment groups ($p = 0.811$ for LTB_4, $p = 0.1323$ for LTC_4).

Clinical signs and symptoms showed improvement with all three treatments after the 30 day trial period, and there were no significant differences in final median composite clinical scores among the three treatments ($p = 0.32$ for signs and $p = 0.09$ for symptoms). For the median composite sign and symptom scores, the reduction became significant after 10 days of treatment with lodoxamide and fluorometholone but not until the 20th day with DCG. Median scores for signs and symptoms are summarised in Figs. 3 and 4.

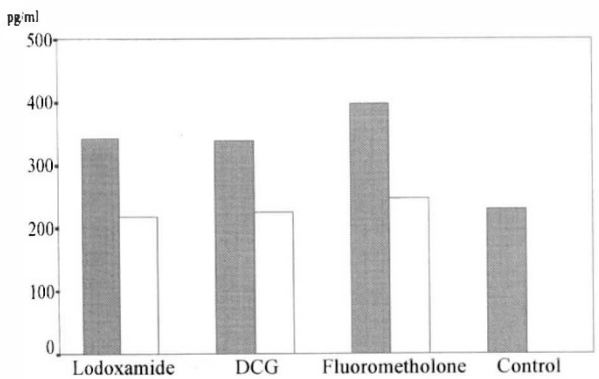

Fig. 1. *Median tear leukotriene B_4 (LTB_4) levels of the control group and vernal keratoconjunctivitis patients in the three treatment groups at baseline (grey bars) and after treatment (open bars).*

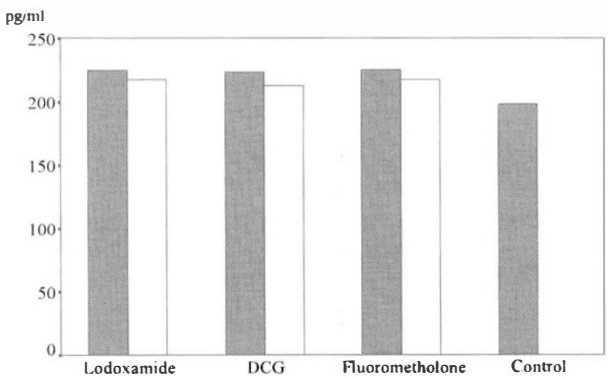

Fig. 2. *Median tear leukotriene C_4 (LTC_4) levels of the control group and vernal keratoconjunctivitis patients in the three treatment groups at baseline (grey bars) and after treatment (open bars).*

Table 3. *Post-treatment median tear leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) levels of vernal keratoconjunctivitis patients in the three treatment groups compared with the controls*

	LTB_4 (pg/ml)			LTC_4 (pg/ml)		
Treatment	After treatment	Control group	p value[a]	After treatment	Control group	p value[a]
Lodoxamide	218.3 (101.6–353.8)	229.9 (155.0–438.6)	$p = 0.744$	217.3 (197.5–229.4)	198.7 (164.1–220.1)	$p = 0.0089$
DCG	225.4 (173.3–271.2)	229.9 (155.0–438.6)	$p = 0.744$	212.8 (187.5–239.7)	198.7 (164.1–220.1)	$p = 0.0499$
Fluorometholone	247.9 (220.4–514.8)	229.9 (155.0–438.6)	$p = 0.0724$	217.6 (198.1–241.1)	198.7 (164.1–220.1)	$p = 0.0089$

Results are expressed as median (range).
DCG, disodium cromoglycate.
[a]Statistical significance was $p < 0.05$.

Discussion

This research was carried out in order to study the roles of LTB_4 and LTC_4 in the pathophysiology of VKC. At the same time we planned to compare the effects of two commonly used mast cell stabilisers and one corticosteroid preparation on the tear LTB_4 and LTC_4 levels of VKC patients.

When compared with the controls, tears of VKC patients had significantly higher levels of LTB_4 and LTC_4. Although the role of lipoxygenase products in ocular inflammation is not yet understood, high levels of LTB_4 and LTC_4 in the tears of VKC patients may indicate the role of lipoxygenase pathway products in the pathophysiology of VKC. LTB_4 has been identified as the lipoxygenase product responsible for the transient aggregation of human polymorphonuclear leucocytes induced by arachidonic acid *in vitro*.[8,16] In addition, LTB_4 exhibits both polymorphonuclear leucocyte and eosinophil chemokinesis and chemotaxis and these cells are characteristic of the inflammatory infiltrate of VKC.[8,11] Besides these activities LTB_4 enhanced adherence, augmented the expression of C3b receptors and initiated limited degranulation of polymorphonuclear leucocytes and eosinophils.[10] Nathan *et al.*[11] hypothesised that LTB_4 is a specific mediator in VKC. In VKC there is an increase in the number of mast cells together with an increased percentage of degranulated mast cells; also mast cells are found in the conjunctival epithelium, which is not seen in normal subjects.[17] It has been stated that in the absence of external arachidonic acid supplement, LTB_4 is generated predominantly by the mast cells and macrophages, which have the capacity to produce at least 10 times more LTB_4 than other inflammatory cells.[10] Furthermore, LTC_4 has a potent bronchoconstrictor activity and is a potent depressant of myocardial contraction force.[18,19] Although LTC_4 has not previously been shown in the tears of VKC patients, it has been identified in tears after conjunctival allergen challenge.[12] The ocular effects of LTC_4 are not fully understood. Topical application of LTC_4 to human eyes did not show any effect, despite the finding of high LTC_4 levels in many ocular inflammatory conditions.[8]

In this study, tear leukotriene levels in normals were measured only once. Since there can be day-by-day variations in tear leukotriene levels, more studies on these variations are needed, to allow more accurate conclusions regarding the role of leukotrienes in the pathophysiology of VKC.

In the second part of the study we showed that treatment with lodoxamide and DCG reduced median tear LTB_4 levels to a level even lower than that in the control group. Previous studies on lodoxamide have shown that the only effect of this drug is to stabilise the mast cell membrane[20] and, as the LTB_4 level after treatment was reduced below the control levels by lodoxamide (which inhibits only mast cells) we can hypothesise that the origin of the increased tear LTB_4 level in VKC was mainly due to mast cells. DCG, apart from mast cell stabilisation, may have a direct inhibitory action on leucocyte activation.[21] In accordance with this hypothesis, one can suggest that DCG and lodoxamide reduced LTB_4 levels below the control group levels by acting on small number of control mast cells present in

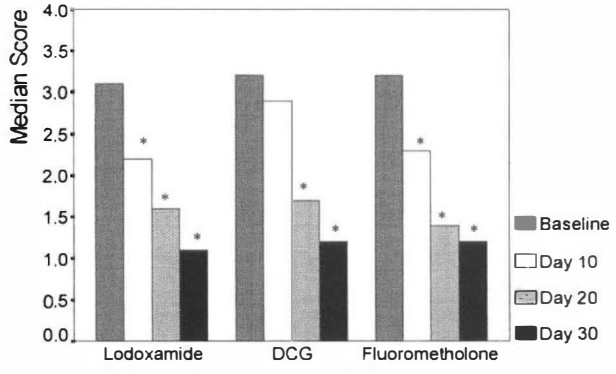

Fig. 3. *Median composite scores for ocular signs in each treatment group (*p < 0.05 when compared with the baseline).*

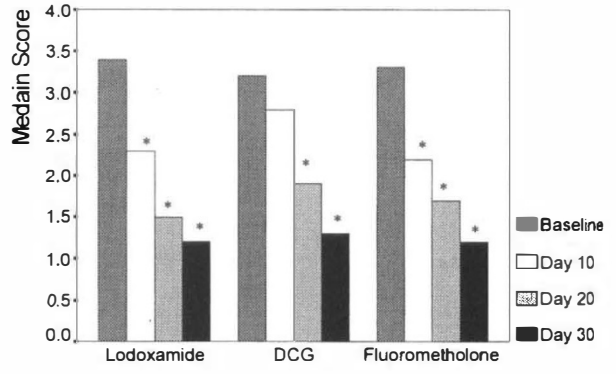

Fig. 4. *Median composite scores for ocular symptoms in each treatment group (*p < 0.05 when compared with the baseline).*

conjunctiva of vernal conjunctivitis patients. On the other hand, this small number of mast cells that are normally found in conjunctiva of control patients continues to produce LTB_4. Hence, higher levels of LTB_4 in the tears of the control group were found when compared with the LTB_4 levels after treatment in the DCG and lodoxamide groups.

Although all three drugs reduced tear LTC_4 levels, these levels after treatment were still higher than in the control group. This finding can be explained by the presence of separate mechanisms in activation and inhibition of production and secretion of different classes of leukotrienes. These mechanisms are currently unclear.

The median clinical scores for signs and symptoms improved during the trial period while the tear LTB_4 and LTC_4 levels decreased in all treatment groups. This improvement in parallel with the decrease in tear leukotriene levels is another indication for the role of these substances in pathophysiology of VKC.

As LTC_4 levels failed to decrease to control levels, we can conclude that over the period of treatment these three drugs could not completely block the pathological process. This may be one of the reasons why, in many clinical studies, treatments fail to resolve the signs and symptoms of VKC completely, although they reduce the signs and symptoms to some extent.[3,22,23]

In our study fluorometholone was selected because of its lower potential for increasing intraocular pressure. Comparing the effectiveness of these drugs, there were no significant differences between them regarding the reduction in LTB_4 and LTC_4 levels, and although lodoxamide and fluorometholone reduced the signs and symptoms scores a few days earlier than DCG there were no differences after the 20th day of treatment. We can therefore conclude that mast cell stabilisers are as effective as steroids, and are without the side-effects of the latter.

In conclusion, high levels of LTB_4 and LTC_4 in the tears of VKC patients may indicate the importance of lipoxygenase pathway products in the pathophysiology of allergic eye disorders, but more studies are required to reveal the complete pathophysiological mechanisms. Better understanding of the role of lipoxygenase pathway products may also help to find a better treatment for VKC, through newer drugs with specific lipoxygenase inhibitory activity or antagonistic activities against lipoxygenase pathway products. Until such time, however, mast cell stabilisers must remain the first choice in the treatment of VKC.

The authors wish to thank Dr Semseddin Ustacelebi for his help in laboratory work.

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