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Effects of Antihistamines on Cutaneous Reactions and Influx of Eosinophils after Local Injection of PAF, Kallikrein, Compound 48/80 and Histamine in Patients with Chronic Urticaria and Healthy Subjects

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The effects of one week's daily treatment with dexchlorpheniramine (3 + 3 mg × 2) and loratadine (10 mg × 2) on the cutaneous reactions to putative mediators of urticarial reactions were studied in healthy subjects and in patients with chronic urticaria. Biopsy specimens were taken from skin with delayed reactions and studied immunohistochemically for the presence of eosinophilic cationic protein (ECP). In healthy subjects both antihistamines significantly decreased the weal and flare induced by histamine and the histamine releaser compound 48/80. They also reduced the flare seen after injection of PAF (platelet activating factor) and kallikrein. In patients with chronic urticaria the delayed reactions to PAF and kallikrein were larger than in healthy subjects. The immediate flare seen after injection of histamine, 48/80 and PAF, and the delayed reaction to 48/80, were significantly decreased by treatment with loratadine. No correlation was found between the clinical response and the test reactions.

In the group of healthy subjects, eosinophils were increased in the skin of all subjects after intradermal injection of 100 μg of PAF and in 50% after 1 μg of PAF, but no eosinophils were seen after injection of 1 ng of PAF, In patients with chronic urticaria the eosinophils were increased at all sites where 1 ng of PAF had been injected and also at a limited number of sites of injection of histamine, 48/80, kallikrein and saline. Treatment with the antihistamines had no effect on the influx of eosinophils in the skin. ***Key words: Eosinophilic cationic protein (ECP); Cold urticaria.***

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The inhibitory effect of antihistamines on the immediate cutaneous reactions to intradermally injected histamine and to the histamine-releasing agent compound 48/80 has mainly been studied in healthy subjects. Patients with chronic urticaria also show a delayed reaction to various mediators 5 to 10 h after the injection (1, 2). These delayed reactions and those induced by antigens in sensitized patients seem to be inhibited by some antihistamines but not by others (3–6). We have therefore studied the inhibitory effect of a classical sedating anti-histamine (dexchlorpheniramine) and of a non-sedating one (loratadine) in healthy subjects and patients with chronic urticaria. Since a dermal influx of eosinophils is a common feature in chronic urticaria (7, 8), biopsy specimens were taken from skin with delayed reactions and the number of eosinophils in sections of the specimens was studied by immunohistochemical staining the eosinophilic cationic protein (ECP).

METHODS

Subjects studied

Ten patients (2 men and 8 women aged 22–66 years) who had had severe chronic urticaria for more than one year were enrolled in the study. One of them was being treated with a beta-blocking antihypertensive. Otherwise they were not taking any drugs regularly except short-acting antihistamines, which were withdrawn at least 3 days before entry into the study. Three women (age 22–30) with cold urticaria and positive ice-cube tests were also investigated. Ten healthy volunteers (3 men and 7 women aged 25–46 years) served as controls. Dose-response tests for PAF were performed in seven other healthy subjects and in five subjects with minor non-urticarial skin problems (aged 25–6 , years). These 12 patients are referred to in the following as dose-response controls.

Agents used for intracutaneous testing

PAF-acether (Sigma, St Louis, MO) dissolved immediately before use in a PBS solution containing 0.05% human serum albumin was injected in doses of 1 ng, 100 ng and 100 μg per site. The diluent was used as control. The injected volume of all agents was 0.02 ml.

Kallikrein (Padutin®, Bayer AG, Leverkusen, Germany). To 40 iu of the dry powder, 1 ml of saline was added.

Compound 48/80 (Wellcome, Research Lab, NY) Two milligrams was dissolved in 1 ml of saline.

Histamine-coated prick needles (Phazet, Pharmacia, Uppsala, Sweden) were used with the recommended technique.

Determination of eosinophils in test sites

Three-millimetre punch biopsy specimens were taken from injection sites 5 h after the injection, snap frozen and kept at −70°C until sectioned for immunohistological examination. The immunoperoxidase (PAP) technique was used as described by Sternberger (9). The monoclonal antibody EG-2 was received from Pharmacia, Uppsala and used in a 1/100 dilution. The number of cells per section was counted (objective 16, ocular 10) and the mean from three sections was calculated. The following grading system for the number of eosinophils was used: occasional = 0–3 eosinophils per section; some increase = 4–10; a clear increase = 11–30; and a pronounced increase = >30. Controls without antibody were always performed.

Test procedure

Prick tests with histamine and intradermal injections of 0.02 ml of the other agents were made on the inner aspect of the forearm. For repeated tests the corresponding site on the other arm was used. The weal and erythematous area were outlined on a piece of transparent plastic foil after 0.2 and 5 h The area was calculated by transferring this to white paper, which was cut out and weighed on a microbalance.

After the initial test, when the patients and volunteers were free from antihistamines, they were allotted to one of two groups, to receive either dexchlorpheniramine (Polarimine® prolongatum 3+3 mg, Schering Plough NJ) or loratadine (Clarityne® 10 mg, Schering Plough NJ) orally twice daily for one week. The intracutaneous tests were then repeated and the effects and side effects recorded. Thereafter the patients were switched to the other antihistamine for a week, after which they were again tested and the effects of the treatment

Table I. *Effects of antihistamines in patients with chronic urticaria.*

	Dexchlorpheniramine	Loratadine
Good effect	10	8
No effect	0	2
Tired	7	1
Preference	2	7

were assessed. The person performing the tests was unaware of the type of antihistamine administered.

Statistical analysis

The mean response area ± its standard error of the mean was calculated for each agent tested. The differences in the sizes of weals and erythematous areas within and between urticaria patients and healthy volunteers were analysed with the two-tailed t-test by comparing the mean individual differences in the cutaneous reactions before and after each treatment.

RESULTS

The clinical effects of the antihistamines in chronic urticaria are shown in Table I. No correlation was found between the clinical response and the skin reactions to the tests. In healthy subjects no tiredness or side effects were reported after treatment with loratadine whereas seven said that they were very tired after receiving dexchlorpheniramine and one also had nausea and vertigo on the first day.

In the ten healthy volunteers the weal and flare seen 15 min after injection of histamine, and compound 48/80 were decreased by both antihistamines (Table II). The erythema seen 15 min after injection of kallikrein and PAF were also decreased. The effects on the reactions to the injected substances in patients with chronic urticaria are shown in Table III. Loratadine caused a significant decrease in the flare observed 15 min after injection of histamine, compound 48/80 and PAF, whereas dexchlorpheniramine only inhibited the PAF-induced erythema. When the immediate reactions in patients with chronic urticaria without any treatment were compared with those in untreated healthy volunteers the histamine ($p<0.001$), the kallikrein- and PAF-induced flares were found to be less pronounced ($p<0.05$) in the patients. The delayed kallikrein- and PAF-induced weals were significantly larger ($p<0.02$–0.01) in patients with chronic urticaria than in healthy volunteers. The dose-response curves for PAF in the dose-response controls and in patients with chronic urticaria are shown in Fig. 1. In patients with chronic urticaria the erythematous weal seen 5 h after injection of PAF was larger with all doses tested compared with than in the dose-response controls. No such difference in weal size was noted after 15 min (Fig. 1).

In patients with chronic urticaria, eosinophils were present in all PAF tests at 5 h and the mean number of eosinophils was the same (10–40 per section) at sites where 1 ng, 100 ng and 100 µg had been injected. In all but one of the 10 healthy volunteers several eosinophils were seen at the site where the only test dose of PAF, 100 µg, had been injected. With 1 µg of PAF six of the 12 dose-response control subjects had 10 eosinophils per section and with 100 ng the eosinophils were increased in one of eight of these controls. No eosinophils were seen in dose-response controls in whom 1 ng of PAF had been injected. Treatment with dexchlorpheniramine or loratadine did not affect the influx of eosinophils either in the patients or in the controls.

In patients with chronic urticaria biopsy specimens were also taken from some sites 5 h after injection of histamine, compound 48/80, kallikrein and saline. Here eosinophils were observed in most patients studied (Fig. 2). The antihistamines showed no tendency to alter the number of eosinophils at the injection sites. Except after injection of the control solution, no indication of a correlation was found between the size of the reaction to the test substances and the number of eosinophils. Of the three patients with cold urticaria the symptoms were improved in two after treatment with dexchlorpheniramine as judged by the ice-cube-test, but these two patients complained of marked drowsiness. After treatment with loratadine one was better and none of them were tired. The reactions to the skin tests were similar to those in healthy subjects. No eosinophils were seen at the drug-induced skin-reaction sites except where 100 µg of PAF had been injected.

Table II. *Mean area (mm^2 of weal and flare (± SEM) before and after one week's treatment with dexchlorpheniramine and loratadine in ten healthy volunteers.*

	Before			Dexchlorpheniramine			Loratadine		
	15 min		5 h	15 min		5 h	15 min		5 h
	weal	flare	weal	weal	flare	weal	weal	flare	weal
Histamine	48	924	0	32**	600**	0	24*(*)	168***	0
	±4	±76	–	±3	±95	–	±4	±34	–
48/80	187	1944	516	127*	1224*(*)	312	108**	852***	240
	±20	±129	±148	±17	±150	±89	±11	±121	±60
PAF 100 µg	276	1332	264	129	708***	144	120	558***	60
	±129	±114	±159	±14	±83	±68	±19	±95	±19
Kallikrein	82	576	72	96	288**	31	103	12**	42
	±14	±186	±23	±9	±156	±9	±8	±15	±11

*$p < 0.05$, *(*)$p < 0.02$, **$p<0.01$, ***$p < 0.001$ compared with pre-treatment values.

Table III. *Mean area (mm²) of wheal and flare (± SEM) before and after one week's treatment with dexchlorpheniramine and loratadine in ten patients with chronic urticaria.*

	Before			Dexchlorpheniramine			Loratadine		
	15 min		5 h	15 min		5 h	15 min		5 h
	weal	flare	weal	weal	flare	weal	weal	flare	weal
Histamine	43 ±5	367 ±98	23 ±14	28 ±4	490 ±120	7 ±7	37 ±5	184* ±94	6 ±4
48/80	192 ±31	1549 ±262	899 ±308	174 ±34	1334 ±274	516 ±133	248 ±139	767** ±230	316*(*) ±89
PAF 100 µg	202 ±37	1034 ±151	896 ±239	156 ±19	677 ±89	671 ±234	148 ±22	511*** ±124	498 ±160
PAF 100 ng	88 ±1	300 ±65	75 ±18	107 ±20	162 ±60	115 ±36	84 ±13	223*(*) ±124	71 ±12
PAF 1 ng	61 ±8	101 ±62	29 ±12	66 ±11	41 ±17	30 ±11	61 ±7	18 ±18	18 ±8
Kallikrein	131 ±17	197 ±66	232 ±77	131 ±19	242 ±148	318 ±103	102 ±16	146 ±68	343 ±91
Control	13 ±5	22 ±22	22 ±13	13 ±5	0	20 ±12	8 ±5	0	34 ±17

* $p<0.05$, *(*) $p<0.02$, ** $p<0.01$ compared with pre-treatment values.

DISCUSSION

In this study the most striking difference between patients with chronic urticaria and healthy subjects was the more pronounced delayed reactions in the former to the various mediators injected, in accordance with previous findings (1–5). In the case of PAF this was evident with all doses used. The parallel log dose-response curves for weal area at 5 h (Fig. 1) indicate that the enhancing factor in chronic urticaria is independent of the dose injected. In biopsy specimens from skin with these delayed reactions in patients with chronic urticaria an increased number of eosinophils was found after injection of all the mediators used. Such an increase has previously been described after PAF and antigen injection in sensitized pa-

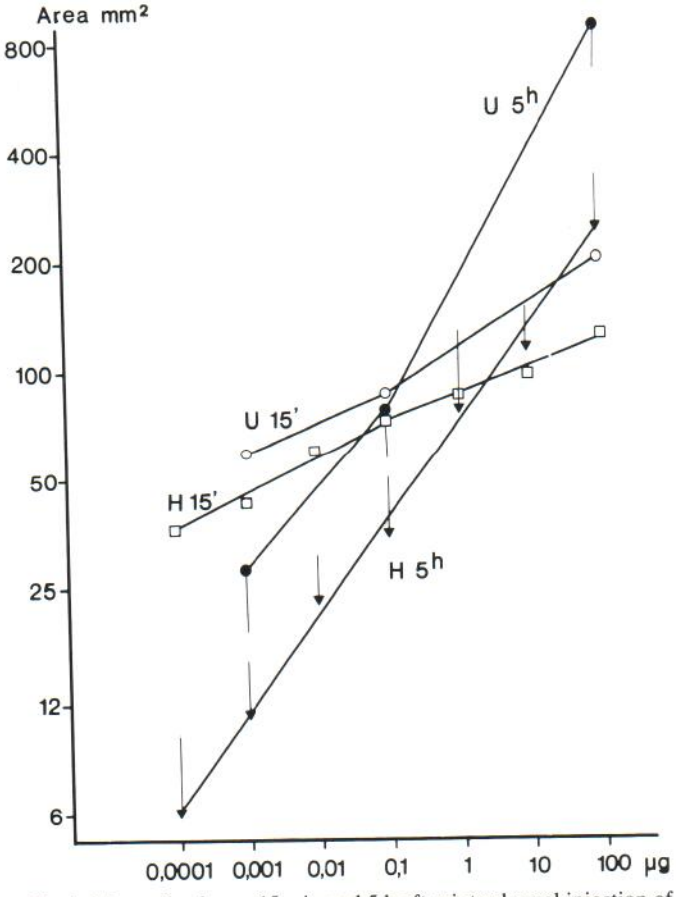

Fig. 1. Mean wheal area 15 min and 5 h after intradermal injection of various doses of PAF in patients with chronic urticaria (U15' ○—○ and U5h ●—●) and in healthy dose-response controls (H15' □—□ and H5h ▼—▼. Vertical lines indicate standard error of the mean.

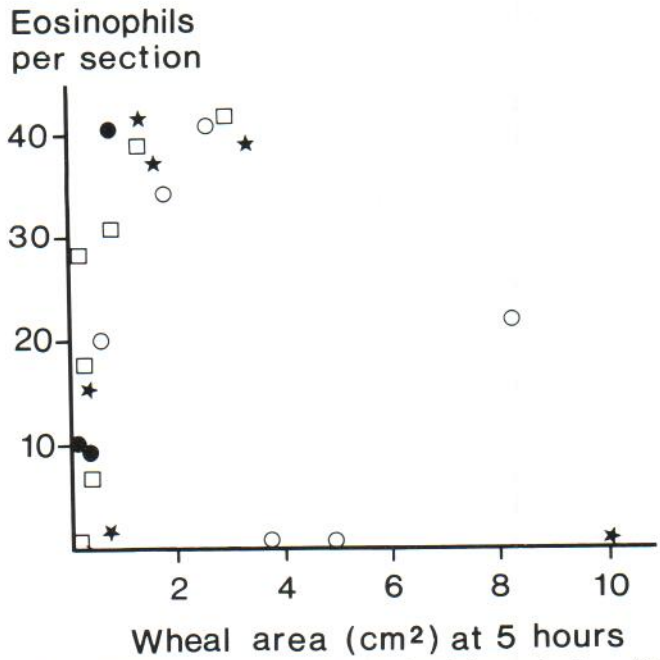

Fig. 2. Number of eosinophils per section in relation to wheal area 5 h after injection of histamine, (●) compound 48/80 (★), kallikrein (○) and saline (□) in patients with chronic urticaria.

tients (10–12). Frew & Kay (13) made similar observations after injections of PAF in patients with asthma, whereas in those with allergic rhinitis the eosinophil increase was only seen after use of high doses. In healthy subjects an eosinophil increase has not previously been reported after injection of PAF but was found to occur here when the dose was increased to 100 μg per site. Histamine injection does not induce an increase in eosinophils in normal skin (14), but when applied to denuded cantharidin blisters in a previous study a marked eosinophilotactic effect was evident (15), indicating that it needs some co-factor. It has been suggested that some patients with urticaria may have a disorder of histamine metabolism with a possible defect of the degrading enzyme diamine oxidase (16).

The lack of correlation between the size of the weal at 5 h and the number of eosinophils at the site of injection conforms with the finding after testing with antigens (12) and shows that eosinophils are not, at least solely, responsible for the delayed reactions but that other factors must also be involved.

The decreased immediate flare in chronic urticaria in response to histamine and kallikrein, compared with that in healthy subjects, deserves a special comment. Since none of the urticaria patients had taken any long-acting antihistamines or short-acting H-1 blockers for the last 72 h, the decreased flare compared with that in healthy subjects should not have been caused by any remaining drug. The mean age of the urticaria patients was only slightly higher and could not have been sufficient to explain the difference. Depletion of histamine after a previous urticarial reaction cannot be ruled out, since tachyphylaxis to histamine has been described (17). Another possible explanation could be an increase in endorphins, which are known to decrease the flare in urticaria (18).

In healthy subjects treatment with dexchlorpheniramine and loratadine diminished both the immediate flare and the weal reaction induced by histamine and compound 48/80 and significantly reduced the PAF-induced flare. These effects are similar to those reported after treatment with chlorpheniramine (4), but differ from the effects of azelastine and ketotifen, which also inhibited the PAF-induced weal (19). The reason for the effect of these latter drugs on the weal formation may be that they are more powerful antihistamines or that they might inhibit mast cell activation. In favour of the latter explanation are the recent findings that PAF does not release histamine from dispersed cutaneous mast cells (20).

In our patients with chronic urticaria only the immediate flare, and not the weal, was inhibited by dexchlorpheniramine and loratadine. This indicates that in addition to histamine other factors could be involved in the reaction as early as 15 min after the injection (21). The augmented response to kallikrein in patients with chronic urticaria was not influenced by the two antihistamines studied indicating that in such patients the kallikrein-kinin system may be overreactive.

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