

Does reversed-type anaphylaxis in healthy subjects mimic a real allergic reaction?

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Summary

Immediate and late cutaneous reactions were induced in healthy and atopic subjects by anti-IgE challenge and a skin-window technique was used in order to verify if the pattern of cells observed at 24 hr was similar in both groups. The study was first performed under basal conditions, and in a second double-blind cross-over step, it was performed again during treatment with cetirizine 10 mg b.i.d. and terfenadine 60 mg b.i.d. Anti-IgE challenge was followed by a significant eosinophil accumulation in atopic subjects only. Cetirizine significantly inhibited this phenomenon while terfenadine showed a mild non-significant inhibitory effect.

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Introduction

Since the demonstration by Ishizaka and Ishizaka [1] that an intracutaneous injection of anti-IgE into monkeys and humans induces a weal-and-flare reaction, it has also been shown that anti-IgE releases histamine from leucocytes of normal human donors [2], allergic subjects [3], intrinsic asthma patients [4] and from basophils of urticarial and control subjects [5]. It was also demonstrated that there is a requirement for two antibody combining sites in anti-IgE for the induction of reversed anaphylactic reactions [6].

Umemoto *et al.* [7] showed that a challenge with a high anti-IgE concentration produced larger weal responses in allergic as well as in healthy subjects and increased frequency and diameters of late cutaneous reactions (LCR) that were very similar, at least morphologically speaking, to antigen-induced LCRs in allergic subjects. The fact that a LCR could be induced in healthy volunteers with anti-IgE was not considered surprising because it was generally accepted that the direct and reversed *in-vitro* anaphylactic reactions both share a common reaction mechanism [2]. The idea that an anti-IgE-induced skin reaction, performed in healthy volunteers, was similar to a real allergic reaction, was so well accepted that some investigators used the reversed-type anaphylaxis model in healthy volunteers in order to test

the anti-allergic properties of drugs such as terbutaline, mepyramine, betamethasone and dazoxiben [8,9]. According to Grönneberg *et al.* [8] the use of healthy volunteers in such studies was an advantage because it allowed avoidance of possible influences of a disease states and of previous or concurrent therapies. Nevertheless, some already ancient works clearly demonstrated that the gross morphological feature of a cutaneous reaction only represents a limited aspect of an allergic phenomenon.

Developed by Rebeck [10] in 1955, the skin-window technique allowed a series of investigators to describe a particular pattern of cells that migrate into the abraded area of the skin after antigen challenge in allergic subjects [11–14]. This pattern is characterized by neutrophils which predominate during the first 20 hr while the mononuclear cells are more frequent afterwards. As far as the eosinophils are concerned, they clearly peak at 24 hr and this eosinophil response of allergic patients is independent of the peripheral eosinophil count [15].

More recently, it was shown with skin biopsies and a modified skin-window technique that an intradermal injection of PAF and FMLP is followed by a local inflammatory process, the pattern of which is completely different when healthy subjects or allergic patients are considered [16,17]. In allergic subjects there is a marked infiltration of the skin by degranulated eosinophils 24 hr after challenge, while these cells are almost absent in healthy volunteers. According to these data, it becomes

clear that the gross morphological feature of a LCR cannot be considered as a reliable indication of the underlying cellular phenomenon.

These data prompted us to reappraise the anti-IgE-induced skin reaction: does such a reaction performed in healthy subjects accurately mimic an antigen-induced reaction performed in atopic patients?

In the present study, performed in 10 healthy volunteers and 10 grass pollen-sensitive subjects, the effect of an intradermal injection of anti-IgE on the migration of inflammatory cells was evaluated 24 hr after cutaneous challenge under control conditions and after H_1 blockade by the specific anti- H_1 cetirizine and by terfenadine.

Subjects and methods

Subjects

Ten healthy volunteers (nine males) and aged 18–36 yr (mean $\pm$ s.d. 25.2 ± 5.5) and ten atopic volunteers (nine males) aged 21–34 yr (mean $\pm$ s.d. 23.9 ± 3.8) were studied.

The healthy subjects had no history of allergy and their grass pollen skin tests were negative. The atopics were grass pollen-sensitive with a typical history of allergic rhinitis during the pollen season and a positive weal response 15 min after a prick test with pollen extract (stallergene 1/100, w/v). All volunteers were duly informed about the experimental conditions and the drugs used, and gave their written consent to enter the study.

Reagents

Goat anti-human IgE (GAHU Nordic Immunology, Tilbury, The Netherlands) was used at 1/1000 concentration. Grass pollen extracts (Stallergene, Fresner, France) was used for pricktest at 1/100, w/v).

Cetirizine dihydrochloride (10 mg capsules) and pulverized terfenadine (60 mg identical capsules) were provided by UCB, Brussels, Belgium. The drugs were given b.i.d. for 4 days under double-blind randomized cross-over conditions.

In order to verify whether the bioavailability of encapsulated pulverized terfenadine was similar to the one of the marketed galenic presentations, a preliminary clinical pharmacology experiment was performed in 10 healthy volunteers which demonstrated that capsules and tablets were equally effective in inhibiting the skin reactivity to histamine (prick method with two histamine concentrations: 10 and 100 mg/ml) 2, 4, 6 and 8 hr after a single p.o. administration of the two galenic forms, and 24 hr and 120 hr during a sub-chronic administration of the recommended dosage (data not shown).

Cutaneous test

Healthy volunteers and atopic subjects were skin tested with anti-IgE on the volar surface of the forearms under control conditions and during the third day of administration of cetirizine (10 mg b.i.d.) and terfenadine (60 mg). Weals and flares were marked on the skin 15 min after i.d. injection of anti-IgE injection and transferred onto transparencies. The areas of the reactions were measured by a computerized planimetry system described earlier [18]. Results were expressed in mm².

The migration of eosinophils, neutrophils and monocytes 24 hr after anti-IgE challenge was evaluated by a modified skin-window technique already described [19]. Results were expressed in percentages of cells.

An interval of 2 weeks was managed between each experimental period, and the atopics were tested outside the pollen season between October 1988 and February 1989.

Statistical analysis

Mean percentages of neutrophils, monocytes and eosinophils, and standard deviations were calculated.

Multivariate analyses of variance for repeated measurements were performed. The replications were the data obtained under control conditions, under cetirizine and under terfenadine. For global differences between control and treatment conditions ($P < 0.05$), pairwise contrasts were used.

Results

Cutaneous response

Under control conditions, anti-IgE-induced weals and flares were very similar in healthy subjects and atopics (Table 1). Cetirizine was superior to terfenadine in inhibiting these reactions.

Leucocyte migration

The cellular response was studied 24 hr after anti-IgE challenge under control conditions and during the fourth day of treatment with cetirizine and terfenadine. This response mainly consisted of neutrophils and monocytes in healthy subjects who presented very few eosinophils (Table 2).

In atopic subjects, there was a significant accumulation of eosinophils with a concomitant decrease in neutrophils (Table 2). Cetirizine significantly inhibited the accumulation of eosinophils while terfenadine induced a non-significant decrease of these same cells.

Late cutaneous reactions

At the height of the response (6–12 hr), the LCR was essentially characterized in both groups by erythema, the

Table 1. Mean weal-and-flare areas 15 min after anti-IgE challenge, under control conditions and after a 4 day treatment with cetirizine and terfenadine

	P-value (pairwise contrasts)					
	Control	Cetirizine (C)	Terfenadine (T)	C vs. control	T vs. control	C vs. T
Healthy subjects						
Weals	97.4 ± 51.1	51.2 ± 27.8	7.03 ± 4.1	0.03	n.s.	0.09
Flares	1063 ± 552	205 ± 114	469 ± 509	0.0006	0.03	0.09
Atopic subjects						
Weals	106.3 ± 56	49.9 ± 30.7	77.5 ± 26.4	0.02	0.07	0.07
Flares	914 ± 526	194 ± 144	228 ± 173	0.003	0.002	n.s.

Results expressed in mm²; means ± s.d. n.s. $P > 0.1$.

Table 2. Percentage of neutrophils (N) eosinophils (EO) and monocytes (M) 24 hr after anti-IgE challenge under control conditions and during a 4-day treatment with cetirizine and terfenadine

	Control	Cetirizine	Terfenadine	P-value of control vs. treatments (global difference)
Healthy subjects				
N	66.1 ± 11.2	61.9 ± 20.8	55.8 ± 19.6	0.19
EO	2 ± 4	3.2 ± 4.1	1.7 ± 1.9	0.71
M	31.8 ± 9.9	34.8 ± 21.8	42.1 ± 19.7	0.22
Atopic subjects				
N	34.2 ± 15	39.7 ± 14.2	45.8 ± 18.1	0.41
EO	19.9 ± 8.1	10.8 ± 6.4*	14.5 ± 12.1	0.08
M	44.8 ± 10.1	49.4 ± 15.3	39.5 ± 14	0.49

* $P = 0.03$; difference between cetirizine and control value calculated with pairwise contrasts.

area of which corresponded to the weal-and-flare response, but, at the concentrations, there was no oedema or tenderness.

Discussion

This clinical pharmacology study performed with a modified skin-window technique clearly demonstrated that the cells recruited during 24 hr after anti-IgE skin challenge are different in atopics compared to healthy subjects. The former presented a significantly elevated percentage of eosinophils at the challenged site while the latter presented only a few eosinophils if any.

These data are in accordance with previous experi-

ments performed with PAF and FMLP which showed that these agonists induced a significant migration of eosinophils *in vivo* only in allergic subjects [16,17] and they strongly suggest that an anti-IgE-induced skin reaction performed in a healthy volunteer does not really mimic an allergic reaction, even when a LCR of similar morphological aspect is present. This is why one should be cautious when assuming that healthy volunteers may be used instead of allergic patients for clinical pharmacology studies designed to test the efficacy of drugs as it has been done several times in the past [8,9,20].

While investigating anti-IgE-induced LCRs in healthy volunteers and atopic subjects, Dolovich *et al.* [21] hypothesized that eosinophils could be involved in the pathogenesis of LCR, but they did not describe any difference between healthy and atopic subjects. Actually, only one normal volunteer and one allergic subject were biopsied after anti-IgE challenge in their study, and this may be why they did not see the differences in cell patterns we could observe in our two groups of 10 individuals. Furthermore, it is also possible that the skin-window technique, which is rather a rough approach, is especially useful for the measurement of the *in-vivo* migration phenomenon because it allows the cells to accumulate during a long period of time (24 hr) in such a way that even a weak cell movement may become significant.

As far as the pharmacological modulation of this anti-IgE cutaneous reaction is concerned, both cetirizine and terfenadine could inhibit weal-and-flare formation and eosinophil accumulation, cetirizine (10 mg b.i.d.) being more effective on both parameters than terfenadine (60 mg b.i.d.) As it is well known that cetirizine is more potent than terfenadine as an anti-H₁ agonist [18], it is difficult to say from the present data whether the inhibiting effect of these two drugs on cell migration is linked to their respective potencies as H₁ blockers.

The inhibiting effect of cetirizine on eosinophil migration *in vitro* and *in vivo* is now well documented [19,22–27]. A possibly similar effect of terfenadine, even if less marked (non-significant in this study) could be of interest from a mechanism of action point of view.

In conclusion, it appears that atopic eosinophils behave differently from normal cells *in vivo* during anti-IgE challenge, as they do with agonists such as PAF and FMLP. An anti-IgE challenge performed in healthy subjects does not mimic a real allergic reaction. The inhibiting effect of cetirizine on eosinophil migration is confirmed once again. Finally, terfenadine might also display some inhibiting effect on eosinophil migration *in vivo* in atopics.

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