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The anti-allergic effects of a cromolyn sodium-chlorpheniramine combination compared to ketotifen in the conjunctival allergen challenge model

A. LEONARDI, F. BUSCA, M. TAVOLATO, A.G. SECCHI

Department of Neuroscience, Ophthalmology and Ocular Inflammation Unit, University of Padova - Italy

PURPOSE. *To compare the inhibitory effects of a topical combination product, cromolyn sodium (DSCG) 4% with the antihistamine, chlorpheniramine, with those of topical ketotifen 0.05% on the clinical allergic reaction induced by the conjunctival allergen challenge (CAC).*
METHODS. *Ten allergic but non-active patients were challenged in both eyes with increasing doses of specific allergen to obtain a positive bilateral reaction (visit 1). They were then rechallenged after 1 week to confirm the allergic threshold dose response (visit 2). After 2 weeks, a third CAC was performed bilaterally 30 minutes after topical application of DSCG-chlorpheniramine in one eye and ketotifen in the contralateral eye in a double-masked fashion (visit 3). Clinical signs and symptoms were registered 5, 10, 15, and 20 minutes after challenge using the standard scoring system. Tear cytology was performed 30 minutes after challenge.*
RESULTS. *Comparing the two drug effects at visit 3, DSCG-chlorpheniramine was shown to be superior to ketotifen at all time points for itching ($p<0.01$) and at 5 minutes for redness ($p<0.01$). For the total signs score, DSCG-chlorpheniramine was shown to be superior to ketotifen at all time points ($p<0.01$), and at 10 and 15 minutes for the total symptoms score ($p<0.05$). Compared to visit 2, DSCG-chlorpheniramine significantly lowered itching ($p<0.001$) and redness ($p<0.05$) at 5, 10, 15, and 20 minutes after challenge. Ketotifen significantly lowered itching at 5 and 10 minutes ($p<0.001$) and redness at 5, 10, and 15 minutes ($p<0.05$). Both drugs reduced the total number of cells evaluated by tear cytology during the early-phase reaction ($p<0.05$).*
CONCLUSIONS. *DSCG-chlorpheniramine was found to be more effective than ketotifen at preventing itching and redness in the CAC model. (Eur J Ophthalmol 2003; 13: 128-33)*

KEY WORDS. *Allergic conjunctivitis, Conjunctival allergen challenge, Chlorpheniramine, Sodium cromolyn, Ketotifen*

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INTRODUCTION

Itching, redness, and lid swelling are the typical inflammatory signs and symptoms of seasonal allergic conjunctivitis (SAC), and are the result of single or repeated natural challenges by environmental allergens (1). The reaction induced by specific conjunctival allergen challenge (CAC) accurately reproduces the signs and symptoms of an acute seasonal allergic reaction. In the CAC model, a single episode is induced in a

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standardized manner that allows for a homogeneous baseline for all patients in the study independent of the type of allergen sensitization (2). This model is a reliable method for quantifying and comparing the efficacy of various therapies in the prophylactic treatment of allergic conjunctivitis. As in SAC, the reaction in the CAC model is characterized by mast cell activation and the release of preformed and newly formed mediators such as histamine, tryptase, prostaglandins, leukotrienes, and cytokines (3, 4), as well as subsequent activation of vascular endothelial cells, expression of adhesion molecules (5), and inflammatory cell infiltration. In the acute allergic reaction, either induced by environmental allergen exposure or by the CAC, most of the signs and symptoms are related to histamine release from mast cells (6). In fact, histamine accounts for 98% of the material released by mast cell degranulation (7). Several topical mast cell stabilizers and antihistamines have been shown to significantly reduce the ocular allergic symptoms in both the SAC and CAC models (8, 9).

The combination product investigated, 4% cromolyn sodium–0.2% chlorpheniramine (DSCG-chlorpheniramine), is an anti-allergic ophthalmic solution that combines the mast cell stabilizing effect of cromolyn sodium (DSCG) with the antihistaminic effect of the H1-receptor antagonist chlorpheniramine. Mast cell stabilizers prevent calcium influx across cell membranes, thereby preventing mast cell degranulation and mediator release (10). Chlorpheniramine is a first-generation anti-H1 alkylamine compound with high H1 receptor affinity (11). Topical application of chlorpheniramine is rapidly effective in reducing itching without systemic side effects (12). Ketotifen fumarate 0.05% ophthalmic solution is a cyproheptadine derived anti-allergic drug that possesses both a mast cell stabilizing effect and H1-receptor antagonistic activity (13). The commercial combination of DSCG-chlorpheniramine has been widely used in Italy since 1985. The efficacy of this combination, however, has not been compared to that of one of the new anti-allergic products that combine in one drug the pharmacologic properties of two, such as ketotifen.

The current study was performed to determine the protective effect of two dual action anti-allergic topical preparations – DSCG-chlorpheniramine and ketotifen – on the onset of the allergic conjunctival reaction induced by the CAC model.

MATERIALS AND METHODS

Ten patients (age range 18–47 years; 6 men, 4 women) with a clinical history of SAC were included in this randomized, double-masked study. All patients were asymptomatic and had positive results on a prick test (wheal diameter >3 mm) as confirmation of their allergic medical history. The allergen that gave the greatest response by prick test and/or that was most clinically correlated with seasonal symptoms was chosen for challenge: seven patients were challenged with rye grass and three with *Parietaria officinalis*. The CAC was performed according to the standardized procedure described by Abelson et al (2).

At visit 1, demographic data, medical and medication history, and informed consent were obtained. Baseline slit-lamp examination and visual acuity (Snellen) were also recorded at each visit before challenge. The allergen threshold dose that induced a positive conjunctival reaction was determined by challenging both eyes with one 20-µL drop of allergen in serial dilutions (10–50–100–200–300 Allergen Unit RAST [AUR] /ml), increasing the dose every 15 minutes until a clinical reaction with a score of 2+ itching and redness was obtained. Seven days later (visit 2), a second challenge with the last threshold dose identified at visit 1 was repeated to confirm the conjunctival reaction. After 2 more weeks (visit 3: drug evaluation day), patients were administered a single dose of ketotifen 0.05% in one eye and DSCG-chlorpheniramine 4% in the contralateral eye in a double-masked fashion 30 minutes prior to CAC using the threshold dose.

Conjunctival signs (redness, chemosis, eyelid swelling) were assessed by the investigators and symptoms (itching, burning, foreign body sensation, tearing) by the patients using a score of 0 (none) to 4 (severe) for each eye before drug administration (time 0), immediately before allergen challenge, and 5, 10, 15, and 20 minutes after challenge.

Tear samples (2 µL) were collected from both eyes with a capillary tube 1 hour before CAC and within 30 minutes after CAC to determine inflammatory cell number. Tears were placed on pre-colored slides (Testsimplets, Roche, Germany) and the numbers of neutrophils, eosinophils, and lymphocytes were immediately counted in five consecutive microscopic fields at 250x magnification power.

The primary efficacy variables were itching and red-

ness. Secondary efficacy variables were the sum scores of signs and symptoms, and tear cytology. The mean change from baseline itching and redness between visit 3 (eyes pretreated with drug and then challenged) and visit 2 (the baseline challenge reaction) provided a measure of clinical efficacy of a drug treatment. Differences in clinical scores between the two active treatments were also determined. The nonparametric Wilcoxon signed-rank test was performed on itching, redness, and total sign and symptom scores at each time point, with significance set at $p<0.05$. Data are presented as mean and standard deviation (±SD). In addition, clinical significance was defined as at least a one-unit difference in the mean score of itching and redness from visit 3 to visit 2.

RESULTS

All the enrolled subjects completed the study and were evaluable for efficacy. No adverse events were reported in this study. There were no significant changes between visual acuity or baseline slit-lamp parameters between visits. The conjunctival signs and symptoms induced by challenge were not statistically different between contralateral eyes of the same subject, and were reproducible from visit 1 to visit 2.

For the efficacy variable itching (Fig. 1), DSCG-chlorpheniramine was statistically significantly superior to ketotifen at all time points (5, 10, 15, and 20 minutes) ($p<0.01$), whereas for redness, DSCG-chlorpheniramine was statistically significantly superior to ketotifen at 5 minutes after challenge ($p<0.01$) (Fig. 2). DSCG-chlorpheniramine eyes demonstrated statistically significantly lower mean itching ($p<0.01$) and redness scores ($p<0.05$) at all time points compared to non-treated eyes (visit 2, the baseline challenge reaction) (Figs. 1 and 2). Ketotifen showed statistically significantly lower mean scores at 5 and 10 minutes for itching ($p<0.01$), and at 10 and 15 minutes for redness ($p<0.05$) (Figs. 1 and 2).

Both DSCG-chlorpheniramine and ketotifen showed a clinically significant reduction in itching after challenge at 5 and 10 minutes (Tab. I), whereas only DSCG-chlorpheniramine showed a clinically significant reduction in conjunctival redness at 10 and 15 minutes (Tab. II).

The mean sum score of signs (Fig. 3) was significantly lower in DSCG-chlorpheniramine–treated eyes than ketotifen-treated eyes at all time points ($p<0.01$), whereas the mean sum score of symptoms (Fig. 4) was significantly lower in DSCG-chlorpheniramine versus ketotifen eyes at 10 and 15 minutes ($p<0.05$). DSCG-chlorpheniramine demonstrated significantly lower mean scores at all time points compared to visit 2, where-

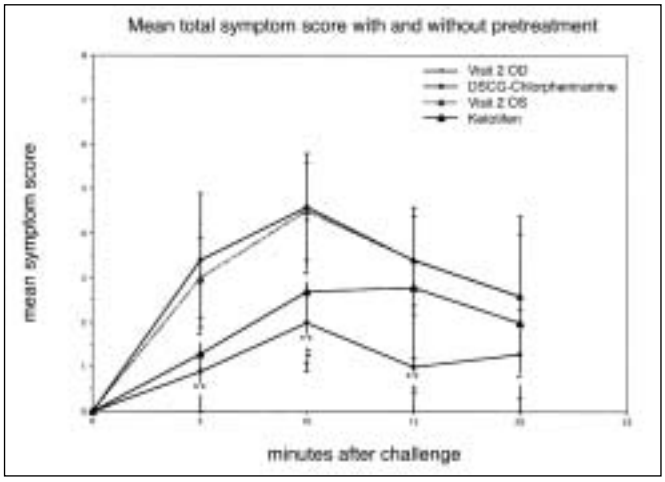

Fig. 1 - *Mean score of the primary variable, itching, after bilateral conjunctival allergen challenge at visit 2, and after pretreatment at visit 3 with either cromolyn sodium (DSCG)-chlorpheniramine in one eye or ketotifen in the contralateral eye. At visit 3, DSCG-chlorpheniramine was statistically superior to ketotifen at all time points (‡p<0.01). Both drugs significantly reduced itching compared to the respective reaction obtained at visit 2 (** p<0.01).*

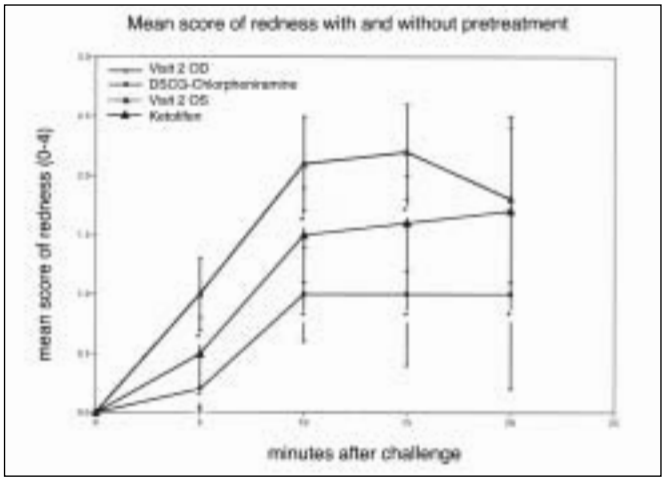

Fig. 2 - *Mean score of the primary variable, redness, after conjunctival allergen challenge. At visit 3, cromolyn sodium (DSCG)-chlorpheniramine was statistically superior to ketotifen at 5 minutes (‡p<0.01). Both drugs significantly reduced redness compared to the respective reaction obtained at visit 2 (*p<0.05).*

TABLE I - MEAN ± STANDARD DEVIATION (SD) OF ITCHING SCORES AT VISIT 2 (no pretreatment) AND VISIT 3 (pretreatment with drugs) AND MEAN DIFFERENCE (D) BETWEEN VISIT 2 AND VISIT 3

	DSCG + chlorpheniramine			Ketotifen		
Minutes after challenge	**Visit 2±SD**	**Visit 3±SD**	**Δ**	**Visit 2±SD**	**Visit 3±SD**	**Δ**
5	1.5±0.7	0.2±0.4	1.3±0.8*	1.5±0.7	0.3±0.4	1.2±0.6*
10	2±0	0.6±0.5	1.4±0.5*	2±0	0.8±0.6	1.2±0.6*
15	1.3±0.3	0.6±0.6	0.7±0.4	1.4±0.5	0.9±0.9	0.5±0.6
20	0.8±0.6	0.1±0.3	0.7±0.4	0.8±0.6	0.4±0.6	0.4±0.5

*Clinically significant (>1-unit difference in itching)

TABLE II - MEAN ± STANDARD DEVIATION (SD) OF REDNESS SCORES AT VISIT 2 (no pretreatment) AND VISIT 3 (pretreatment with drugs) AND MEAN DIFFERENCE (D) BETWEEN VISIT 2 AND VISIT 3

	DSCG + chlorpheniramine			Ketotifen		
Minutes after challenge	**Visit 2±SD**	**Visit 3±SD**	**Δ**	**Visit 2±SD**	**Visit 3±SD**	**Δ**
5	1±0	0.2±0.4	0.8±0.4	1±0	0.5±0.7	0.5±0.5
10	2.1±0.3	1±0.4	1.1±0.5*	2.1±0.3	1.5±0.6	0.6±0.5
15	2.2±0.4	1±0.6	1.2±0.6*	2.2±0.4	1.6±0.6	0.6±0.5
20	1.8±0.7	1±0.8	0.8±0.6	1.8±0.7	1.6±0.9	0.2±0.5

*Clinically significant (>1-unit difference in redness)

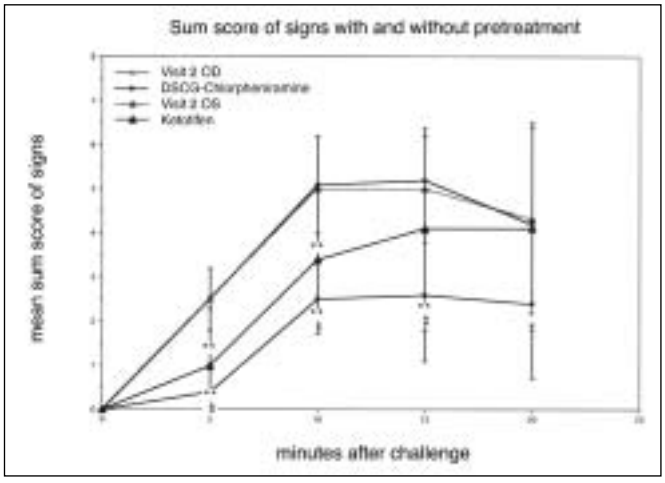

Fig. 3 - *Mean sum score of signs (redness, chemosis, eyelid swelling) after challenge in eyes pretreated with either cromolyn sodium (DSCG)-chlorpheniramine or ketotifen compared with the respective reaction at visit 2 (**p<0.01, *p<0.05). At visit 3, DSCG-chlorpheniramine was statistically superior to ketotifen at all time points (‡p<0.01).*

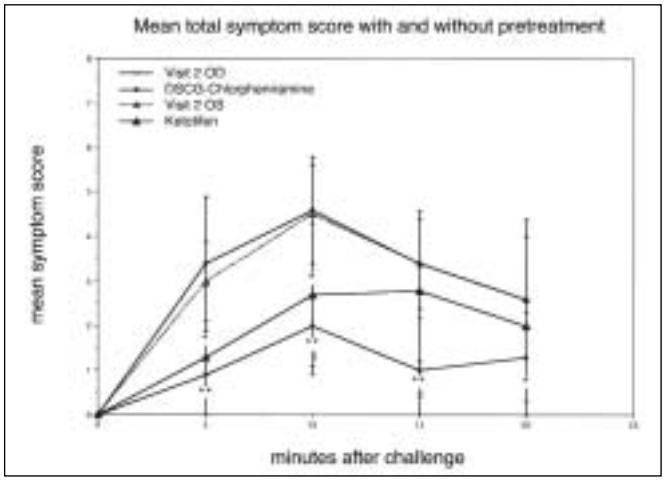

Fig. 4 - *Mean sum score of symptoms (itching, burning, foreign body sensation, tearing) after challenge in eyes pretreated with either cromolyn sodium (DSCG)-chlorpheniramine or ketotifen compared with the respective control reaction without pretreatment at visit 2 (**p<0.005, *p<0.05). At visit 3, DSCG-chlorpheniramine was statistically superior to ketotifen at 10 and 15 minutes (‡p<0.05).*

as these scores in ketotifen-treated eyes were significantly lower only at 5 and 10 minutes after challenge.

Tear cytology performed 30 minutes after CAC showed a statistically significant reduction in the total number of inflammatory cells in the eyes pretreated with each drug (visit 3) compared to visit 2 (DSCG-chlorpheniramine: 12.5±19 versus no pretreatment, 27±35, $p<0.05$; ketotifen: 11±22 versus no pretreatment, 28±30, $p<0.05$). The numbers of single cell populations of eosinophils, neutrophils, and lymphocytes were also reduced at visit 3 compared to visit 2; however, these differences were not statistically significant because of the high SD around the mean (data not shown).

DISCUSSION

The clinical hallmarks of allergic conjunctivitis are ocular itching and conjunctival redness. The CAC model allows for the precise evaluation of itching and redness in a controlled environment (14). In this study, DSCG-chlorpheniramine was statistically significantly superior to ketotifen in the prevention of itching and redness. Efficacy scores of both agents were clinically equivalent up to the first 10 minutes of the reaction. DSCG-chlorpheniramine appeared to be clinically more effective than ketotifen at 15 and 20 minutes after challenge. These results indicate that the antihistaminic effect of the two drugs immediately after challenge is of equal potency; however, DSCG-chlorpheniramine may have a longer duration of action.

In a previous CAC study, ketotifen was reported to be as effective as another topical antihistamine, emedastine, for inhibition of itching (15). The antihistaminic compounds chlorpheniramine and ketotifen have been proven to have an equal H1-receptor affinity in an *in vitro* receptor binding study (16). The present results suggest that the first-generation histamine-receptor antagonist chlorpheniramine is highly effective in reducing not only ocular itching but also redness in the allergic conjunctivitis model. However, it is also possible that the effect on redness was a result of mast cell stabilization provided by cromolyn. Sodium cromolyn has been proven to interfere with the influx of calcium and to inhibit mast cell degranulation and histamine release (10,17,18). Mast cell stabilizers may also act through other mechanisms. Cromolyn sodium is known to inhibit chemotaxis, activation, degranulation, and cytotoxicity of neutrophils, eosinophils, and monocytes (19, 20). Similar properties have also been shown for ketotifen (13, 21). DSCG-chlorpheniramine has been shown to prevent tear histamine release in a CAC model, with a 2-week pretreatment before challenge (22). Results of the present study suggest some effect on mast cell stabilization, even after only one drop. In fact, both drugs also reduced the total number of inflammatory cells 30 minutes after challenge, suggesting an inhibitory effect of other chemotactic mediators besides histamine.

In conclusion, one would expect the dual mast cell stabilization and antihistaminic properties of ketotifen to be superior in anti-allergic efficacy compared to an older combination product such as DSCG with the first-generation antihistamine chlorpheniramine. However, this was not the case. In the present model, DSCG-chlorpheniramine and ketotifen were both effective at preventing the onset of the clinical response, and reducing the cytologic reaction, but DSCG-chlorpheniramine was more effective in reducing itching and redness.

Reprint requests to:
Andrea Leonardi, MD
Department of Ophthalmology
University of Padova
Via Giustiniani 2
35128 Padova, Italy
andrea.leonardi@unipd.it

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