

Clinical trial

Effects of loratadine and cetirizine on serum levels of neuropeptides in patients with chronic urticaria

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Abstract

H1-receptor inhibiting drugs, namely loratadine and cetirizine, were frequently used in treatment of chronic urticaria. Urticarial weal and flare reactions, a neurogenic reflex due to neuropeptides, were reported to be more effectively inhibited by cetirizine than loratadine. The aim of this study was to determine and compare the effects of systemic loratadine and cetirizine treatments on serum levels of selected neuropeptides in chronic urticaria. Treatment groups of either systemic loratadine or cetirizine (10 mg/d), consisting of 16 and 22 patients, respectively, were included. Serum levels of stem cell factor (SCF), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), nerve growth factor (NGF), vasoactive intestinal peptide (VIP), and substance P (SP) were detected before and after one week of treatment with antihistamines. Serum NPY and VIP levels were significantly decreased when compared before and after treatment with antihistamines ($P < 0.001$ and $P < 0.01$, respectively). SCF and NGF values were also decreased after antihistamine treatment ($P < 0.05$). Post-treatment levels of CGRP were significantly higher compared with pretreatment values, while no significant difference was detected between pre and post treatment levels of SP. Cetirizine was significantly more effective than loratadine on lowering serum levels of SCF among the other neuropeptides. Systemic loratadine and cetirizine treatments in patients with chronic urticaria precisely caused variations in serum levels of neuropeptides. The predominant effect of cetirizine compared to loratadine on reducing serum SCF levels might be explained with anti-inflammatory properties of cetirizine.

Introduction

Neuropeptides (NPs) released from cutaneous sensory nerves react by modulating immune responses and neurogenic inflammation. Psychological stress activates an organized process of events in which NPs such as substance P (SP), calcitonin gene-related peptide (CGRP), nerve growth factor (NGF), neuropeptide Y (NPY), stem cell factor (SCF), and mast cells predominantly have the key roles.^{1–3} These peptides are potentially implicated in mast cell activation and degranulation (Table 1).^{1,3,4}

Recent investigations focus attention on the new aspects of NPs in the pathogenesis of some common dermatoses as well as urticaria.^{4,5} Mast cells secrete vasodilatory and proinflammatory mediators, such as histamine, vasoactive intestinal polypeptide (VIP), and cytokines as a response of interaction with NPs.^{1,4,5} Along with NPs, such as SP, and other vasoactive substances, histamine leads to increased vascular permeability, vasodilatation, weals, and flares. Histamine, acting through H₁ receptors,

is the main mediator of pruritus in chronic urticaria (CU).³ Loratadine and cetirizine are well-known H₁ receptor inhibiting drugs frequently used in the treatment of CU.⁶ Cetirizine was reported to inhibit urticarial reactions due to SP and CGRP.⁷ It was also reported that urticarial weal and axon reflex-mediated flare reactions were more effectively inhibited by cetirizine than loratadine.⁸ While loratadine and cetirizine possess their effects targeting histamine, there were limited data evaluating the effects of these antihistamines (AHs) on NPs. In the present study, any differentiation in serum levels of selected NPs due to systemic loratadine and cetirizine treatments in patients with CU were documented.

Materials and Methods**Patients**

Thirty-eight patients with CU who have not been using systemic therapy for the preceding four weeks were enrolled. Duration of the disease, etiological factors, and any associated systemic and

Table 1 Key neuropeptides in response to psychological stress

Neuropeptide	Initials	Mode of action
Substance P	SP	Activates mast cells, provokes itch and elements of neurogenic inflammation such as erythema and weal-and-flare reaction Induces lymphocyte proliferation and inflammation
Calcitonin gene-related peptide	CGRP	Activates mast cells, induces vascular permeability Influences cellular proliferation and differentiation
Nerve growth factor	NGF	Acts in the development and regulation of immune cells Acts as a signaling molecule between neuronal and immune cells Activates mast cells Promotes monocyte and macrophage migration through vascular endothelium
Neuropeptide Y	NPY	Activates mast cells Induces adherence, chemotaxis, phagocytosis, lymphocyte, antibody and cytokine production Activates mast cells, induces vascular permeability
Stem cell factor	SCF	Promotes mast cell proliferation and migration Activates mast cells
Vasoactive intestinal peptide	VIP	Causes cutaneous weal response Modulates immune and inflammatory responses

autoimmune diseases were recorded. Clinical assessments were performed, and severity of the disease was determined by using urticarial activity score (UAS) described previously. Sum of the UAS was noted numerously to be between 0 and 6.⁹ All patients underwent intradermal autologous plasma skin test (APST) with sterile autologous sodium citrate–anticoagulated plasma, saline as negative control, and histamine as positive control.¹⁰

Patients were classified into two groups of either loratadine or cetirizine therapy. The first group consisted of 22 patients treated with systemic cetirizine 10 mg/d, and the remaining 16 patients were treated with loratadine 10 mg/d, during a period of one week. Peripheral venous blood was drawn twice, before and after one week of treatment with either loratadine or cetirizine for detecting serum levels of SCF, NPY, CGRP, NGF, VIP, and SP. The study protocol was approved by the Medical School Ethics Committee.

Sample preparation

The samples were centrifuged at 1650 g for 10 minutes (Nüve A.Ş. NF1200R, Turkey), and the serums were separated and stored at -80°C . The parameters were measured using a commercially available kit by enzyme linked immunosorbent assay. Serum SCF levels were measured by Assaypro (Assaypro, St. Charles, MO, USA), NGF levels by Chemicon International ChemoKine (Chemicon Int, Billerica, MA, USA), NPY and CGRP levels by Phoenix Pharmaceuticals (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA), SP levels by Cayman Chemical Company (Cayman Chemical Company, Ann Arbor, MI, USA), and VIP levels by Peninsula Laboratories (Peninsula Laboratories, San Carlos, CA, USA) kits. All samples were double studied, and the concentrations were calculated according to standard curves by using standard serums available in the kits.

Statistical analysis

All data collected were analyzed using SPSS for Windows Version 15 (Chicago, IL, USA). Normality of variables was

detected by the Kolmogorov–Smirnov and Shapiro–Wilk tests. Chi-squared, paired sample *t* test and Wilcoxon signed ranks tests were used for comparing pretreatment and post-treatment values. The Mann–Whitney *U*-test and chi-squared tests were used to compare the effects of loratadine and cetirizine on serum NP levels. $P < 0.05$ was considered to show a statistically significant result.

Results

The study group consisted of 27 (71.1%) women and 11 (28.9%) men with a mean age of 39.39 ± 17.74 years. Duration of disease ranged from two months to 15 years (median: 12 months). APST was positive in 18 (47.40%) patients. There was significant difference between pretreatment (1.87 ± 1.67) and post-treatment values (0.84 ± 1.07) of UAS (Wilcoxon signed ranks test, $P < 0.001$).

Serum NPY and VIP levels were significantly decreased after treatment with both of the AHs ($P < 0.001$ and $P < 0.01$, respectively). Post-treatment values of SCF and NGF were significantly decreased, while CGRP levels were significantly increased compared to pretreatment values. There was no statistically significant difference between pre- and post-treatment levels of SP (Table 2).

When treatment groups of loratadine and cetirizine were compared, no difference was detected between pre- and post-treatment serum CGRP, VIP, SP, NPY, and NGF levels. However, cetirizine was significantly more effective than loratadine at reducing serum levels of SCF (Table 3). There was no difference between the effects of loratadine and cetirizine on UAS and APST.

Discussion

The mediator mechanisms of cutaneous weal and flare response, which form the origin of urticarial conditions,

Table 2 Comparison of pre- and post-treatment levels of selected neuropeptides in 38 patients (mean $\pm$ SD)

	SCF (ng/ml)	CGRP (ng/ml)	VIP (ng/ml)	SP (pg/ml)	NPY (ng/ml)	NGF (pg/ml)
Pretreatment	3.06 $\pm$ 1.93	0.40 $\pm$ 0.18	0.58 $\pm$ 0.17	30.61 $\pm$ 7.37	0.71 $\pm$ 0.36	15.49 $\pm$ 4.02
Post-treatment	2.03 $\pm$ 1.54	0.51 $\pm$ 0.23	0.50 $\pm$ 0.07	29.45 $\pm$ 6.08	0.36 $\pm$ 0.08	14.05 $\pm$ 2.59
<i>P</i> ^a	0.018	0.012	0.006	0.500	0.000	0.042

CGRP, calcitonin gene-related peptide; NGF, nerve growth factor; NPY, neuropeptide Y; SCF, stem cell factor; SP, substance P; VIP, vasoactive intestinal peptide.

^aPaired samples two test.

Table 3 Effects of cetirizine and loratadine on serum neuropeptide levels (mean $\pm$ SD)

Group (no.)	SCF (ng/ml)	CGRP (ng/ml)	VIP (ng/ml)	SP (ng/ml)	NPY (ng/ml)	NGF (ng/ml)
Cetirizine (22)	1.59 $\pm$ 1.22	0.44 $\pm$ 0.18	0.51 $\pm$ 0.084	27.34 $\pm$ 5.76	0.35 $\pm$ 0.062	14.42 $\pm$ 3.20
Loratadine (16)	2.63 $\pm$ 1.75	0.47 $\pm$ 0.20	0.49 $\pm$ 0.06	27.30 $\pm$ 5.09	0.36 $\pm$ 0.11	13.54 $\pm$ 1.34
<i>P</i> ^a	0.042	0.895	0.549	0.492	0.759	0.234

CGRP, calcitonin gene-related peptide; NGF, nerve growth factor; NPY, neuropeptide Y; SCF, stem cell factor; SP, substance P; VIP, vasoactive intestinal peptide.

^aMann-Whitney *U*-test.

are controversial. The question whether NPs cause vasodilatation directly or they induce further release of histamine was pointed out by Petersen *et al.*¹¹ and revealed that the flare reaction to focal histamine release was a neurogenic reflex. The role of H₁-antagonists in suppressing cutaneous responses to SP and CGRP in healthy volunteers was reported.¹² H₁-antagonists have also been reported to inhibit histamine secretion. SP-induced flare reduction by cetirizine was suggested to result from H₁ receptor blockage in patients with CU. Moreover, the reduction of an SP-induced weal might be explained by anti-inflammatory properties of cetirizine,¹³ and a direct anti-NP effect could be proposed to account for inhibition of the NP-induced weal.⁷ Despite a number of studies evaluating NPs, such as SP and CGRP in skin, little is known about the relevance of AHs with circulating NPs in patients with CU. To our knowledge, there were no previous data comprising the effects of AHs on serum NPs and whether this mechanism has a role in the treatment of CU.

Neuropeptide Y is released locally and into the circulation, activates mast cells and induces vascular permeability, while the central anxiolytic neuromodulator effect of this peptide results in inactivation of NPY during stress.¹ Recent evidence revealed gene expression of NPY is dependent on the histaminergic neuronal system and seems to involve an H₁ receptor mechanism in mice.¹⁴ It may be suggested that significantly downregulated serum NPY levels with H₁-antagonists were in accordance with

this previously reported observation. Knowing that stress was mainly implicated in the pathogenesis of CU, the contribution of this important effect of H₁-antagonists on NPY was an impressive finding about the participation of AHs in CU treatment.

Smith *et al.*¹⁵ reported a significant increase in VIP-induced weal in urticarial plaques and increased sensitivity of microvasculature to VIP in patients with CU. It was also eliminated previously that adrenal VIP was mainly coregulated by histaminergic control and decreased with chlorpheniramine maleate.¹⁶ Despite a number of studies evaluating local effects of VIP, our investigation revealed, for the first time, that serum VIP levels were also significantly affected by H₁-antagonists.

Histamine can be released from mast cells upon activation of the KIT receptor for SCF and receptors for NGF and SP. NGF is comparable to SCF with regard to its *in vitro* effects on mast cell differentiation, whereas SCF also induces mast cell migration.³ Recent work revealed NGF to induce an increase in airway responsiveness via the histamine receptor-dependent pathway in guinea-pig tracheal rings *in vitro*.¹⁷ SCF and p75NGF receptor expressions were downregulated in the skin of patients with CU, and this might represent a negative feedback to increased mast cell reactivity and proliferation.¹⁸ A single investigation consisting of a group of patients with allergic diseases revealed elevated serum NGF levels in patients with CU.¹⁹ While significantly decreased tissue NGF levels were reported at two weeks of treatment for

atopic dermatitis with an H_1 receptor antagonist,²⁰ any previous studies evaluating the effect of H_1 -antagonists on skin or serum SCF and NGF in CU did not exist. Therefore, the observation attained by this investigation that H_1 -antagonists significantly inhibited serum SCF and NGF levels might suggest a possible pathogenetic effect of these AHs in the treatment of CU.

In addition to their antihistaminergic properties, a number of H_1 -antagonists have been described that partially exert antiallergic and anti-inflammatory effects. The inhibition of SP-induced weals was explained by the anti-inflammatory properties of cetirizine, and a direct negative impact on SP was proposed in tissue.⁷ On the other hand, histamine release from mast cells was inhibited by an H_1 -antagonist, although SP-induced activation and SCF priming were not.²¹ Although the previous data were not comparable to ours, the tendency of declining in post-treatment serum SP levels with both of the AHs was observed in this investigation. Nevertheless, no detection of statistical significance made us consider that, even if cetirizine exerts a direct negative effect on SP in skin, this impact is not necessarily observed in serum SP levels. It may also be suggested that higher than normally achieved tissue levels of NPs might be required to induce SP release to peripheral blood.

In patients with CU, CGRP was reported to elicit an enhanced weal and prolonged flare response directly or via histamine liberation. Only histamine induced the flare but not the weal as it was inhibited by H_1 -antagonists, namely cetirizine.⁷ Previous data indicating that H_1 -antagonists increase CGRP secretion from sensory neurons or skin accompanied by an increase in serum CGRP concentration was lacking. Our results suggest that H_1 -antagonists might augment significant CGRP release from skin to peripheral blood in patients with CU. Though considered a novel observation of the present study, this result requires further investigation as to whether it is dependent on direct effects of H_1 -antagonists on CGRP.

The anti- H_1 effect of cetirizine proved to be significantly more pronounced and longer lasting than that of loratadine when given in a similar single dose of 10 mg orally.^{8,22} Inhibition of histamine and SP-induced reactions were less marked after loratadine compared with cetirizine.¹² The percentage inhibition of weal or flare area was greater for cetirizine than loratadine after one week's treatment with a standard dose.⁶ Cetirizine, in a quarter dose, was as potent in inhibiting the histamine skin reactivity as 10 mg loratadine.²³ Although cetirizine was reported to reduce cutaneous responses to SP, there were no previous data comparing direct effects of cetirizine and loratadine on the activity of NPs. Differing from loratadine, cetirizine has been shown to decrease the release of platelet-activating factor (PAF) and reduce the

PAF-induced influx of eosinophils independent of its specific H_1 -blocking activity. Cetirizine appears to have more anti-inflammatory properties than loratadine, causing a reduction in the inflammatory cell infiltrate, which mainly consisted of eosinophils, neutrophils, and basophils.^{6,24} SCF is a pleiotropic cytokine exerting its role in regulating mast cell growth and differentiation and inducing the migration of mast cells, which is also stimulated by PAF and histamine. SCF is a potent eosinophil degranulator and activator, and SCF-induced eosinophil activation may play a complimentary role during inflammatory and immune response.²⁵ While SCF and PAF exert similar effects targeting mast cells and eosinophils, it may be suggested that these mediators might share a common pathway and that cetirizine could have been effective probably by modulating SCF, like for the PAF. This possible mechanism might account for the documented greater efficacy of cetirizine over loratadine in reducing serum SCF levels in the present investigation.

Conclusions

This study demonstrated the interference of H_1 -antagonists with NPs, particularly NPY, VIP, SCF, and NGF in CU, for the first time. H_1 -antagonists also facilitated significant enhancement of CGRP in peripheral blood of patients with CU. We also propose that anti-inflammatory properties of cetirizine might account for the significantly reducing serum SCF levels, compared to loratadine. While further experimental studies and clinical confirmation are needed, our hypothesis may provide new insights into the interaction of NPs and H_1 -antagonists of which treatment in CU largely depends on. These data have to be elucidated, by which mechanisms of H_1 -antagonists with different doses and treatment periods exert their effects on NPs.

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