

Protective effect of different doses of terfenadine on the conjunctival provocation test

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The protective effect of terfenadine on inflammatory processes following the early phase of conjunctival provocation tests by specific allergen was assessed in 24 patients suffering from seasonal allergic rhinoconjunctivitis, in a single-blind, randomized, placebo-controlled, parallel-group study. Patients were randomly assigned to four treatment groups, each being given terfenadine 60, 120, 180 mg daily, or placebo, respectively, for 7 days, out of the pollen season. Clinical severity (burning, itching, lacrimation and hyperemia), and number of inflammatory cells obtained by conjunctival scraping (neutrophils, eosinophils, lymphocytes, and monocytes) were evaluated before and after treatment. Pre-treatment with terfenadine resulted in a significantly higher allergen threshold dose than placebo ($p < 0.01$), regardless of drug dose. Patients given terfenadine experienced a significant reduction ($p < 0.03$) in conjunctival symptom severity, as compared with placebo, following conjunctival challenge. Accordingly, the number of inflammatory cells in terfenadine-treated patients was significantly lower ($p < 0.01$) than in the placebo-treated after the conjunctival provocation test. The results of this study suggest that terfenadine has a significant protective effect on the early-phase cellular and clinical events of conjunctival reaction induced by allergen challenge in atopic patients.

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The conjunctival provocation test with specific allergen (CPT) in atopic subjects represents a simple, safe, reliable and reproducible tool to evaluate the pathogenetic mechanisms of allergic reactions (6, 9, 14, 15). Cytological evaluation of conjunctiva as well as measuring the concentration of the main mediators in the lacrimal fluid released during the allergic reaction (2, 6, 11) was performed.

A pattern of conjunctival events following CPT characterizes the early and late phase of allergic response: the cytological evaluation of conjunctiva after CPT shows that signs and symptoms appear simultaneously with local infiltration of inflammatory cells, namely neutrophils, eosinophils, lymphocytes and monocytes. The cellular pattern varies among subjects according to time of observation and allergen concentration (1, 2, 3, 4, 5).

Terfenadine is a specific and selective H_1 receptor antagonist with additional anti-allergic properties; it has also been shown to protect against several challenges, such as conjunctival, skin, nasal and bronchial (7, 8, 13). Thus, the aim of this study was to assess more precisely the efficacy of terfenadine, at the three more commonly employed dosages, in

preventing allergic conjunctival changes following CPT with specific allergen in sensitized patients.

Patients and methods

The study, designed as single-blind, randomized, and parallel-group treatment, was carried out in 24 patients (17 men and 7 women) aged between 17 and 59 years (mean age 34), suffering from seasonal allergic rhinoconjunctivitis (*Parietaria judaica*), in the off-pollen season.

Patients were randomly assigned to four parallel groups of treatment, each receiving for 1 week a single daily morning dose of terfenadine 60, 120 and 180 mg, or placebo, respectively. The inclusion criteria were history of pollen allergy for at least one season, positive skin prick test, and positive RAST with the specific pollen. Patients with previous documented allergy to the study drug, patients with eye diseases, women of childbearing potential, or lactating, and patients suffering from diseases able to alter drug kinetics and metabolism were excluded. No concomitant medication was allowed for at least 4 weeks before and during the study.

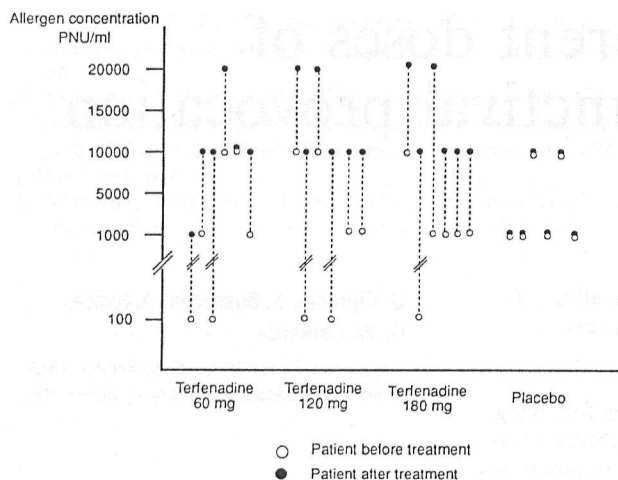


Fig. 1. Allergen concentration in CPT, expressed in PNU/ml. Statistical analysis: Intragroup comparison (before treatment vs after treatment) performed by Wilcoxon Signed Rank Test: -terfenadine (60 mg, 120 mg and 180 mg) $p < 0.05$; - placebo NS. Intergroup comparison performed by Mann-Whitney test for independent samples: - terfenadine (60 mg, 120 mg and 180 mg) vs placebo $p < 0.01$.

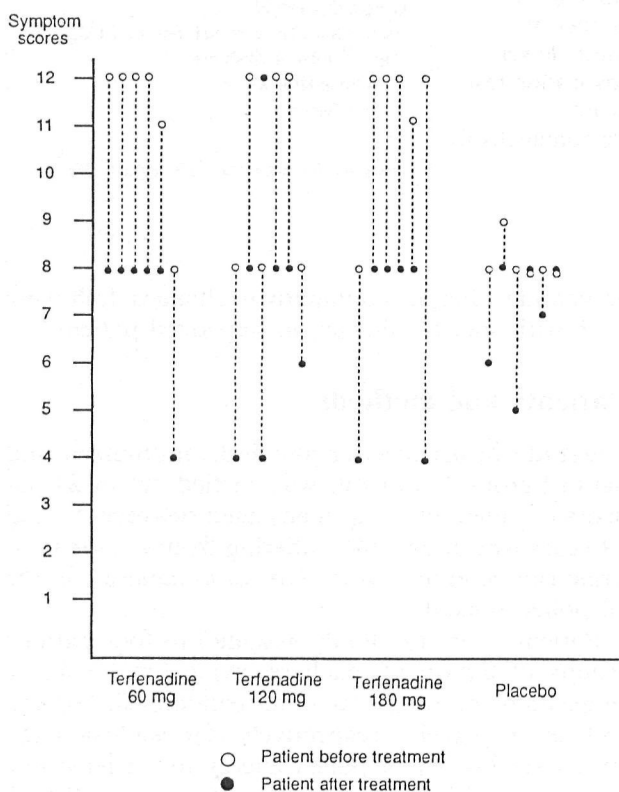


Fig. 2. Conjunctival clinical scores after CPT, before and after treatment.

Statistical analysis: Intragroup comparison (before treatment vs after treatment) performed by Wilcoxon Signed Rank Test: -terfenadine (60 mg, 120 mg and 180 mg) $p < 0.03$; - placebo NS. Intergroup comparison performed by Mann-Whitney test for independent samples: - terfenadine (60 mg, 120 mg and 180 mg) vs placebo $p < 0.01$.

All patients gave their informed consent to participate in the study. They were initially submitted to an open CPT to screen positive subjects: 10 μ l of the lowest allergen concentration (kindly provided by Bayropharm-DHS, Milan, Italy) was instilled into the lower conjunctival sac of the right eye. The first allergen concentration tested was the lowest allergen solution that elicited a positive skin reaction. Ten milliliters of pure diluent (saline albumin) was instilled into the left eye, as control. Afterwards, patients were challenged with 10 μ l of allergen at a time, in increasing concentrations (100, 500, 1000, 5000, 10 000, 15 000, 20 000 PNU/ml), into the right eye every 20 min, until allergic symptoms occurred, achieving an allergen threshold dose (ATD). Similarly, pure diluent was instilled into the left eye, every 20 min. Conjunctival scrapings were obtained from both eyes at baseline and at the end of the challenge.

All selected patients underwent CPT with ATD into the right eye and received pure diluent into the left eye; 20 min after CPT the clinical reaction to the allergen (observed as conjunctival hyperemia, lacrimation, itching and burning) was assessed according to an arbitrary 4-point rating scale, from 0

Table 1. Total number of inflammatory cells before and after CPT, at baseline and after treatment

Patient	Drug	Before treatment		After treatment	
		Baseline	After CPT	Baseline	After CPT
1	Terfenadine 60 mg	3	5	3	5
6		0	5	0	4
7		0	4	0	2
9		2	4	1	2
13		0	6	0	2
16	Terfenadine 120 mg	0	4	0	4
2		0	6	0	3
3		0	3	0	2
8		0	5	0	3
11		0	4	0	2
14		0	4	0	3
17		1	5	0	5
4	Terfenadine 180 mg	0	6	0	5
5		0	4	0	2
10		2	5	0	3
12		1	6	1	3
15		1	7	0	3
18	Placebo	0	4	0	2
19		1	7	0	6
20		0	6	0	5
21		0	6	0	5
22		1	5	1	3
23		1	7	1	3
24		0	6	0	6

Statistical analysis: Intragroup comparison (before treatment vs after treatment) performed by Student's t-test: terfenadine (60 mg, 120 mg and 180 mg) $p < 0.001$; placebo $p = \text{NS}$. Intergroup comparison performed by Student's t-test: for independent samples: terfenadine (60 mg, 120 mg and 180 mg) vs placebo $p < 0.01$.

to 3, increasing for severity (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Concomitantly, a conjunctival scraping for cytological determinations was obtained from each patient at baseline and 20 min after CPT or pure diluent from both eyes. Specimens were spread on glass slides, air-dried, stained with May-Grunwald Giemsa dye and read by microscope (Leitz Laborlux D microscope, 25 ×, 50 × focus). The slides were examined separately by two investigators blind to the identity of the samples. The number of inflammatory cells, i.e. neutrophils, eosinophils, lymphocytes, and monocytes, was considered to be total number of cells/each microscope field; the data are expressed as a mean of 10 fields.

The next day patients were randomly assigned to the 7-day treatment with terfenadine or placebo at different doses. At the end of the treatment period, the patients underwent another CPT 3 h after the last drug intake, starting with ATD and, if possible, progressively increasing the allergen concentration, every 20 min, reaching a new ATD. Conjunctival symptoms and cell count were also evaluated and rated in both eyes at baseline and 20-min challenge. In addition, all possible adverse effects were spontaneously reported by the patients and were carefully noted and evaluated.

Statistical analysis of results was performed by Wilcoxon's signed ranks test for paired data to test differences within groups; by Mann-Whitney test for independent data to test differences among groups; and by Student's t-test for parametric data to test differences among groups.

Results

All patients completed the study and were therefore included in the final analysis. Before treatment, patients showed an early conjunctival reaction within 20 min after allergen challenge, at concentrations ranging between 100 and 10 000 PNU/ml, characterized by hyperemia, lacrimation, itching, and burning. In conjunctival scrapings a significantly greater number of inflammatory cells were detected after challenge, as compared with the prechallenge observation and with the contralateral eye challenged with pure diluent. No statistically significant differences in clinical and cellular responses were observed among the four treatment groups.

After treatment, patients were rechallenged with allergen concentrations ranging between 1000 and 20 000 PNU/ml. Pretreatment with terfenadine (all doses) resulted in a significantly higher ATD than placebo ($p < 0.01$), as shown in Fig. 1. After chal-

Table 2. Cell number of each single cell group before and after CPT, at baseline and after treatment

Patient	Drug	Before treatment								After treatment							
		Baseline				After CPT				Baseline				After CPT			
		N	E	L	M	N	E	L	M	N	E	L	M	N	E	L	M
1	Terfenadine 60 mg	0	1	1	1	2	1	1	1	0	1	1	1	2	1	1	1
6		0	0	0	0	2	1	1	1	0	0	0	0	2	1	0	1
7		0	0	0	0	1	1	1	1	0	0	0	0	1	0	1	0
9		0	0	1	1	1	1	1	1	0	0	1	0	1	0	1	0
13		0	0	0	0	2	2	1	1	0	0	0	0	1	1	0	0
16	Terfenadine 120 mg	0	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1
2		0	0	0	0	3	1	1	1	0	0	0	0	1	1	0	1
3		0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0
8		0	0	0	0	2	1	1	1	0	0	0	0	1	1	1	0
11		0	0	0	0	1	1	1	1	0	0	0	0	1	0	0	1
14	Terfenadine 180 mg	0	0	0	0	2	1	1	0	0	0	0	0	1	1	0	1
17		0	0	0	1	2	1	1	1	0	0	0	0	1	1	2	1
4		0	0	0	0	2	1	2	1	0	0	0	0	2	1	1	1
5		0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0
10		0	0	1	1	2	1	1	1	0	0	0	0	1	0	1	1
12	Placebo	0	0	1	0	2	1	2	1	0	0	1	0	1	0	2	0
15		0	0	1	0	2	2	2	1	0	0	0	0	1	1	1	0
18		0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0
19		0	1	0	0	2	2	2	1	0	0	0	0	2	1	2	1
20		0	0	0	0	2	1	2	1	0	0	0	0	2	1	1	1
21		0	0	0	0	2	2	1	1	0	0	0	0	1	1	2	1
22		0	0	0	1	2	1	1	1	0	0	0	1	0	1	1	1
23		0	0	1	0	2	2	2	1	0	0	1	0	2	2	1	1
24		0	0	0	0	2	2	1	1	0	0	0	0	2	2	1	1

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

lenge, conjunctival changes in patients treated with placebo did not significantly differ from those observed before treatment, as regards clinical evidence and cellular scrapings. Compared with pretreatment values, clinical signs after challenge were significantly less severe after terfenadine (all doses) than after placebo ($p < 0.03$), as reported in Fig. 2. Similarly, the total number of inflammatory cells increased after challenge, but was significantly less after terfenadine pretreatment (all doses) than with placebo ($p < 0.001$) (Table 1). The number of each inflammatory cell type (i.e. neutrophils, eosinophils, lymphocytes and monocytes) is reported in Table 2.

As assessed by conjunctival symptoms and cytological evaluation, no significant differences were found among the different daily doses (60, 120 and 180 mg) of terfenadine. Terfenadine was well tolerated and no adverse effects were reported after taking terfenadine or placebo.

Discussion

The conjunctival provocation test is a simple and reliable method for evaluating drugs intended for treatment of allergic conjunctivitis (2, 3, 4, 5, 6, 9, 11, 14, 15). In the present study, CPT allowed evaluation of the protective effect of terfenadine both on histamine-mediated conjunctival changes and on inflammatory cells present in conjunctiva after the specific challenge. Our results in adults are consistent with those reported in children by Kjellman & Andersson (7), showing an increased tolerance to conjunctival provocation with specific allergen after terfenadine treatment. On the other hand, recent evidence has shown the additional protective effect of terfenadine on the cell-mediated events during allergic reactions (10) such as asthma (8, 13); this evidence seems to be consistent with terfenadine, which appears to be able to prevent the appearance of early-phase cellular infiltration of conjunctiva after CPT. This protective effect of terfenadine was shared equally by the three dose regimens tested, thus indicating that the treatment duration over 1 week is long enough to show therapeutic effects even with the lowest dose of the drug tested.

In this human model of allergic reaction the administration of a highly selective H_1 antagonist had a clearly protective effect on the early-phase cellular and clinical events of conjunctival reaction induced

by allergen challenge, in spite of the higher concentration of allergen in atotics than in controls. Moreover, this result underlines the predominant role of histamine in the pathogeny of allergic conjunctivitis.

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