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Antihistamines do not inhibit the flare induced by the intradermal injection of autologous plasma in chronic urticaria patients

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Key words

Chronic urticaria, autoreactivity, skin testing, histamine

Summary

Background: *There is some evidence suggesting that factors other than autoantibodies to FceRI or IgE and histamine released from mast cells may play a role in skin autoreactivity that characterizes many patients with chronic urticaria (CU) and, possibly, in the pathogenesis of this disease.* ***Objective:*** *The effect of antihistamine treatment on autologous plasma skin test (APST) in patients with CU was assessed.* ***Methods:*** *24 patients with CU underwent autologous plasma skin test (APST) as well as SPT with histamine 10 mg/ml while taking antihistamines. In 6 cases the same tests had been carried out also before the start of antihistamine treatment. Plasma levels of D-dimer, prothrombin F1+2 fragment, and vascular endothelial growth factor (VEGF) were measured in 21 patients.* ***Results:*** *21/24 (87%) patients showed a large flare on APST while taking antihistamines while the skin reaction to histamine 10 mg/ml was abolished or negligible. Little difference in the autologous plasma-induced flare was seen before and after the start of cetirizine therapy in 6 cases, whereas the drug exerted a marked effect on the histamine SPT as well as on the autologous plasma-induced wheal. The APST-induced flare was not associated with patients' response to antihistamine. Plasma levels of VEGF, prothrombin F 1+2 fragment, and D-dimer were increased in plasmas from 8, 9, and 2 patients, respectively.* ***Conclusions:*** *Factors other than histamine are probably involved in the flare following APST in CU; such factors might play a pathogenic role particularly in patients not responding to standard antihistamine treatments.*

Introduction

Chronic urticaria (CU), defined as the recurrent occurrence of short-lived wheals with or without angioedema for more than 6 weeks, has remained an obscure disorder until Grattan and co-workers observed that the intradermal injection of autologous serum (ASST, autologous serum skin test) caused a wheal-and-flare reaction in a proportion of patients (1). This prompted the presence of circulating histamine-releasing factors, which was confirmed by the following detection of functional IgG autoantibodies to IgE (2) and/or to the high affinity IgE receptor, FcεRI (3-5). This finding provided an immunological pathogenic basis for at least a proportion of patients with this disease. Although some scientists suspect that all CUs might be autoimmune in origin (6), autoantibodies

can be detected in less than 50% of sera from CU patients (5-12), and a number of observations seem to put their clinical role into question. FcεRI autoantibodies can be detected also in clinical conditions other than CU, such as autoimmune diseases and bullous dermatoses (13), and even in normal subjects (14, 15), although in these cases they seem not functional. Further, sera from CU patients containing FcεRI autoantibodies are still able to induce a wheal-and-flare reaction upon ASST after depletion of IgG (7). Finally, while CU sera causing histamine release from cultured human basophils in-vitro score regularly positive on ASST, only a proportion (about 50%) of ASST-positive sera induce histamine release in-vitro (12). All these observations suggest that skin autoreactivity occurs also in the absence of circulating autoantibodies and point to the possible involvement of factors other than autoantibodies in the pathogenesis of CU.
Recent observations that in CU patients the intradermal injection of autologous plasma anticoagulated with Na citrate (APST, autologous plasma skin test) produces a wheal and flare reaction much more frequently than ASST (16) led to detect an activation of the coagulation cascade via the extrinsic pathway in this disease (16-19). In view of these findings, factors other than histamine, such as thrombin, have been suggested as potential mediators of vasodilatation in CU. The present study adds further evidence to this concept showing that in CU the skin reaction produced by the intradermal injection of autologous plasma is only partially inhibited by histamine.

Methods

Patients

24 patients (M/F 5/19; mean age 51,8 years, range 27-85 years) with chronic urticaria seen at the allergy department of the Clinica San Carlo were studied. The diagnosis of CU was based on the presence of recurrent wheals with or without angioedema for more than 6 weeks. Eighteen patients were taking antihistamines (cetirizine 10 mg daily in all cases but 2 that were taking desloratadine 5 mg daily) at the time of the first visit and were unable to discontinue the treatment due to the immediate relapse of their disease. The remaining 6 patients were not taking antihistamines at the time of the first visit. Clinical activity of CU was assessed according to Sabroe et al.: 1-10 small (< 3 cm in diameter) wheals = grade 1 (slight); 10-50 small wheals or 1-10 large wheals = grade 2 (moderate); > 50 small wheals or > 10 large wheals = grade 3 (severe) (20).

Skin tests

All 24 patients underwent intradermal testing with 0.05 ml of fresh autologous plasma anticoagulated with Na citrate (APST, autologous plasma skin test) as previously described (16); an intradermal test with 0.05 ml of saline as well as a SPT with histamine 10 mg/ml were carried out in all cases as negative and positive control, respectively. The 18 patients who were unable to stop antihistamine treatment underwent skin tests while taking their therapies, whereas the remaining 6 underwent the skin tests both at the first visit and 7 days after the start of antihistamine treatment (cetirizine 10 mg daily). All patients gave an informed consent before the skin tests.
Readings were taken at 15 minutes when the wheal-and/or-flare skin reaction diameters were measured. Even though a 30 minutes reading has become a standard practice, the wheal-and-flare response usually appears within 10 minutes (21) and we have taken readings at 15 minutes also in previous studies with excellent results (12, 22). Further, although a wheal-and-flare reaction is generally needed to regard as positive the skin response following an autologous serum skin test (23), in view of the antihistamine treatment taken by our study patients, the clinical criteria for a positive skin test were slightly changed, and a clear-cut flare in absence of a palpable wheal was considered as a positive skin response if the intradermal injection of saline did not produce any appreciable skin reaction.

In-vitro tests

Plasma levels of D-dimer, prothrombin F1+2 fragment, and vascular endothelial growth factor (VEGF) were measured in 21/24 patients.
D-dimer levels were measured by ELISA (Enzygnost D-dimer; Behring Diagnostics GmbH) according to manufacturer's instructions. The intra- and inter-assay coefficients of variation were 10% and 15%, respectively.
Prothrombin fragment F_{1+2}, a marker of thrombin generation, was measured by a sandwich immunoenzymatic assay (Enzygnost F_{1+2}; Behring Diagnostics GmbH, Frankfurt, Germany) according to manufacturer's instructions. Intra-assay and inter-assay coefficient of variations were 5% and 8% respectively. The measuring range of the assay is between 20 and 1200 pmol/L.

Table 1 - Patients, skin tests without and with antihistamines, and response to treatment

Patient	Sex/age	APST (no therapy)	SPT H (no therapy)	APST (therapy)	SPT H (therapy)	Response
1	F/34	ND		flare 20 x 20 mm	Negative	Absent
2	F/37	flare 20 x 20 mm	12mm	flare 20 x 20 mm	1mm	Good
3	F/76	ND		flare 10 x 10 mm	Negative	Good
4	F/69	flare 15 x 15 mm	10 mm	flare 10 x 10 mm	2 mm	Good
5	F/62	flare 20 x 20 mm	12 mm	flare 15 x 10 mm	5 mm	Good
6	F/51	ND		flare 30 x 30 mm	2mm	Poor
7	F/29	ND		flare 40 x 40 mm	4 mm	Poor
8	F/41	ND		flare 20 x 20 mm	2 mm	Good
9	F/27	ND		flare 15 x 15 mm	2 mm	Good
10	F/51	ND		flare 12 x 12 mm	2 mm	Poor
11	M/54	ND		flare 8 x 8 mm	2 mm	Sufficient
12	F/32	ND		flare 10 x 10 mm	2 mm	Sufficient
13	F/64	ND		flare 10 x 10 mm	2 mm	Good
14	F/85	ND		flare 14 x 14 mm	4 mm	Sufficient
15	F/60	ND		flare 18 x 10 mm	3 mm	Good
16	F/27	flare 15 x 15 mm	11 mm	Negative	2 mm	Good
17	F/65	ND		flare 10 x 10 mm	3 mm	Good
18	M/36	ND		flare 12 x 12 mm	Negative	Good
19	F/47	flare 25 x 20 mm	14 mm	flare 20 x 20 mm	3 mm	Sufficient
20	F/62	ND		Negative	Negative	Poor
21	M/59	flare 20 x 20 mm	14 mm	flare 8 x 8 mm	5 mm	Poor
22	M/44	ND		flare 14 x 12 mm	4 mm	Poor
23	M/67	ND		Negative	2mm	Sufficient
24	F/64	ND		flare 12 x 10 mm	5 mm	Sufficient

The response to antihistamine treatment was considered good if the drug fully controlled the disease, sufficient in case of a significant reduction but not of complete disappearance of wheals, and poor in case of a lack of response.

VEGF concentration was measured by a sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN, USA), according to manufacturer's instructions. Intra-assay and inter-assay coefficients of variation were 5% and 7%, respectively. The detection limit of the assay is less than 0.1 pmol/L and the upper limit is 22.2 pmol/L. The assay employs a monoclonal antibody, pre-coated onto a microplate, and an enzyme-linked polyclonal antibody conjugated to horseradish peroxidase, both specific for VEGF. After drawing venous blood from the subjects under examination, plasma was frozen at –80°C until assayed for VEGF concentration. Mean plasma VEGF level in 53 normal subjects was 0.54±0.08 pmol/l (range 0.1-2.11).

Statistics

Proportions were compared by X^2-test with Yates' corrections. Probability (p) values less than 5% were considered statistically significant.

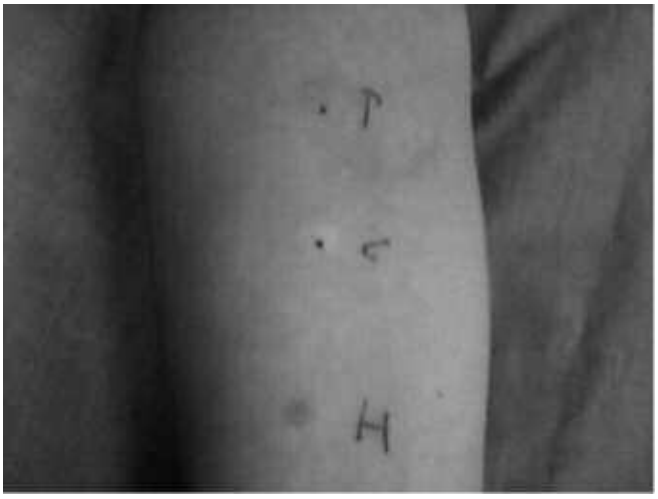
Results

Skin tests

At the time of skin testing and blood drawing, all patients were under antihistamine treatment and the clinical score ranged between 0 and 1. Twenty-one out of 24 (87%) patients showed a marked skin reaction on APST while taking antihistamines. Interestingly, the skin reaction induced by autologous plasma consisted of a large flare (diameter range 8 x 8 mm - 40 x 40 mm) with little or no wheal; in contrast, not surprisingly, the skin reaction to histamine 10 mg/ml was abolished or negligible (diameter < 3 mm) under antihistamine therapy in most cases (Tab. 1). A typical case is shown in figure 1.

The 6 patients who were examined both before and after the start of cetirizine treatment showed a marked wheal-and-flare reaction upon intradermal injection of autologous plasma while off antihistamine treatment (the flare

Figure 1 - A typical case of persistence of APST-induced flare in a patient treated with cetirizine 10 mg daily. Autologous plasma (P) induces a flare that largely exceeds that induced by a SPT with histamine 10 mg/ml (H). The intradermal injection of saline does not cause any visible skin reaction



diameter is shown in table 1); in these patients histamine SPT induced an intense skin reaction as well. Cetirizine treatment abolished or markedly reduced the skin response to histamine. Interestingly, the drug abolished the palpable wheal in all 6 patients but exerted a variable effect on the flare induced by autologous plasma which was abolished in 1 case (no. 16), slightly reduced in 3 patients (4, 5 and 21), and virtually unchanged in 2 cases (no. 2 and 19).

The intradermal injection of saline did not cause any skin reaction in all patients either taking or not taking antihistamines.

The flare response induced by autologous plasma was not significantly associated with patients' response to antihistamine treatment (Tab. 1).

Plasma measurements

Levels of VEGF, prothrombin F 1+2 fragment, and D-dimer were increased in plasmas from 8, 9, and 2 patients, respectively. Interestingly the two patients showing elevated D-dimer levels showed increased plasma levels of both VEGF and F 1+2 as well. Elevated plasma levels of both VEGF and F 1+2 were observed only in 2 further cases (Tab. 2).

Table 3 - Plasma VEGF, F 1+2, and D-dimer levels in the 24 study patients

Patient	VEGF (pmol/l)	F 1+2 (pmol/L)	D-dimer (pmol/l)
1	5.390	119.82	0.17
2	0.37	152.24	0.63
3	2.9	138.82	0.69
4	0.33	173.61	0.88
5	8.11	145.25	0.75
6	0.1	121.53	1.62
7	6.97	134.31	0.34
8	0.66	247.84	1.49
9	0.1	233.72	0.85
10	43.1	422.13	10.39
12	0.32	280.42	0.69
13	1.55	214.69	2.02
14	5.66	358.43	5.19
15	2.45	304.65	2.01
16	0.80	387.25	0.24
19	0.30	83.14	0.78
20	4.43	309.98	1.79
21	1.09	163.82	1.14
22	2.39	110.22	0.38
23	5.46	179.86	0.37
24	0.1	293.82	0.50
Mean	4.88	217.88	1.56
SD	9.66	98.69	2.29
SEM	2.1	21.53	0.50
Median	1.55	179.86	0.78

Mean VEGF plasma level in 53 healthy subjects was 0.54±0.08 pmol/l (range 0.1-2.11).
F 1+2 normal range: 69 - 229 pmol/L.
D-dimer normal range: 0.5-4 pmol/l.

Discussion

In this study we found that in most patients with chronic urticaria treated with antihistamines the intradermal injection of autologous plasma still induces a clear-cut flare. The clinical effect of antihistamine therapy was shown by the markedly reduced or absent skin reaction to histamine 10 mg/ml. Further, although few patients were studied in this sense, cetririzine treatment caused the disappearance of the palpable wheal induced by the intradermal injection of autologous plasma, whereas the APST-induced flare persisted. This observation suggests that the flare

(vasodilation) elicited by the intradermal injection of autologous plasma is only partially dependent on histamine release from skin mast cells. The vasodilation induced by autologous plasma upon intradermal injection was not associated with patient's better or worse response to antihistamine treatment nor was associated with disease severity. This finding supports a role played by vasoactive factor(s) other than histamine which are present in plasma of most patients with chronic urticaria; it is possible that this substance plays a role in amplifying the histamine-induced vasodilation in CU and may be responsible for the limited response to antihistamine treatment that characterizes some CU patients. We recently found that both F 1+2 prothrombin fragment and D-dimer plasma levels may be increased in patients with CU as a result of a (sometimes) massive activation of the coagulation cascade by the extrinsic pathway (16-18), and that CU is frequently associated with elevated VEGF levels, possibly as a result of the activation of eosinophils (19, 24). All these phenomena are associated with disease severity. Thus, in view of their potential vasoactive properties, we investigated whether one of these 3 substances was associated with the flare induced by autologous plasma on intradermal injection in patients unable to stop antihistamine treatment. Our plasma measurements showed that VEGF and F 1+2 were frequently elevated, however it was not possible to detect a clear association with the skin reaction induced by autologous plasma. Other potential candidates responsible for histamine-independent vasodilation induced by the intradermal injection of autologous plasma include neuropeptides released from sensory nerves of the skin. However, in a previous study we were unable to detect any increase in circulating substance P in most patients with CU (25), and other groups have shown that the wheal-and-flare reaction induced by substance P is inhibited by cetirizine (26), although this effect was not observed with hydroxyzine (27). Interestingly, it has been shown that CU serum is able to induce de-novo synthesis of sulfidoleukotrienes (28), however, in the same study, such effect was inhibited by the anti-histamine mizolastine which does not seem to correspond to our present observations.

Previous studies showed that in CU patients positive on autologous serum skin test (ASST) the intradermal tests with heparin-anticoagulated plasma always scores negative, suggesting that heparin inhibits autoreactivity in-vivo (11, 12). Interestingly, heparin exerted its inhibitory effect in all ASST-positive patients irrespective of the ability of their sera to induce histamine release in-vitro on cultured basophils, but did not inhibit the wheal-and-flare reaction induced by a SPT with histamine nor the skin reaction induced by a SPT with a specific allergen extract in an allergic subject (12). These observations prompted that histamine is not the target of heparin, at least in-vivo; in effect, besides its well-known anticoagulant activities, heparin is able to interact with a number of plasma proteins and mast cell surface components. These previous findings fit rather well with the recent observation of an activation of the coagulation cascade with generation of thrombin in CU patients (16-19) as, in experimental models, thrombin has been shown to induce edema through an increase in vascular permeability, to trigger mast cell degranulation, to activate protease activated receptor-1 on mast cells, and to generate C5a in the absence of C3, thus bypassing the whole first part of the complement cascade (29-33).

In conclusion, along with histamine, vasoactive substances other than histamine seem to be involved in the marked vasodilation induced by autologous plasma upon intradermal injection in CU; it is possible that such substances play a pathogenic role in the disease and may particularly relevant in patients showing a poor response to antihistamines.

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