

Deflazacort protects against late-phase but not early-phase reactions induced by the allergen-specific conjunctival provocation test

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The protective effects of deflazacort against the inflammation that follows the conjunctival provocation test (CPT) by specific allergen were assessed in 24 patients with rhinoconjunctivitis caused by *Parietaria judaica* in a double-blind study. After a screening CPT, patients were randomized into four treatment groups, each being given deflazacort (oral tablets) at 6, 30, and 60 mg once daily, or matching placebo, for 3 d, outside the pollen season. Clinical evaluation (itching, hyperemia, lacrimation, and swelling of eyelids) and cytologic assessment (number of inflammatory cells in conjunctival scraping and evaluation of ICAM (intercellular adhesion molecule)-1/CD54 expression on epithelial cells) were performed at base line, 30 min (early-phase reaction (EPR), 6 h and 24 h (late-phase reaction (LPR)) after specific CPT, and before and after treatment. Neither the EPR clinical reactions nor the EPR total number of inflammatory cells was modified by deflazacort. However, the LPR clinical effects were significantly reduced by deflazacort at 30 or 60 mg/d ($P < 0.01$), as compared with placebo. The total number of inflammatory cells during LPR was significantly reduced by deflazacort at 30 or 60 mg/d ($P < 0.01$), as compared with placebo. Furthermore, CD54 expression was significantly reduced by deflazacort at 30 or 60 mg/d both in the EPR ($P < 0.01$) and LPR ($P < 0.01$), as compared with placebo. None of the studied indicators were modified at the 6 mg/d dose. This study shows that deflazacort has a highly protective action against clinical and cellular LPR effects induced by the specific CPT. In addition, deflazacort markedly reduces CD54 expression on the conjunctival epithelium during both EPR and LPR.

Clinical aspects

We have investigated the possible effects of deflazacort, a new heterocyclic derivative of prednisolone, on the changes occurring after conjunctival challenge; that is, the development of an early-phase reaction and the maintenance of the inflammatory process in the late-phase reaction. We evaluate the ocular response according to clinical and cytologic criteria. Particularly, several findings indicate that the adhesion machinery plays a central role in allergic inflammation and suggest that maneuvers designed to decrease the adhesion of inflammatory cells (such as lymphocytes, eosinophils, etc., expressing LFA-1) may be a useful therapeutic approach. As expected, deflazacort exerted a significant protective action against clinical and cellular LPR effects but also a mild effect on CD54 expression on the conjunctival epithelium in EPR. This finding is of interest and may reflect the immediate anti-inflammatory effect of deflazacort, an effect which could in turn interfere with interactions between surface molecules on the inflammatory cells and epithelium. Therefore, we may hypothesize that a surface molecule, i.e. CD54 on various epithelial cells, might be one of the primary targets, along with lymphocytes and monocytes, for the actions of glucocorticosteroids. Moreover, our results suggest that CD54 expression on epithelium could be considered to be another indicator for evaluating pharmacologic modulation of the allergic response.

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Several experiments have already used the conjunctival provocation test (CPT) with allergens as a fruitful *in vivo* model with which to assess pharmacologic modulation of allergic response: in fact, the CPT is safe, reliable, and reproducible (4, 9, 10, 11–13, 23, 36, 44). CPT evaluation may be performed by clinical examination, cytologic evaluation, and/or analysis of mediator release in the lachrymal fluid (6, 22, 42). Moreover, we have recently used a sensitive immunocytochemical APAAP (alkaline phosphatase-monoclonal antialkaline phosphatase) method to detect the possible expression of CD54 on the conjunctival epithelium in allergic subjects as an additional indicator of the allergic reaction (13).

The exposure of the conjunctival mucosa to specific allergen results in an immediate hypersensitivity reaction which occurs 20–30 min after challenge and quickly subsides. This early-phase reaction (EPR), like allergen provocation in the skin (22), lung (20, 33), and nose (38), is characterized by clinical symptoms and local infiltration of inflammatory cells, mainly neutrophils (6, 7). Bonini et al. described a second clinical reaction, termed the late-phase reaction (LPR), appearing 1 h later, peaking at 6–8 h after exposure, and slowly (24 h or more) subsiding. The LPR shows a predominance of eosinophils and the presence of lymphocytes (5–7). No quiescent (symptomless) pause exists at the conjunctival level; instead, a “continuous” reaction is observed. This evidence is probably due to the high allergen dose employed in the CPT, as compared with tests for other organs, such as the nose, bronchus, and skin (20, 31, 38). Both clinical evidence and cellular infiltration may vary according to the allergen dose employed. At high allergen doses a constant cellular late phase and a frequent clinical late phase predominate (6, 7, 31), whereas only a cellular infiltrate is observed at low allergen doses.

In the treatment of allergic diseases, the effectiveness and possible mechanism(s) of action of the drugs employed might be identified by monitoring several indicators after an allergen-specific CPT (9–12, 44).

Deflazacort, an oxazoline derivative of prednisolone, is a new, synthetic, calcium- and glucose-sparing, bone-saving corticosteroid agent (1). Its anti-inflammatory activity has been shown both in *in vitro* (8, 18) and *in vivo* models (1, 18). The present study was designed to investigate the possible effects of deflazacort on clinical and cytologic changes following specific CPT in sensitized subjects, since corticosteroids (topical and systemic) have already been shown to exert a significant protective effect on LPR events and a weak effect on EPR events in the skin and in the nose (41, 46, 50).

Patients and methods

Study design

The study was designed as a placebo-controlled, double-blind, randomized, four-parallel-groups trial.

Conjunctival challenge, clinical evaluation, and cytologic and immunocytochemical assessment were performed according to the methods of our previous reports (9–13). A preliminary CPT (“screening test”) was performed weekly, for 3 weeks, to screen patients positive for LPR with *Parietaria judaica* extract at different dilutions. *P. judaica*-sensitive patients with allergic rhinoconjunctivitis were studied outside the pollen season (December–February), during remission of symptoms.

P. judaica extract (kindly provided by Bayropharm-DHS, Milan, Italy), used for skin prick tests (43) and stored as a lyophilized powder at 4°C, was reconstituted in the diluent, saline human albumin (0.03%) (kindly provided by Bayropharm-DHS, Milan, Italy), within 24 h before the test. The concentration was expressed as AUR/ml (AUR (Activity Units by RAST) is the standardized Bayropharm-DHS unit), and seven dilutions were employed for the “screening” CPT: 20, 60, 200, 600, 1200, 2000, and 5000 AUR/ml. The same batch of allergen was used throughout the study. Saline albumin diluent was administered into the contralateral eye to exclude possible nonspecific reactivity.

An amount of 10 µl of diluted allergen was instilled into the lower conjunctival sac in the right eye. The initial allergen concentration was the one identified by the skin end-point test; the skin end-point test was performed according to the protocol established by the “Memorandum della diagnostica allergologica della Società Italiana di Allergologia e Immunologia Clinica” (47). During the following weeks, the next more concentrated allergen dilutions were used until a late clinical reaction was elicited (a total symptom score of more than 7 was considered to be positive for LPR). In the left eye 10 µl of diluent was instilled as control.

After preliminary screening, selected patients (24 out of 29 of those who showed LPR) were randomly assigned to four parallel treatment groups receiving an oral daily dose of deflazacort at 6, 30, and 60 mg/d, or placebo, in the morning for 3 d. Patients underwent CPT at the LPR provoking dose (PD), before and after treatment. The last medication dose was administered in the morning, 3 h before the CPT. The PD was defined as the allergen dilution eliciting a clinical LPR.

Clinical conjunctival evaluation and conjunctival scrapings were performed at base line, 30 min, 6 h, and 24 h after challenge. The clinical reaction to the allergen was evaluated by conjunctival hyperemia,

lacrimation, itching-burning, and swelling of eyelids; the score was assigned according to an arbitrary four-point rating scale, from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). A total symptom score was calculated as the sum of individual symptom scores, as previously described (9–11, 13).

Concomitantly, a conjunctival scraping for cytologic and immunocytochemical assessment was obtained from both eyes immediately before and 30 min, 6 h, and 24 h after CPT. After topical anesthesia (ossibuprocaine 4 mg/ml, one drop each eye), the upper tarsal conjunctiva was scraped with a sterile Kimura spatula. Specimens were spread on glass slides, air-dried, stained with May-Grunwald Giemsa dye, and read by microscope (Leitz Laborlux D microscope, $\times 250$ and $\times 500$ focus). The slides were separately examined by two investigators blind to the identity of the samples. The number of inflammatory cells, i.e. neutrophils, eosinophils, and lymphocytes, was counted separately and as a total number of cells in each microscopic field; the data are expressed as a mean of 10 fields (9–13).

An immunoenzymatic APAAP complex procedure modified from Cordell et al. was employed (17). Specimens were air-dried at room temperature for 30 min and incubated with appropriate dilution of purified CD54 mAb (1 mg/ml, 84H10, IgG1 – Immunotech, Marseille, France) (39) or anticytokeratin mAb (α CK19, DAKO, Milan, Italy). After washing in phosphate-buffered saline (PBS) pH = 7.6, samples were incubated with rabbit antimouse Ig, followed by the APAAP complex. The specimens were then incubated with a substrate solution containing basic new fuchsin, naphthol As-biphosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma, St Louis, MO, USA). In control samples, either mAb or antimouse Ig was omitted. As negative isotype-control for CD54 staining on epithelial cells, an anti-T-lymphocyte (CD3) mAb OKT3 IgG1 (Ortho Diagnostic, Raritan, NJ, USA) at 1:20 dilution of the stock solution (provided by the manufacturer) was used. The dilutions were established on the basis of previous titration experiments. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators blind to the identity of the samples (13).

CD54 expression on epithelial cells was graded according to a rating scale from 0 to 4, where 0 = no positive cells, 1 = mild positivity on 25% of epithelial cells, 2 = mild positivity on 75% of epithelial cells, 3 = intense positivity on 75% of epithelial cells, and 4 = very intense positivity on all epithelial cells, as previously described (13).

All possibly adverse effects spontaneously reported by the patients were carefully noted and evaluated.

Study subjects

Twenty-nine *P. judaica*-sensitive subjects suffering from seasonal allergic rhinoconjunctivitis (aged 18–63 years, 16 men and 13 women) were screened outside the pollen season. The patients had a history of pollen allergy for at least two previous seasons, positive skin prick test, and RAST for the specific pollen. They had no other ocular diseases and did not wear contact lenses. Patients with previously documented allergy to the studied drug, women of childbearing potential, and lactating women were excluded.

After screening, 24 patients (those who showed LPR), 13 men and 11 women, aged 18–61 years (mean age 30.3), were included in the trial and randomized to double-blind treatment (Table 1). No topical or systemic drugs were allowed for 1 month before and during the study period. All subjects gave informed consent, and the trial was approved by the department ethical committee.

Statistical analysis

Statistical analysis of the results was performed by the Wilcoxon signed rank test for paired data to test differences within groups, by the Mann-Whitney test for independent data to test differences between

Table 1. Allergen concentration employed before and after treatment in CPT: concentration is expressed as AUR/ml

	Patient	Allergen concentration
Deflazacort 6 mg/d	1	2000
	2	2000
	5	5000
	7	2000
	11	5000
	12	5000
Deflazacort 30 mg/d	14	5000
	15	2000
	17	2000
	20	2000
	23	5000
	24	5000
Deflazacort 60 mg/d	3	5000
	4	5000
	6	2000
	8	2000
	9	2000
	10	5000
Placebo	13	2000
	16	5000
	18	5000
	19	2000
	21	2000
	22	2000

groups, and by Student's *t* test for independent samples. A probability of 0.05 or less was considered to be statistically significant.

Results

Pretreatment CPT

Base line. All patients were symptom-free. A few inflammatory cells (mainly neutrophils, one or two in each microscope field) were detected in 11 out of 24 included subjects, and no CD54 expression on the conjunctival epithelium was shown in any patient. Therefore, at base line, the deflazacort groups and the placebo group did not show any difference in clinical, cellular, and immunocytochemical effects.

EPR. All subjects showed an immediate hypersensitivity conjunctival reaction within 5 min after allergen challenge, at concentrations ranging from 2000 to 5000 AUR/ml (Table 1). At 30 min the EPR was characterized by conjunctival hyperemia, lacrimation, burning and itching, and swelling of eyelids; the reaction was significant, as compared with base line and the contralateral eye given diluent ($P < 0.0005$) (Fig. 1A). Significant numbers of inflammatory cells (i.e. 11–20 neutrophils in each microscopic field, 2–4 eosinophils, and 2–5 lymphocytes) were detected in challenged eyes, as compared with control eyes and base-line conditions ($P < 0.001$) (Fig. 2A).

As far as the epithelium is concerned, firstly, a relevant number of epithelial cells was obtained by

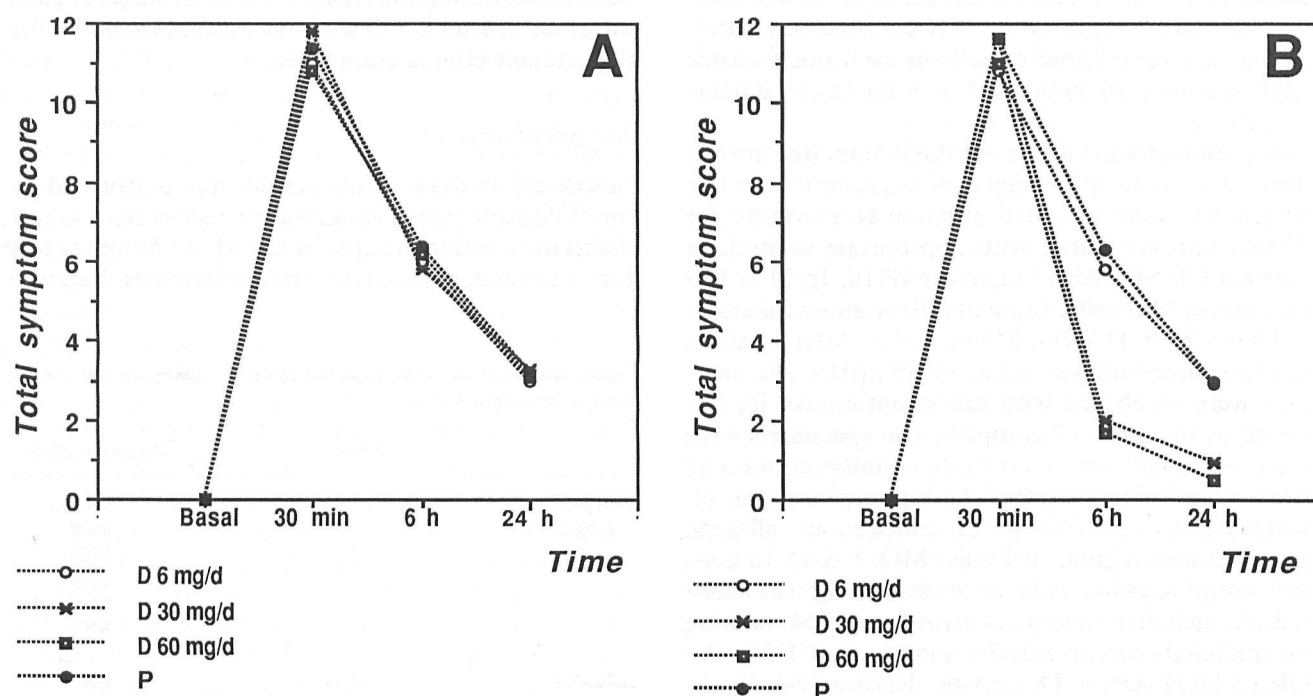


Fig. 1. Median values of total symptom scores before (A) and after (B) treatment (D = deflazacort, P = placebo), before and after CPT. Before treatment (A)

All subjects were symptom-free. Comparison between base line and EPR (30 min), and base line and LPR (6 h and 24 h) performed by Wilcoxon signed rank test:

30 min	$P < 0.0005$
6 h	$P < 0.001$
24 h	$P < 0.01$

After treatment (B)

1) Intragroup comparison (before and after treatment) performed by Wilcoxon signed rank test:

- D (30 and 60 mg/d)	30 min	NS
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d)	all times	NS
- P	all times	NS

2) Intergroup comparison performed by Mann-Whitney test for independent samples:

- D (30 and 60 mg/d) compared with P	30 min	NS
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d) compared with	all times	NS

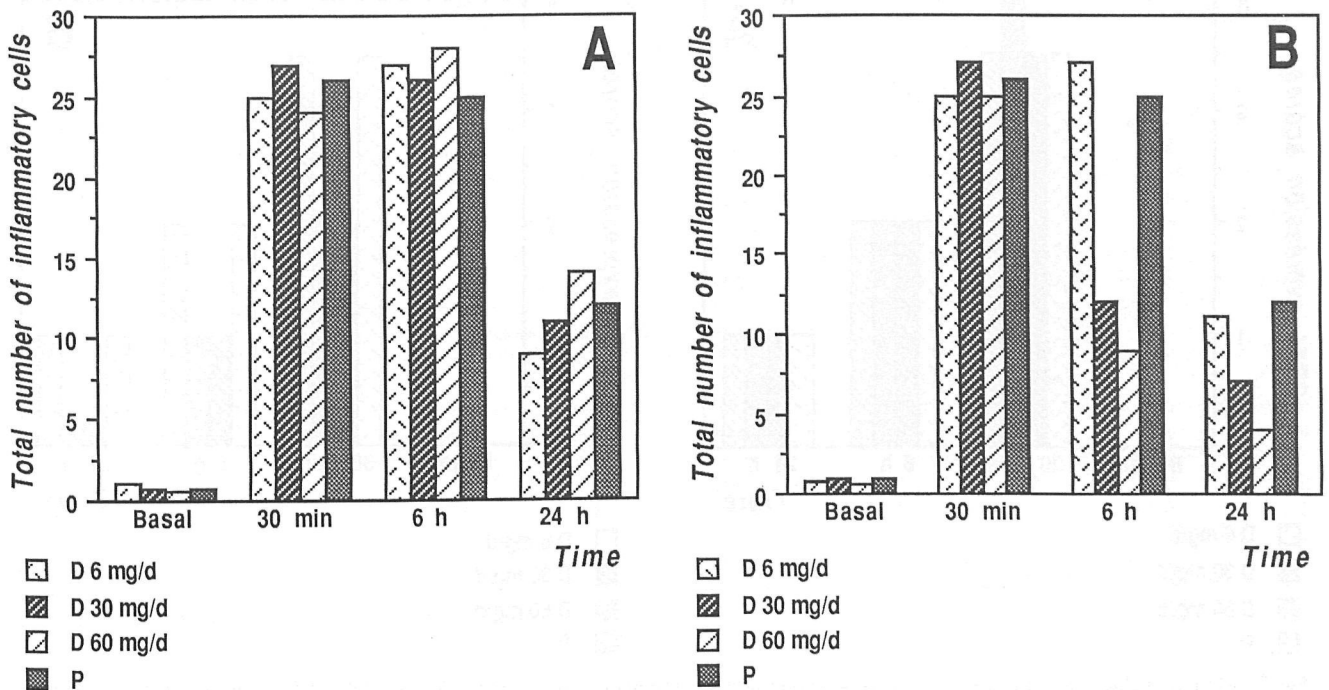


Fig. 2. Median values of total number of inflammatory cells before (A) and after (B) treatment (D = deflazacort, P = placebo), before and after CPT.

Before treatment* (A)

Comparison between base line and EPR (30 min), and base line LPR (6 h and 24 h) performed by Wilcoxon signed rank test:

30 min	$P < 0.001$
6 h	$P < 0.001$
24 h	$P < 0.01$

* Occasionally, a few subjects showed some inflammatory cells (1–2 each microscope field).

After treatment (B)

1) Intragroup comparison (before and after treatment) performed with Wilcoxon signed rank test:

- D (30 and 60 mg/d)	30 min	NS
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d)	all times	NS
- P	all times	NS

2) Intergroup comparison performed with Student's *t* test for independent samples:

- D (30 and 60 mg/d) compared with P	30 min	NS
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d) compared with	all times	NS

conjunctival scrapings (up to 15–30 cells in each microscopic field at $\times 250$). All exhibited positive staining with anticytokeratin mAb (α CK19, DAKO, Milan, Italy). Thirty minutes after challenge, all allergic subjects showed a remarkable CD54 expression, as compared with base line and the contralateral eye ($P < 0.0005$) (Fig. 3A).

LPR (6 h). Six hours after challenge, the intensity of the ocular reaction was decreased and was characterized mainly by a hyperemia and a swelling of eyelids that were significantly evident, as compared with the contralateral eye and base line ($P < 0.001$) (Fig. 1A). Together with neutrophils (7–12 cells each microscopic field), a significant infiltration of eosi-

nophils (5–9 cells) and lymphocytes (3–10 cells) was detected, as compared with base line ($P < 0.001$) (Fig. 2A). CD54 expression on epithelial cells was present; its intensity was significantly lower than during the 30-min observation ($P < 0.05$), but still significantly higher than base line ($P < 0.001$) (Fig. 3A).

LPR (24 h). Twenty-four hours after challenge, a swelling of eyelids and a mild hyperemia that were significant, as compared with base line ($P < 0.01$), persisted in all allergic subjects (Fig. 1A). Neutrophils (2–5 each microscope field), lymphocytes (3–7), and eosinophils (2–4) also persisted in conjunctival scrapings ($P < 0.01$) (Fig. 2A). CD54 expression

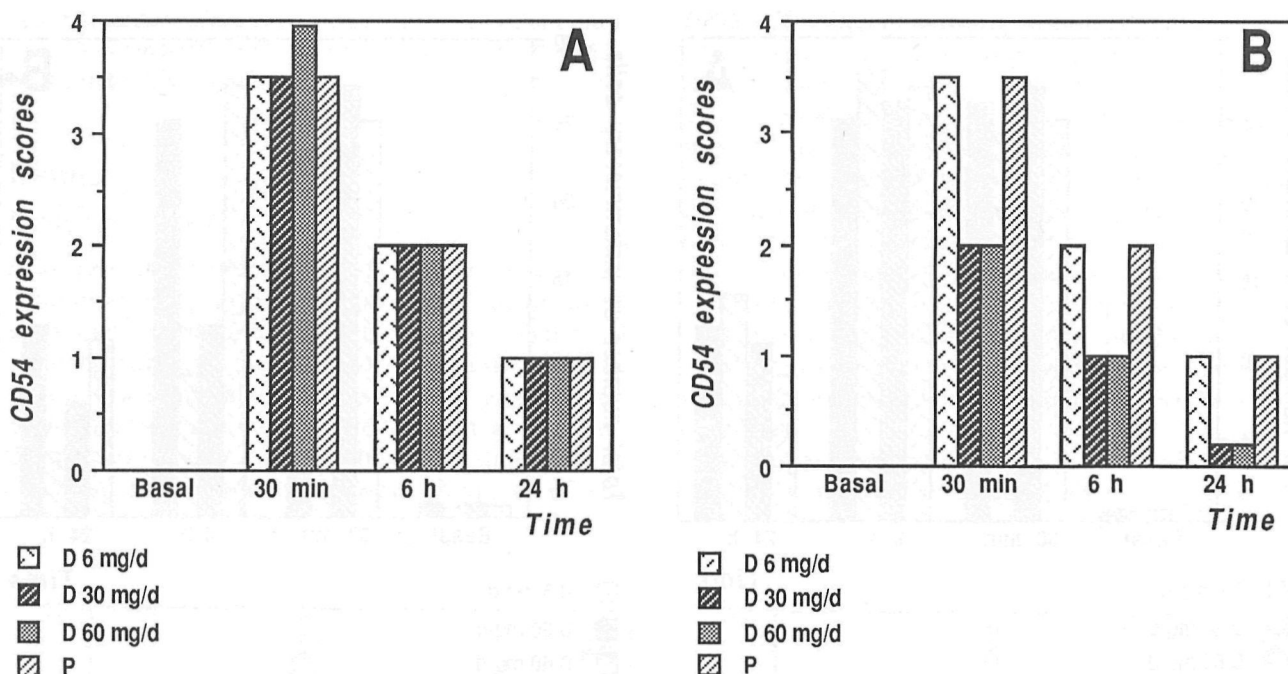


Fig. 3. Median values of total scores of CD54 expression on epithelial cells before (A) and after (B) treatment (D = deflazacort, P = placebo), before and after CPT. Deviation of individual values from the median was ≤ 1 .

Before treatment (A)

No subjects showed CD54 expression on epithelial cells. Comparison between base line and EPR (30 min), and base line and LPR (6 h and 24 h) performed by Wilcoxon signed rank test:

30 min	$P < 0.0005$
6 h	$P < 0.001$
24 h	$P < 0.01$

After treatment (B)

1) Intragroup comparison (before and after treatment) performed with Wilcoxon signed rank test:

- D (30 and 60 mg/d)	30 min	$P < 0.01$
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d)	all times	NS
- P	all times	NS

2) Intergroup comparison performed with Student's *t* test for independent samples:

- D (30 and 60 mg/d) compared with P	30 min	$P < 0.01$
	6 h	$P < 0.01$
	24 h	$P < 0.01$
- D (6 mg/d) compared with P	all times	NS

on epithelial cells was decreased but still present, unlike base-line conditions and the contralateral eye ($P < 0.01$) (Fig. 3A).

No difference was shown in healthy volunteers after allergen challenge (both in EPR and LPR), as compared with base-line condition. In allergic subjects included in the trial, no difference was evident between screening CPT and before-treatment CPT. The data with individual signs and symptoms of two representative patients are reported in Table 2. Individual inflammatory cell proportions were not modified by the treatment. The data for individual inflammatory cells of two representative patients are reported in Table 3.

Posttreatment CPT

After treatment, all patients were rechallenged by the same PD (ranging from 2000 to 5000 AUR/ml) employed before treatment. The placebo-treated group did not evince any statistically significant difference, as compared with base line. Clinical, cellular, and immunocytochemical indicators were not modified at the 6 mg/d dose, as compared with base line and the placebo-treated group (Figs. 1B, 2B, and 3B).

EPR. Compared with pretreatment values, the deflazacort groups and the placebo group did not show any statistically significant difference as far as EPR clinical and cellular effects are concerned (Figs. 1B

Table 2. Individual signs and symptoms of two representative patients receiving 30 mg/d deflazacort

Patient no. 15 (according to randomization list)									
Before treatment					After treatment				
Time	Hyp	Lac	Itch	Sw	Time	Hyp	Lac	Itch	Sw
Basal	0	0	0	0	Basal	0	0	0	0
30 min	4	3	3	2	30 min	3	4	3	2
6 h	2	2	1	2	6 h	0	0	1	1
24 h	0	0	1	3	24 h	0	0	0	0

Patient no. 23 (according to randomization list)									
Before treatment					After treatment				
Time	Hyp	Lac	Itch	Sw	Time	Hyp	Lac	Itch	Sw
Basal	0	0	0	0	Basal	0	0	0	0
30 min	4	2	4	2	30 min	3	3	3	2
6 h	2	1	1	2	6 h	0	0	1	1
24 h	0	0	2	2	24 h	0	0	0	1

Hyp=hyperemia; Lac=lacrimation; Itch=itching; Sw=swelling of eyelids.

and 2B). The CD54 expression was significantly reduced by deflazacort at 30 and 60 mg/d in the EPR, as compared with pretreatment values ($P < 0.01$) and the placebo-treated group ($P < 0.01$) (Fig. 3B). No effect was observed with deflazacort 6 mg/d.

LPR. Clinical LPR, at both 6 and 24 h, was significantly less severe in the deflazacort-treated group at 30 and 60 mg/d, as compared with both pretreatment values ($P < 0.01$) and the placebo-treated group ($P < 0.01$) (Fig. 1B).

LPR cellular infiltration, at both 6 and 24 h, was significantly reduced by deflazacort at 30 and 60 mg/d, as compared with both pretreatment values ($P < 0.01$) and the placebo-treated group ($P < 0.01$) (Fig. 2B). CD54 expression was significantly reduced by deflazacort at either 30 or 60 mg/d in the LPR, as compared with pretreatment values ($P < 0.01$) and the placebo-treated group ($P < 0.01$) (Fig. 3B). No statistically significant difference in any indicator considered was found between deflazacort at 30 and 60 mg/d.

Table 3. Individual inflammatory cells of two representative patients receiving 30 mg/d deflazacort

Patient no. 15 (according to randomization list)									
Before treatment					After treatment				
Time	N	E	L	Mo	Time	N	E	L	Mo
Basal	1	0	0	0	Basal	2	0	0	0
30 min	24	9	4	5	30 min	20	10	5	4
6 h	15	16	4	5	6 h	8	4	7	0
24 h	9	7	3	2	24 h	3	2	3	0

Patient no. 23 (according to randomization list)									
Before treatment					After treatment				
Time	N	E	L	Mo	Time	N	E	L	Mo
Basal	0	0	1	0	Basal	1	0	0	0
30 min	15	6	4	5	30 min	18	7	3	5
6 h	11	13	3	2	6 h	6	4	2	0
24 h	4	7	3	2	24 h	3	1	2	0

N=neutrophils; E=eosinophils; L=lymphocytes; Mo=monocytes.

Side-effects

Deflazacort was well tolerated and no adverse effects were reported at any time during the study.

Discussion

The efficacy and therapeutic value of glucocorticoids in the treatment of allergic diseases have been extensively demonstrated for both oral and topical glucocorticoid preparations (1, 3, 12, 14, 15, 18). In experimental allergen challenges in man, glucocorticoids are effective in dampening or blocking the appearance of the LPR following challenge in skin, upper airways, and the lung (41, 43, 50). On the other hand, most studies have shown a failure of oral glucocorticoids to block the acute phase of the experimental allergen-challenge response in the skin, nose, and lung (1, 16, 18, 26, 41). At these sites, topically applied compounds blunt the EPR; this effect is probably multifactorial, involving more than one cell type (1, 18, 40, 44, 46).

Possible mechanisms of action of glucocorticosteroids include reduction in neutrophil, monocyte, and eosinophil accumulation and decrease of cell activation and chemotaxis (16, 25, 28, 43). Low concentrations of dexamethasone reduce T-cell proliferation, with decreased cytokine production and IL-2 receptor expression (25). We have compared the *in vitro* effects of prednisone, dexamethasone, and deflazacort, a new heterocyclic derivative of prednisolone with calcium- and glucose-sparing effects, on T-cell activation through different pathways and on γ -interferon secretion (1). All compared glucocorticosteroids inhibited the immunologic indicators; however, deflazacort showed a stronger inhibition of the immune response than the other compounds (49). *In vivo* studies in the nose show reduction of mediator release after topical steroid treatment (41). In other studies, topical treatment of the skin with glucocorticoids showed evidence of reduced HLA-DR expression and cytokine production (40).

For these reasons, we have investigated the possible effects of deflazacort on the changes occurring after conjunctival challenge. The conjunctival changes following specific CPT represent a safe and reproducible model of experimental allergic conjunctivitis. As described by several authors, including ourselves (5, 9–13, 23, 24, 49), the pathologic events occurring after CPT include the development of a clinical EPR and the maintenance of the inflammatory process in the LPR. Although recruitment of inflammatory cells to the site of allergic reaction is well documented (5, 29, 31, 37), their activation of such cells and the interaction with epithelial cells appear to be crucial; the pathogenesis of allergic

reaction involves a large number of morphologic and functional changes, including adhesion machinery (21, 48, 51).

Our recent data have demonstrated the first evidence of CD54 expression on the conjunctival epithelium after conjunctival challenge (12, 13). CD54 is a surface molecule with immunoglobulin-like structure involved in intercellular adhesion via interaction with the β_2 integrin LFA-1 (CD11a/CD18) (19, 30, 32, 34, 35).

CD54 expression occurs early and simultaneously with the appearance of the inflammatory infiltrate; persists throughout the allergic reaction, i.e. during LPR; and vanishes 24 h after the challenge (13). The occurrence of this phenomenon following allergen exposure is restricted to allergic subjects (13). Moreover, several findings indicate that the adhesion machinery plays a central role in allergic inflammation and suggest that maneuvers designed to decrease adhesion of inflammatory cells (such as lymphocytes, eosinophils, etc., expressing LFA-1) may be a useful therapeutic approach (27, 32, 51). These findings prompted us to evaluate not only clinical and cellular events, but also CD54 expression on conjunctival epithelium in the present study.

Firstly, deflazacort is effective at either 30 or 60 mg/d; the ineffectiveness of the 6-mg dose might be related to the shortness of the treatment period. As expected, deflazacort exerts a protective action selectively against late effects (6 h and 24 h after challenge), resulting in a decrease of symptom severity and cellular changes. In particular, CD54 expression on epithelial cells appears to be reduced in the EPR as well as in the LPR. This finding is of interest and may reflect an immediate anti-inflammatory effect of deflazacort, an effect which could in turn interfere with interactions between surface molecules on the inflammatory cells and epithelium. This effect exerted even on EPR may suggest a new possible mechanism of action of corticosteroid.

Furthermore, as recently shown, epithelial cells should no longer be considered to be merely bystanders in the development of allergic reaction; they play an active role in the activation and migration of inflammatory cells (2). Moreover, *in vitro* data have recently shown that deflazacort can exert an inhibitory effect on CD54 expression on epithelial cell lines in culture (8). Further elucidation of such a complex mechanism of action involving cells and surface molecules would require the aid of double-staining methods and time-course studies. The former would provide evidence of the possible co-expression of CD54 on the conjunctival epithelium and of its natural ligand LFA-1 (CD11a/CD18) on inflammatory cells and its modulation by steroids. We have obtained preliminary evidence of CD11a (α

chain of LFA-1) expression on the conjunctival epithelium in allergic subjects after specific challenge. A similar phenomenon was also observed on thyroid epithelium in autoimmune thyroiditis (paper in preparation). The last-named would allow drawing of a correct dose-response curve for deflazacort.

In conclusion, it is possible to hypothesize that a surface molecule, such as CD54 and, more extensively, cellular adhesion effects leading to an inflammatory infiltrate, might be among the possible targets of glucocorticosteroid action. Our results strongly support the use of immunocytochemical evaluation (evaluation of CD54 on epithelium) as another indicator for evaluating pharmacologic modulation of the allergic response.

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