

Cellular adhesion molecules in chronic urticaria: modulation of serum levels occurs during levocetirizine treatment

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Summary

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Key words

cellular adhesion molecule, chronic autoimmune urticaria, chronic urticaria, enzyme-linked immunosorbent assay, levocetirizine

Conflicts of interest

None declared.

Background Some antihistamines are capable of reducing levels of adhesion molecules in wealing tissues of patients with chronic urticaria (CU).

Objectives To determine if 6 weeks of therapy with levocetirizine 5 mg once daily would also induce any decrease in serum levels of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, endothelial leucocyte adhesion molecule-1 (ELAM-1) or P-selectin in subjects with CU and chronic autoimmune urticaria.

Methods Thirty-six patients with CU (18 with positive and 18 with negative autologous serum skin test) were studied, together with 10 control healthy subjects. All patients received levocetirizine 5 mg daily. Serum soluble cellular adhesion molecule (CAM) levels were determined by immunoenzymatic assay before and after the end of the study period. Disease activity was recorded according to the EAACI/GA²LEN/EDF scoring system.

Results After levocetirizine therapy CAM levels decreased in patients with CU, significantly in the cases of ELAM-1 and P-selectin. Patients' clinical scores improved during regular antihistamine therapy.

Conclusions Levocetirizine 5 mg daily demonstrated a broad anti-inflammatory effect in patients with CU. The significant decrease in serum levels of ELAM-1 and P-selectin might reflect the inhibitory activity on neutrophil rolling and extravasation towards inflamed skin.

The pathogenesis of chronic urticaria (CU) is still partially unknown, and in 70–80% of cases clinical manifestations occur daily for longer than 6 weeks without any apparent causative agent.¹ Recently there has been considerable interest in the presence of circulating functional IgG antibodies in patients with CU, leading to the possibility of autoimmune mechanisms underlying the disease.²

These autoantibodies, directed against the high-affinity IgE receptor of mast cells and basophils (FcεRI) or against IgE itself,³ seem to be responsible for positivity to the intradermal autologous serum skin test (ASST), that is largely used to identify the supposedly autoimmune subset of CU (chronic autoimmune urticaria, CAIU).⁴

Although mast cells, which release histamine and other mediators after IgE binding, remain the primary trigger of early-phase wealing response, a wide variety of molecules is thought to sustain the late phase of CU, in selective recruitment and activation of inflammatory cells leading to tissue damage. Thus, a large set of literature on CU has investigated

the pathogenic involvement of cellular adhesion molecules (CAMs),^{1,5,6} a system that controls leucocyte migration and selective activation at the site of inflammation. CAMs are a heterogeneous group of inducible immunoglobulins, integrins and selectins sharing functional properties as regards cell-to-cell adhesion, cellular recruitment, homing and healing.⁷

It has been demonstrated that CAMs show a selective pattern in both asthma and rhinitis, where the immunoglobulin-like molecule intercellular adhesion molecule-1 (ICAM-1) is the hallmark of allergic inflammation. ICAM-1 is promptly produced on nasal and conjunctival epithelia after allergen exposure,^{8,9} but is also detected when patients are symptom free, therefore representing a reliable marker of both clinical and minimal persistent inflammation (MPI).¹⁰ Moreover, it seems clear that CAM upregulation also plays a pivotal role in cutaneous allergic diseases^{11–13} and that newer antihistamines such as fexofenadine, cetirizine, loratadine and desloratadine can efficaciously modulate this part of the immunological mechanisms involved in disease pathogenesis.^{14–18}

Levocetirizine is the R-enantiomer or active isomer of the racemate cetirizine. It has twice the affinity for the H1 receptor compared with cetirizine and its potency as an antihistamine has been investigated by inhibition of histamine-induced weal and flare reactions.¹⁹ A recently published double-blind, placebo-controlled trial²⁰ has demonstrated that levocetirizine 5 mg once daily is an effective agent in patients with CU, as its action provides a rapid and satisfactory control of the symptoms and subjective improvement, although this is limited to the duration of treatment.

The aim of the present study was to investigate further the efficacy of levocetirizine in CU, focusing on the modulation of serological activity of CAMs, that reflects cell recruitment to the inflammatory site. The ASST was used in this series as screening tool for patients with CAIU, in order to highlight possible differences in comparison with the 'idiopathic' CU group.

Materials and methods

Patients

Patients were recruited from the urticaria registry of the Dermatological Clinic of the University of Florence, Italy, that includes subjects diagnosed with CU and screened with the ASST, routinely performed in our department according to the standardized technique of Grattan *et al.*²¹

We included in the study 36 subjects (25 women and 11 men; age range 17–76 years, mean 44; positive ASST 18, negative ASST 18). All subjects had a disease duration of more than 3 months, with recurrent wealing at least twice a week, and neither signs of vasculitis nor drugs, foods or physical factors could be identified as causative for urticaria. Exclusion criteria were C₁-esterase inhibitor deficiency, alcohol abuse, pregnancy and lactation.

Patients had discontinued any treatment (antihistamines, corticosteroids, other) 3 weeks before enrolment. At baseline they were prescribed levocetirizine (Xyzal®; UCB Pharma, Brussels, Belgium) 5 mg daily for 6 weeks. Ten age- and sex-matched healthy volunteers acted as controls.

Clinical score

Specific clinical features were recorded from patients at both baseline (during the week before levocetirizine administration) and at the end of the trial (during the last week of treatment). Urticarial activity was evaluated according to the European Academy of Allergology and Clinical Immunology Dermatology Section/European Union-funded Network of Excellence/European Dermatology Forum (EAACI/GA²LEN/EDF) guideline, which considers intensity of wealing and severity of itching.²²

Enzyme-linked immunosorbent assay technique

Fifty millilitres of peripheral blood was collected from patients before the beginning of treatment and at the end of the trial.

Sera obtained by centrifugation at 160 g were employed for a solid-phase enzyme-linked immunosorbent assay (ELISA; Bender MedSystems Diagnostic, Vienna, Austria), which was performed according to the manufacturer's instructions to determine endothelial leucocyte adhesion molecule-1 (ELAM-1), ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) and P-selectin levels (all in ng mL⁻¹). CAM concentrations are presented as mean values $\pm$ SD. ELISA for CAMs was also performed on control sera.

Statistical analysis

Statistical evaluation was carried out using a Wilcoxon rank sum test. Mean serum levels of CAMs were compared in subjects with CU vs. CAIU before treatment; in subjects with CU and with CAIU before vs. after treatment; in subjects with CU before and after treatment vs. healthy controls; and in subjects with CAIU before and after treatment vs. healthy controls.

The Wilcoxon rank sum test was also used to compare the EAACI/GA²LEN/EDF score²² before and after therapy in both groups. Statistical significance was considered to be $P \leq 0.05$.

Results

Clinical score

Urticaria activity was expressed as mean $\pm$ SD score in subjects with CU and CAIU, as shown in Table 1.

Serum soluble cellular adhesion molecule levels

Results of ELISA are summarized in Table 2. Briefly, all studied molecules were detected in blood samples of patients and controls. Highest concentrations were shown by VCAM-1, whereas ELAM-1 showed the lowest serum levels. Concentrations in healthy subjects were lower than in patients with urticaria pretreatment, except for ICAM-1, whose concentration in controls was slightly greater than in subjects with CU. Levocetirizine therapy provided a serum level decrease for all investigated CAMs in all groups.

As shown in Table 3, statistical comparison did not supply significant results for the two molecules ICAM-1 and VCAM-1, whereas ELAM-1 and P-selectin showed stronger differences

Table 1 Clinical evaluation and comparison between subjects with chronic urticaria (CU) and subjects with chronic autoimmune urticaria (CAIU)²² (mean $\pm$ SD severity score)

	EAACI/GA ² LEN/EDF score (t = 0)	EAACI/GA ² LEN/EDF score (t = 6 weeks)	P-value
CU subjects	5.0 $\pm$ 0.82	0.2 $\pm$ 0.63	< 0.0001
CAIU subjects	5.43 $\pm$ 0.85	0.79 $\pm$ 1.53	< 0.0001
Range of possible scores: 0–6.			

Table 2 Mean $\pm$ SD serum concentrations of cellular adhesion molecules in subjects with chronic urticaria (CU) and chronic autoimmune urticaria (CAIU) before and after treatment, and in healthy controls

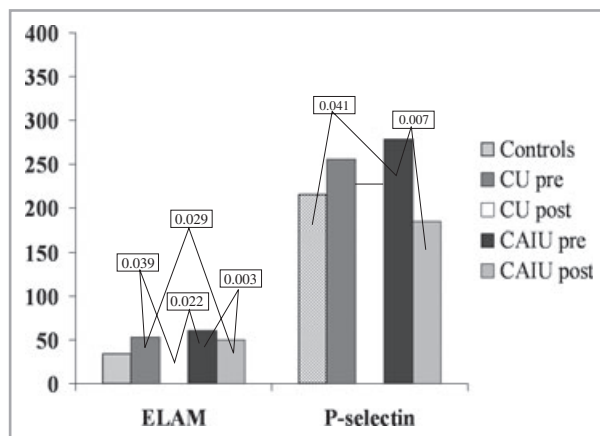
	ELAM-1 (ng mL ⁻¹)	ICAM-1 (ng mL ⁻¹)	VCAM-1 (ng mL ⁻¹)	P-selectin (ng mL ⁻¹)
CU pretreatment	53.52 $\pm$ 29.70	459.43 $\pm$ 228.38	729.67 $\pm$ 248.90	255.41 $\pm$ 72.50
CU post-treatment	37.98 $\pm$ 19.76	428.49 $\pm$ 165.70	695.83 $\pm$ 162.46	228.22 $\pm$ 84.22
CAIU pretreatment	60.42 $\pm$ 34.87	564.83 $\pm$ 193.51	774.17 $\pm$ 214.45	278.63 $\pm$ 87.66
CAIU post-treatment	49.97 $\pm$ 37.22	535.47 $\pm$ 167.26	730.58 $\pm$ 185.43	184.18 $\pm$ 56.68
Healthy controls	34.83 $\pm$ 9.77	469.45 $\pm$ 186.46	649.83 $\pm$ 211.42	215.98 $\pm$ 61.16

ELAM-1, endothelial leucocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

Table 3 Statistical comparison (Wilcoxon rank sum test) between serum levels of cellular adhesion molecules in chronic urticaria (CU) and chronic autoimmune urticaria (CAIU) before (pre) and after (post) treatment, and in healthy controls

	ELAM-1	ICAM-1	VCAM-1	P-selectin
CU pre vs. CAIU pre	NS	NS	NS	NS
CU pre vs. CU post	0.02	NS	NS	NS
CAIU pre vs. CAIU post	0.003	NS	NS	0.007
CU pre vs. controls	0.04	NS	NS	NS
CU post vs. controls	NS	NS	NS	NS
CAIU pre vs. controls	0.03	NS	NS	0.04
CAIU post vs. controls	NS	NS	NS	NS

ELAM-1, endothelial leucocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NS, not significant.

**Fig 1.** Endothelial leucocyte adhesion molecule-1 (ELAM) and P-selectin concentration (ng mL⁻¹) in the studied groups before (pre) and after (post) treatment with levocetirizine 5 mg daily for 6 weeks, and statistical significance. CU, chronic urticaria; CAIU, chronic autoimmune urticaria.

among the groups. In particular, subjects with CU and CAIU at pretreatment had significantly greater ELAM-1 concentrations than controls, and the molecule was significantly decreased following treatment in both groups (Fig. 1). P-se-

lectin levels were significantly higher in patients with CAIU than in healthy subjects, and a significant decrease was seen in this group after levocetirizine therapy.

Discussion

In this study, the level of adhesion molecules as immunoinflammatory parameters in patients with CU was evaluated before and after treatment with levocetirizine, better to understand the pathogenic mechanism of the disease and the possible effects of therapy with the newer antihistamines. The upregulation of adhesion molecules has already been reported in some forms of urticaria. At the serological level, previous investigations have revealed the existence of relevant amounts of P-selectin²³ in patients with CU, supporting the findings observed with this molecule in sera of our patients with CAIU when compared with controls. However, contradictory results were reported in the literature^{11,23} for ICAM-1, VCAM-1 and ELAM-1, which were not always significantly elevated in CU sera. In the present study, VCAM-1 and ICAM-1 serum levels at baseline were not significantly different from those in healthy controls, while ELAM-1 levels were significantly higher in patients with CU and CAIU and this same molecule was particularly affected by levocetirizine treatment. As is known, ELAM-1 and P-selectin belong to the selectin subgroup of CAMs and favour the rolling and extravasation of lymphocytes and neutrophils into inflamed skin.²⁴ These molecules are produced on endothelia and can also be found in serum and plasma, as the consequence of a systemic 'shedding' from the cell surface.²⁴ In the light of this, their increase in CU and CAIU sera should reflect a potential role for leucocytes in the pathomechanism of CU, and this hypothesis also agrees with the numerous reports of a significant lymphocyte and neutrophil infiltration in lesional skin.^{1,13}

Some studies have also detected CAMs in skin specimens from patients with CU.^{12,13,17,25} ICAM-1, ELAM-1 and VCAM-1 showed an upregulation in wealing skin, but the most relevant finding at the cutaneous level seemed to be the strong production of CAMs also in apparently unaffected skin, that one could interpret as a sign of the latent MPI in patients with urticaria. As we could deduce from the present study, it is likely that CAMs spread through blood vessels from the primary wealing site to the entire skin surface, possibly to trigger

subsequent weals. This diffusion might explain the increased serum levels of such molecules in CU, that in turn could be regarded as a marker of disease activity and efficacy of treatment.

Treatment with levocetirizine 5 mg daily for 6 weeks produced in all subjects with urticaria a decrease in serum CAMs that paralleled a subjective improvement in these same patients. The inhibitory effects of antihistamines on CAMs have already been established in allergic diseases,^{17,18,26} and our results seem to confirm this role for the new molecule levocetirizine. The mechanism by which anti-H1 drugs counteract the effects of histamine is mainly dependent on binding and stabilizing the inactive form of the receptor, but recent studies also suggest that desloratadine and loratadine directly influence ICAM-1 mRNA production via inhibition of nuclear factor κ B (NF- κ B) in target cells during rhinovirus infection.²⁷ Similarly, it has been demonstrated that levocetirizine inhibits eotaxin, interleukin-1, tumour necrosis factor- α and VCAM-1 production in endothelial cells through blocking the transcriptional complex NF- κ B.²⁸ These and similar observations have shed new light on broader antiallergic actions of levocetirizine involving gene regulation and expression, and it cannot be excluded that these same mechanisms were also working in our subjects with CU and CAIU.

Finally, it is remarkable that the patients with CU and CAIU had similar trends in CAM concentration and variation in our study, although mean levels were slightly different in the groups. In particular, the investigated molecules showed higher concentrations in subjects with CAIU, and this correlated with a higher clinical score observed in this subgroup. This finding further agrees with the hypothesis of a more severe form of the disease in the 'autoimmune' group,²⁹ both clinically and immunologically characterized.

As it emerges from our results, levocetirizine produced a significant clinical improvement in this series. Interestingly, both the ASST-positive and ASST-negative subjects experienced a reduction in size, number and duration of urticarial lesions, but many patients still complained of itching at the end of the trial (Caproni M *et al.* unpublished data). In other words, it seemed that levocetirizine was very effective in the reduction of signs, with a lesser influence on symptomatic relief. Therefore, it could be confirmed that this molecule possesses 'anti-allergic' properties that bypass the antipruritic action mediated by the H1 receptor, and that, for optimal effectiveness in CU, levocetirizine should be given on a regular basis rather than as needed. Clarification of its precise action is also needed, with an aim of opening novel perspectives in the antihistamine treatment of inflammatory dermatoses.

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