

Effects of systemic corticosteroids on cutaneous histamine secretion and histamine-releasing factor in patients with chronic idiopathic urticaria

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Summary

Background Enhanced skin mast cell releasability of histamine, increased production of histamine releasing factor (HRF), and cutaneous inflammatory process are the hallmarks of chronic idiopathic urticaria (CU). Although H₁-antihistamines are known to alleviate the symptoms effectively in most cases, systemic corticosteroids (CS) are given in more resistant patients. Their mode of action remains a matter of controversy.

Objectives In the present study, the effects of a 7-day course of CS or placebo on histamine content and HRF production in non-lesional skin of 19 CU patients were examined.

Methods Using the skin chamber technique, HRF production and histamine content were assessed in normal-appearing skin of patients with CU over a 2-h observation period. Those two parameters were measured before and after treatment, in a double-blind fashion.

Results No significant changes occurred in any parameters after placebo treatment. In contrast with this, significant decrease of HRF activity was observed after 1 week of oral methylprednisolone while no change was documented for histamine secretion.

Conclusion These data suggest that CS therapy improves symptoms of CU in association with a decreased production of HRF in uninvolved skin.

Keywords: urticaria, histamine, HRF, corticosteroids, mast cell

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Introduction

Chronic idiopathic urticaria (CU) is mainly characterized by abnormal histamine release (HR) [1] and more specifically by an increase in skin mast cell releasability of histamine [2]. The disease is usually managed with various anti-H₁ and anti-H₂ agents which provide symptomatic relief and even complete remission. We have previously studied the influence of antihistamine drugs on the profile of cutaneous HR in patients with CU: no effect on skin mast cell releasability of histamine after 1

month of treatment was demonstrated despite distinct clinical improvement [3]. In severe cases, more potent agents, such as systemic glucocorticosteroids (CS), may be required to control the disease, in combination with antihistamines [4]. The exact mechanisms by which CS or other anti-inflammatory drugs act in CU disease is not entirely understood. It is clear that an inflammatory process is involved in this disorder as demonstrated by dermal infiltration of activated CD4 positive lymphocytes [5] and increased cutaneous production of histamine-releasing factor (HRF) [6]. These two phenomena are indeed closely linked [6,7] and it is interesting to speculate that CS may act, at least in part, at this level.

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In order to further delineate the physiopathology of CU and to better understand the mechanism of action of CS in this disease, we used a double-blind protocol to study the production of HRF and the secretion of histamine from non-lesional skin of CU patients, before and after a 7-day course of oral methylprednisolone (Mo) or placebo. The results were correlated with the clinical symptomatology.

Materials and methods

Study group

Nineteen patients were selected in this study, 14 women and five men, with a mean age of 43 years (Table 1). All patients had classic CU, as defined by symptoms of at least 6-week duration, with all known causes ruled out. All subjects had a normal physical exam with minimal dermographism, and normal laboratory tests including: complete blood count, sedimentation rate, SMA 12/60, antinuclear antibody, complement profile, thyroid function test, and urinalysis. Upon entry in the study, all patients were symptomatic, and had not received CS for at least 1 month, classic H₁ and H₂-blockers for 72 h and astemizole for 6 weeks. The study was approved by the Ethics Committee of our institution and a written informed consent was obtained from all subjects.

Design of the study

We performed a double-blind, randomized, placebo-controlled study where the patients were designated to receive either oral Mo, 32 mg once daily in the morning, or a placebo, for a 7-day period. The placebo capsules were prepared to look similar and indistinguishable from the active drug. Patients were blindly assessed by an investigator on day 0, before starting the study, and on day 7, after the treatment was completed.

The therapeutic response was assessed by the patients; each subject had to complete a daily card and report the highest score for the past 24 h for each of the following

parameters namely, the number of hives, the severity of itching and the degree of interference with daily activities and/or sleep, using a defined scoring system: 0, no itching, no hives, no discomfort, no interference with daily activity and/or sleep; 1, mild itching, 1–6 hives, mild discomfort with no interference with activity and/or sleep; 2, moderate itching, 7–12 hives, moderate discomfort with some interference with daily activities and/or sleep; 3, severe itching, more than 12 hives, severe discomfort and considerable interference with daily activity/sleep. A total 'symptom score' between 0 and 9 was then available for each CU patient.

Skin chamber technique

Skin chamber studies were conducted as previously reported [2]. The technique was performed at baseline, day 0, and on the seventh day of treatment, 2 h after the last morning dose of CS or placebo. The profile of HRF and histamine secretion was obtained by the skin chamber technique, as adapted in our laboratory [2, 6]. In short, two blisters of a 1 cm diameter base were raised on normal-appearing skin of the forearm using a heat and suction device. The blister fluid was aspirated with a 1 mL plastic syringe utilizing a 27-gauge needle and was kept frozen until assayed. Then, the collapsed blister was unroofed to expose its denuded base. After irrigation with PBS, a collection chamber was appended over the abraded skin areas, and each chamber was filled with PBS. The skin chamber fluids were then replaced with fresh PBS at 0.5, 1 and 2 h after the end of the blister formation. Samples were recovered on each occasion and stored at -70°C until histamine and HRF determinations were carried out.

Mediator assays

Measurement of histamine in the chamber and blister fluids was carried out by a radioenzymatic assay, as previously reported by our group [2]. This assay can detect histamine concentration at levels as low as

Table 1. Characteristics of the study patients

	Age (years)	Sex (F/M)	Mean duration of disease (years)	Sx score* Day 0	Sx score** Day 7	P
Placebo (n = 9)	38.7 $\pm$ 3.9	7/2	4.0 $\pm$ 2.1	6.4 $\pm$ 0.5	5.5 $\pm$ 0.8	0.2
CS (n = 10)	47.3 $\pm$ 5.5	7/3	4.7 $\pm$ 2.1	6.2 $\pm$ 0.8	2.6 $\pm$ 1.0	<0.01

* Day 0 = baseline, before treatment.

** Day 7 = after 7 days of treatment.

100 pg/mL in all biological fluids. All samples obtained were assayed on one and the same occasion, at the end of the study.

HRF activity was assessed using the *in vitro* basophil histamine release assay previously adapted by our group [8]. Briefly, heparinized blood was collected from a single atopic donor and leucocyte-enriched pellets containing basophils were obtained after sedimentation of whole blood with Dextran 6% (Dextran T-500, Pharmacia). After enrichment of leucocytes by Dextran sedimentation, the leucocyte-enriched pellet containing basophils was washed in Tris buffer (25 mmol/L, pH 7.4, 4°C) containing albumin 0.03% and ethylenediaminetetraacetic (EDTA, 8 mmol/L). After centrifugation, the pellet was washed with the same buffer without EDTA and supplemented with CaCl₂ (1 mmol/L) and MgCl₂ (0.6 mmol/L), and centrifugated. The washing buffer was then removed by aspiration, and cells were resuspended in 50 µL of cold buffer containing Ca⁺⁺ and Mg⁺⁺ at a final concentration of 2×10^6 cells/mL, and added to either 100 µL of PBS only or with skin chamber fluids. After an incubation of 20 min at 37°C, the reaction was stopped by cooling, and the cell-free supernatants were recovered for histamine determination.

The supernatants were then used for histamine determination and the HRF activity from skin chamber fluids was expressed as percentage of HR and calculated as follows:

$$\%HR = \frac{A - B}{T} \times 100$$

A = HRF-induced HR; B = Buffer-induced HR; T = Total histamine content of basophils.

Statistical analysis

The data were tested for normality using the *t* distribution test, and no significant deviations were found. Therefore the data have been summarized using means and SEMs. Differences within a group have been tested

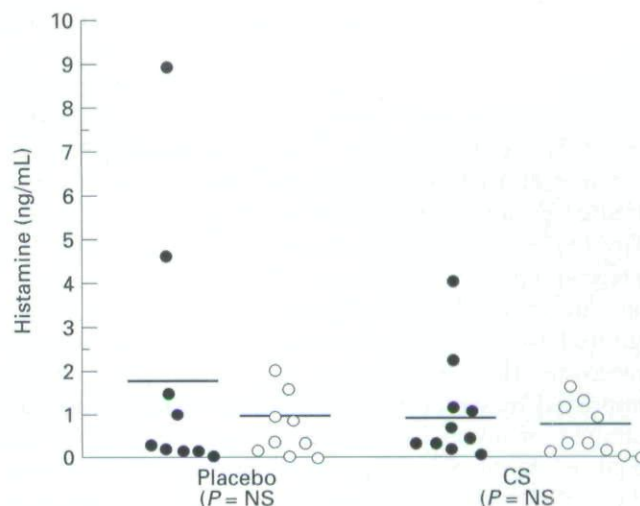


Fig. 1. Histamine concentration in skin blister fluids for each subject of the placebo and CS groups, and on day 0 and day 7. Means of histamine concentration are indicated by a solid bar (—). ●, Day 0. ○, Day 7.

using a paired *t*-test, and differences between groups with an unpaired *t*-test. All tests were one-sided and difference were considered to be significant at a *P*-value of less than 0.05. The power of the study was 73% for the steroid-treated group vs the placebo-treated group, on day 7. When steroid-treated patients were examined on day 0 and day 7, the power of the test reached 93%.

Results

Nineteen adult patients with active CU were assigned to randomly receive Mo 32 mg daily, or placebo for a period of 7 days. The mean duration of the disease was 4.0 ± 2.1 years for the placebo group and 4.7 ± 2.1 years for the CS group. The demographic distribution was comparable for the two groups and data are summarized in Table 1.

Table 2. Histamine secretion in skin chamber fluids during 2-h observation for the placebo and CS groups, before and after treatment

Time (h)	Histamine (ng/mL $\pm$ SEM)					
	Placebo			CS		
	Day 0	Day 7	<i>P</i>	Day 0	Day 7	<i>P</i>
0.5	1.0 $\pm$ 0.6	0.6 $\pm$ 0.5	0.1	1.1 $\pm$ 0.6	0.6 $\pm$ 0.5	0.1
1	2.6 $\pm$ 1.9	0.5 $\pm$ 0.3	0.1	2.2 $\pm$ 1.6	0.2 $\pm$ 0.2	0.1
2	3.0 $\pm$ 2.8	0.9 $\pm$ 0.6	0.2	0.9 $\pm$ 0.7	0.3 $\pm$ 0.3	0.2

The mean symptom score before treatment, on day 0, and after treatment, on day 7, is reported for each group in Table 1. At baseline, symptom scores were comparable for the two treated groups (6.4 ± 0.5 vs 6.2 ± 0.8 , $P = 0.5$). However, after 7 days of medication, the mean symptom score decreased only in the steroid-treated group, from 6.2 ± 0.8 on day 0 to 2.6 ± 1.0 on day 7 ($P < 0.01$). No significant change was seen for the placebo group (6.4 ± 0.5 vs 5.5 ± 0.8 , $P = 0.2$). More specifically, in the steroid group, six out of 10 patients showed marked clinical improvement within 48 h of receiving the drug while two additional subjects improved by day 4 or 5; finally, only two patients had minimal or no change in their cutaneous symptoms. In contrast, in the placebo group, eight patients out of nine did not manifest any improvement of their urticaria during the treatment phase. These results correlate with the significant decrease of symptoms in the CS group after the 7-day treatment as compared with baseline, and also when the steroid-treated patients are compared with the placebo group, on day 7 ($P < 0.01$).

Levels of histamine in blister fluids were measured for each patient both on day 0 and 7 of the study (Fig. 1). In both groups, no statistical difference in the histamine content was measured at baseline and at the end of the study. Indeed, the histamine concentration in blister fluids of the placebo group was 1.9 ± 0.9 ng/mL on day 0, and 0.8 ± 0.2 ng/mL on day 7 ($P = \text{NS}$) while in the CS group, the histamine content was at 1.1 ± 0.4 ng/mL on day 0, and 0.7 ± 0.2 ng/mL on day 7 ($P = \text{NS}$). When we looked at spontaneous HR in the skin chamber throughout the 2-h observation (Table 2), no significant difference between the two groups was seen at each point of time, and on day 0 and day 7. As a whole, treatment with systemic steroids did not alter the spontaneous histamine secretion in non-lesional skin of CU patients.

On the other hand, the HRF activity in skin blisters was significantly decreased in the steroid-treated group

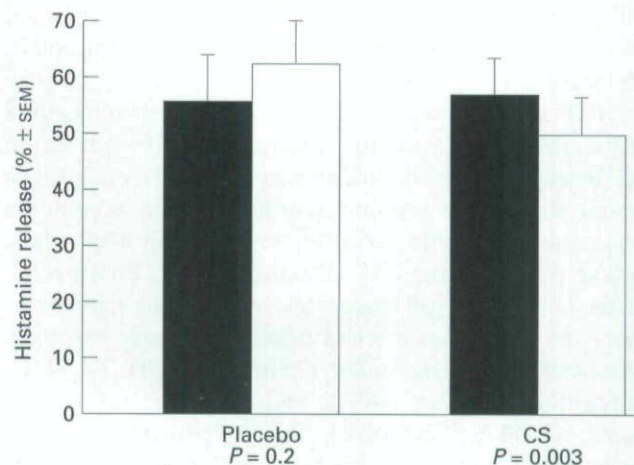


Fig. 2. The pre-and post-treatment levels of HRF, in skin blister fluids, for both placebo and CS-treated subjects. ■. Day 0. □. Day 7.

as opposed to placebo-treated patients as shown in Fig. 2. Specifically, HRF levels went from a mean of 57% at baseline to 50% after a week of Mo ($P = 0.003$) while no significant change was demonstrated in the placebo group ($P = 0.2$). Moreover, when we studied the profile of HRF production in skin chamber fluids during 2 h, the same pattern was observed. Indeed, HRF activity was definitely lower in the CS group at 0.5, 1 and 2 h as compared with the pretreatment levels (Table 3); however, statistical significance was reached only for the 30 min interval ($P = 0.007$) and for the weighted average value ($P = 0.05$) which represents the mean of HRF activity during the 2 h observation period according to the time. For the placebo group, the profile of spontaneous HRF production remained the same throughout the study ($P > 0.3$). In brief, when the skin chamber model was used, HRF recovered from non-affected skin of CU patients treated with systemic CS for a week,

Table 3. Spontaneous HRF activity in skin chamber fluids during 2 h-observation of placebo and CS groups, before and after treatment

Time (h)	HRF (% ± SEM)					
	Placebo			CS		
	Day 0	Day 7	P	Day 0	Day 7	P
0.5	48 ± 6	42 ± 6	0.3	52 ± 6	40 ± 4	0.007
1	41 ± 3	39 ± 4	0.4	42 ± 5	35 ± 3	0.06
2	53 ± 4	52 ± 4	0.5	48 ± 5	41 ± 5	0.05
Weighted average	48.75 ± 4	46.25 ± 5	NS	47.5 ± 5	39.2 ± 4	0.05

decreased significantly not only in blister fluids but over a 2 h observation period as well.

Discussion

This study shows that in CU patients, 1 week of treatment with oral Mo, at a dosage of 32 mg daily, significantly decreases the symptoms of urticaria and HRF production in non-lesional skin, when compared with a placebo-treated group. In contrast with this, histamine secretion is not influenced by the same regimen of CS. These two different effects of systemic CS on histamine and HRF in CU should be placed within the perspective of the multifactorial aspects involved in the pathophysiology of the disease.

Most reports agree that the final common pathway in the formation of hives is histamine liberation from cutaneous mast cells [9]. More importantly, non-lesional skin of CU patients displays a state of mast cell hyperactivity [2] which is present only during active disease and not in the remission phase [10]. In the present study, the profile of spontaneous histamine release remains unaltered by CS, at least on a short-term basis, in spite of clear clinical improvement. However, some patients continued to show some signs of disease activity. To achieve better control of the symptoms or even a remission, a longer or a more intensive treatment, such as one involving the addition of an anti-H₁ drug, might have been required. In fact, it would be interesting to evaluate the effect of a prolonged course of CS, perhaps as long as 1 month, on cutaneous histamine secretion and determine if that regimen could modify the state of increased mast cell releasability. In addition, however, the absence of effects of CS on histamine release by mast cells has been reported by others. As a matter of fact, Dunskey *et al.* [11] showed in atopic volunteers that one dose of intravenous methylprednisolone had no effect on skin mast cells and their histamine content, in contrast with a significant decrease not only in the total basophil counts of peripheral blood but also in the histamine content of circulating leucocytes. The same group of investigators [12] has also reported that CS exert no effect in atopics on weal-and-flare responses to either allergen, histamine, or compound 48/80 intradermal injections. On the other hand, different results were observed when topical steroids were studied. Indeed, two studies have shown that the use of topical CS, as a different route of administration, can decrease the histamine content of the skin or the nasal mucosa. First, Lavker *et al.* [13] demonstrated that the use of topical steroids on the skin of 17 normal volunteers significantly decreased the number of cutaneous mast cells and, consequently, reduced the skin histamine content, after a 6-week test period. This long-

term toxic effect of topical CS on skin mast cells was a reversible phenomenon since mast cell number returned to normal levels within 2–3 weeks after discontinuation of the treatment. Not only does their study design diverge from our own in respect of the route of administration, it also presents two other major differences: the duration of therapy and the characteristics of subjects.

In a later study from Johns Hopkins University [14], there was a clear inhibition of histamine release in nasal lavages of allergic rhinitis patients challenged with pollen extracts when they were pretreated with topical glucocorticosteroids for one week. However, comparisons with our model should take into account the differences in many variables such as the ones cited for the Lavker's study, in addition to the non IgE-mediated nature of CU for many patients as opposed to common allergic diseases. Of significant interest, the Johns Hopkins group [15] also showed a lack of effect on histamine secretion during early phase reaction, in the same nasal-challenge model, when patients were pre-treated with systemic CS for 2 days, whereas during the late reaction, prednisone reduced the histamine recovered in lavages.

The second part of our study relates to an important pathogenic feature of CU, which is the increased cutaneous production of HRF as a modulator of skin mast cell and a possible marker of cutaneous inflammation in this disease [6,7]. In contrast to histamine liberation, CS treatment significantly decreases the production of HRF in non-lesional skin, whereas no changes were observed in the placebo group. Lower HRF activity followed the clinical improvement of patients. However, the changes in HRF were small and levels did not reach normal range, which is in agreement with an improved clinical condition but still active. As mentioned earlier, a longer period of treatment and/or a higher dose of CS might have been needed to achieve complete disappearance of symptoms and a greater change in HRF concentration. Also, a more pronounced decrease in HRF levels might be necessary in order to further modify histamine secretion. Another possibility which might explain those findings could be that HRF molecules do not play a major role in CU disease; this, however, seems less likely since our previous work [6] has shown that increased concentration of HRF were present not only in normal-appearing skin of CU patients but also in the urticarial lesions, making HRF a possible modulatory factor of histamine secretion in this disorder. In fact, this observation does not exclude the participation of other molecules or co-factors for histamine release in CU. In other models, mainly seasonal allergic rhinitis [8] and asthma [16], it has been proven that successful treatment with specific immunotherapy has been followed by a decrease of HRF activity as well. Although CU does not represent

an allergen IgE-mediated disorder, the reduction of HRF levels after successful treatment strengthens the importance of HRF molecules in the pathogenesis of many mast cell-related diseases. In the present experiment, the exact nature of HRF recovered has not been further characterized in regard to chemokines such as RANTES and MIP-1 (MCP-1). Moreover, our data do not provide us with the exact mechanisms of action by which CS can modify HRF production. Knowing the action of CS on cellular inflammatory responses [17], and the dermal inflammatory cell infiltrates found in urticarial lesions [5], one could speculate that CS express their anti-inflammatory effect by preventing the accumulation of T cells in the affected skin and/or by a direct decrease of cytokine production, including HRF. This point needs further evaluation since the cell source of HRF in CU has not been precisely identified.

Other considerations have also to be discussed in regard to the study protocol. Although our clinical trial was performed according to a double-blind design, the patient's compliance with medication regimens was assessed only by medication count. Additional tests such as lymphocyte counts, cortisol levels or eosinophilia have not been completed but should be included in future studies. Finally, before completely ruling out any direct effect of systemic CS on cutaneous mast cell histamine secretion in CU patients, we would like, in the future, to look at kinetics of histamine liberation in skin chambers after challenge with compound 48/80 or anti-IgE in patients treated with oral steroids or placebo. Furthermore, it would be interesting to measure both HRF and histamine in blister fluids obtained from residual hives after 1 week of Mo treatment and compare it to blister fluid histamine and HRF recovered from fresh hives of comparable size before treatment.

To summarize, at this time, our studies lead us to believe that glucocorticoids administered systemically can, in the short term, successfully treat moderate to severe chronic idiopathic urticaria. It also suggests that the activity of these drugs is directed at first toward the inhibition of the inflammatory events rather than to mast cell histamine release.

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