Allergy 1979, 34, 121–124

Effects of Ketotifen and Clemastine on Passive Transfer of Reaginic Reaction

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Passive transfer (PK) tests were performed with a reaginic serum on a recipient reacting with an immediate and a more prolonged reaction when specifically challenged. Both reactions are thought to be mediated by IgE immunology. Ketotifen, a cycloheptathiophene derivative, and clemastine, given to the recipient in maximal clinical doses for 3 days, inhibited the immediate reaction. Ketotifen had a very slight effect also on the prolonged reaction.

The results indicate that the *in vivo* effects of ketotifen in the human system are due not so much to mast cell inhibitory mechanisms, but more to post-release antihistaminic and some anti-inflammatory properties.

Key words: allergy; immediate hypersensitivity; ketotifen; mast cell; reaginic reaction.

Accepted for publication 19 September 1978

Ketotifen has been stated to have a comparable effect on the inhibition of allergic mast cell release of mediator agents as locally applied cromolyn sodium, even when used perorally (9, 10, 11, 14). The effects on allergic reactions are suggested to be due to more than the antihistaminic action as such.

The present study was undertaken to test the validity of this suggestion. For this purpose the passive transfer test, with a purified codfish allergen, and a purified grass pollen allergen with a complementary human allergic serum, was found suitable as it was known to give both an immediate wheal and a more prolonged inflammatory reaction in the skin of a given non-atopic volunteer. Since both types of reaction are thought to be the result of the mast cell release of a number of biochemically active agents, this test system appeared suitable for the present investigation.

MATERIAL AND METHODS

Human reaginic serum (DE) was collected by venipuncture from a non-hyposensitized patient allergic to codfish and grass pollen (1, 5). The serum contained high levels of IgE- antibodies to codfish (radioallergosorbent class 4) and timothy grass pollen (radioallergosorbent test class 3) (5). It has been used several times in passive transfer experiments (4, 5), and the passive transfer tests have regularly elicited both whealing and a prolonged, slowly developing inflammatory response in the recipient used. One aliquot of the DE serum was heated at +56°C for 5 h in order to inactivate its reaginic sensitizing ability.

The specific allergens used were a purified major allergen from codfish (DS 22), used in a number of previous experiments (4, 5), and a highly purified allergen preparation of

0105–4538/79/020121-04$02.50/0

timothy pollen (8). Both were supplied by Nyegaard & Co. (Oslo) as freeze-dried and easily soluble preparations. The material was dissolved in sterile saline shortly before use.

Ketotifen (Zasten®) was kindly supplied by Sandoz in 1 mg tablets. Clemastine (Tavegyl®) was purchased and used as the antihistaminic control agent. Both drugs were transferred, in 0.5 mg aliquots, into opaque gelatine capsules, ordinarily used for food provocation tests (2), to facilitate a double-blind medication system, and the capsules were coded.

Passive transfer tests were performed and evaluated as previously described in detail (4,5). Following allergen challenge the wheal was recorded after 20 min and the deeper inflammatory reaction was recorded after 120 min. The outlines of the tissue reactions were marked with a soft ballpoint pen and the marks were transferred to squared paper with translucent Scotch tape. The largest diameter (D) and the diameter vertical to D (d) were measured, and the results were expressed as (D + d) : 2.

Identical experiments were performed both before and after medication as outlined under Experiments and Results. They were performed in duplicate each time in two series of experiments with an interval of 2 months.

EXPERIMENTS AND RESULTS

Passive transfer sensitization was initially done when the recipient was using no medication. Sensitization was performed on the volar surfaces of the underarms by injecting approximately 0.02 ml of the sera diluted 1:100 and 1:400, respectively.

Heat-inactivated serum DE in dilutions 1:2, 1:10 and 1:100 was injected likewise. Only injections raising initial blebs of at least 4 mm ± 0.5 mm in diameter were accepted for further use. The following day (22–26 h after sensitization) all sites were challenged by an i.c. injection of approximately 0.01 ml of DS 22 in a 0.1 μg/ml concentration in the first series of experiments, and i.c. injection of the timothy pollen allergen preparation in a 0.1 μg/ml (10 BU/ml) concentration (3, 4) in the second series. All reactions were recorded after 20 and 120 min. Histamine HCl 0.1 mg/ml was used as a positive control for the whealing reactivity (3).

With intervals of 7–10 days identical experiments were performed, but this time the recipient was pretreated with clemastine (2 mg three times daily) or ketotifen (2 mg three times daily), respectively, in a double-blind system. Each drug was given for 3 days prior to the challenge, and an additional 2 mg was given on an empty stomach 1 h before the allergen was injected.

In the initial (untreated) trials the allergen challenge resulted in quite a large wheal (Table 1) at the 1:100 serum site, but quite a small wheal at the 1:400 serum site, while the reaction to histamine was fairly constant during all experiments. Duplicate tests showed no more than a 2-mm variation with respect to the mean diameter of the wheals. No reaction whatsoever could be observed at the sites injected with heat-inactivated serum and allergen. The wheals subsided and disappeared within an hour, but before this a

Table 1

	*Wheal following histamine test		Wheal following allergen test Site 1:100		Site 1:400		Inflammation Site 1:100		Site 1:400	
			C	T	C	T	C	T	C	T
No medication	16–16,	15–17	17–15,	20–18	5–5,	6–5	36–40,	30–34	28–28,	12–12
Clemastine	10–9 ,	7–7	7–5,	10–10	2–2,	0–2	30–38,	24–20	26–20,	14–9
Ketotifen	9–9 ,	10–8	7–6,	8–6	2–1,	0–0	28–18,	17–12	20–22,	11–9

*All figures indicate (D + d):2 in mm. Results of two series of duplicate tests are given.
C, codfish allergen challenge; T, timothy pollen allergen challenge.

deeper swollenness with erythema and a slight tenderness developed at the same sites. A similar but slightly more moderate inflammatory type of reaction occurred also at the sites sensitized with the 1:400 serum dilution in spite of minimal or no immediate whealing reactions at these sites. No prolonged inflammatory reactions were observed at the histamine test sites.

The outlines of the inflammatory and prolonged reactions were quite diffuse and difficult to mark exactly, making the measurements less precise, and there were larger variations in the mean diameters measured in the duplicate tests (Table 1).

When the recipient was on antihistamine or ketotifen the reactions from the histamine test, and to the allergen challenges recorded after 20 min (wheals), were markedly reduced. The effect on the prolonged inflammatory reaction was minimal, however, although somewhat more diminished after treatment of the recipient with ketotifen than with antihistamine, or no treatment (Table 1). The recipient reported considerable dryness of the mouth, and sedation with feelings of dizziness with both drugs.

DISCUSSION

The immediate whealing reaction to direct skin tests in the allergic individual, and to allergen challenge in passively sensitized skin sites, are thought to be mediated mainly by histamine released from the mast cells. This reaction as well as the reaction to the injection of histamine itself were depressed by both drugs used in the trial, thus confirming an approximately equal antihistaminic activity for the two drugs.

The prolonged inflammatory type of reaction resulting from the allergen challenge at the sensitized sites but not the histamine test site, is thought to be a secondary stage reaction to mediators released or activated following the allergen IgE-antibody reaction on the mast cell surface (6). The same kind of reaction occurred regularly in the recipient used in this study after the described passive transfer tests with different sera and various allergens.

This indicates that the reaction is not related to various concentrations of non-IgE or "precipitating" antibodies to the allergenic preparations, but is more likely a distinct pharmacochemical reaction pattern in the recipient. The use in this study of highly purified allergenic material and the lack of reaction at the sites injected with heat-inactivated allergic serum supports this, since heating to +56°C destroys the ability of reaginic antibodies to sensitize mast cells, while not significantly affecting the activity of non-reaginic antibodies.

The prolonged inflammatory reactions are likely to be a result of mediators released by the interaction on the mast cell surface of allergens and reaginic (IgE)-antibodies, although this is not proven in this study. Dolovich et al. (7) and Solley et al. (15) have reported the same kind of reaction, and showed that it was mediated by IgE antibodies. Austen et al. (6) and Lichtenstein (12) have outlined the biochemistry involved in this type of inflammatory reaction induced by mast cell release of mediators and enzymes.

Reports that rat passive cutaneous anaphylaxis may be inhibited by ketotifen could suggest that this compound was a mast cell blocker comparable to locally applied cromoglycate in that species (13). The present experiments indicate that ketotifen does not exert mast cell blocking effects of any significance on the human reaginic system. In the passive transfer system used, direct comparison between ketotifen and cromoglycate is not possible due to the low solubility of the latter making it less suitable for systemic use.

If the results of the prolonged and late reactions are studied, ketotifen produced a 36%–55%, 43%–60% degree of inhibition for the inflammatory reaction using a serum dilution of 1:100. This compares with a degree of inhibition of 17%–5%, 20%–41% for clemastine. Using the 1:400 dilution, the

figures were 0–30%, 0–25% for clemastine and 13%–21%, 8%–25% for ketotifen. These figures can possibly be taken to indicate that ketotifin has a slight anti-inflammatory action, but the limitations of the method do not allow any firm conclusion to be made.

The marginal ability of ketotifen to inhibit the prolonged and late reaction, while depressing the whealing reaction, suggests that the main effect of ketotifen is that of an antihistaminic agent and not so much that of a mast cell inhibitor. It appears, however, that ketotifen may have an effect on the response to the reaginic reaction in addition to that exerted by the antihistamine. This may be due to some effects on the post-release biochemistry and not to the direct inhibitory effects on the human mast cell *in vivo*.

The doses used were quite high from a clinical viewpoint, and the side effects reported prohibited experiments with still higher doses.

Since the total amount of mast cells in the body is rather impressive, one would expect that also large amounts of systemic drugs would have to be used in order to block all the mast cells in question. However, as long as the physiological role of mast cells remains unknown, it may be as well to leave most of them functionally undisturbed.

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