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Increased serum soluble vascular endothelial cadherin levels in patients with chronic spontaneous urticaria

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ABSTRACT

Background: Chronic spontaneous urticaria (CSU) is a common skin disease characterized by recurrent itchy wheals with or without angioedema that lasts longer than 6 weeks. Vascular endothelial (VE)-cadherin is an endothelial cell-specific adhesion molecule that plays critical roles in angiogenesis and endothelial permeability.

Objective: To investigate serum levels of soluble VE (sVE)-cadherin in patients with CSU.

Methods: Serum levels of sVE-cadherin in patients with CSU, patients with atopic dermatitis, and healthy controls were determined by enzyme-linked immunosorbent assay. In addition, changes in sVE-cadherin serum levels were compared in patients with CSU before and after H_1 antihistamine treatment. Furthermore, the effects of histamine on sVE-cadherin release by HMEC-1 cells were determined by enzyme-linked immunosorbent assay. The inhibition effects of H_1 antihistamine and H_2 antihistamine on sVE-cadherin release, VE-cadherin phosphorylation, and VE-cadherin disruption were evaluated in histamine-treated HMEC-1 cells by western blot and immunofluorescence.

Results: Serum levels of sVE-cadherin in patients with CSU were significantly higher than those in patients with atopic dermatitis and healthy controls. Serum sVE-cadherin levels in patients with CSU were correlated with the severity of CSU according to Urticaria Activity Scores. Furthermore, serum sVE-cadherin levels in patients with CSU at pretreatment decreased after H_1 antihistamine treatment. In addition, histamine markedly induced sVE-cadherin release in HMEC-1 cells. Moreover, H_1 antihistamine, but not H_2 antihistamine, significantly inhibited sVE-cadherin release in histamine-treated HMEC-1 cells. Western blot data showed that histamine induced phosphorylation of VE-cadherin in HMEC-1 cells, which was blocked by H_1 antihistamine.

Conclusion: The present data showed serum levels of sVE-cadherin are increased in patients with CSU. Histamine-induced sVE-cadherin release from endothelial cells could play a role in the pathogenesis of CSU.

Introduction

According to the guideline of the European Academy of Allergy and Clinical Immunology (EAACI), the Global Allergy and Asthma European Network (GA[2]LEN), the European Dermatology Forum (EDF), and the World Allergy Organization (WAO),[1] urticaria can be divided into 2 major types: spontaneous urticaria and inducible urticaria. Chronic spontaneous urticaria (CSU) is defined as the spontaneous appearance of itchy wheals with or without angioedema that lasts longer than 6 weeks. Although the actual pathogenesis of CSU is far from clear, abundant evidence has indicated that the release of histamine from activated mast cells and basophils and increased microvascular permeability induced by vascular active substances, including histamine, play crucial roles in the development of this disease.

The integrity of the vascular barrier is dependent on organized tight and adherens junctions in endothelial cells. Vascular endothelial (VE)-cadherin is an endothelial cell-specific adhesion molecule located at the junctions between endothelial cells and is involved in angiogenesis and vascular permeability. It has been reported that vascular active substances (such as vascular endothelial growth factor), proinflammatory cytokines (such as tumor necrosis factor-α), chemokines, and oxidative stress can increase microvascular permeability by targeting VE-cadherin.[2–5] A previous study also indicated that histamine can induce the tyrosine phosphorylation of VE-cadherin, lead to the dissociation of this

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Table 1
Cohort Demographics[a]

	CSU	AD	Controls
Patients, n	54	28	42
Age (y), mean (range)	24.5 (11–41)	21 (13–35)	22 (10–39)
Male/female, n	25/29	13/15	20/22
Disease activity, median (IQR)	3 (1–6)[b]	34.6 (18.8–82.5)[c]	NA
ASST, median (IQR)	1 (1–2)	NA	NA

Abbreviations: AD, atopic dermatitis; ASST, autologous serum skin test; CSU, chronic spontaneous urticaria; IQR, interquartile range; NA, not applicable.
[a]Data in the table showed no significant difference among groups.
[b]Disease activity in patients with CSU was assessed according to daily Urticaria Activity Scores.[9]
[c]Disease activity in patients with AD was assessed by the Scoring of Atopic Dermatitis index.[10]

protein, and increase endothelial permeability in human dermal microvascular endothelial cell line (HMEC-1) cells.[6] In addition, in some immune and inflammatory conditions, soluble VE (sVE)-cadherin can be released into the circulation because of the proteases of the extracellular domain of VE-cadherin. Increased serum sVE-cadherin levels have been reported in patients with Henoch-Schönlein purpura, urticarial vasculitis, allergic vasculitis, and Behcet disease.[7,8] To explore whether histamine-induced sVE-cadherin release might be associated with the pathogenesis of CSU, this study investigated serum levels of sVE-cadherin in patients with CSU and compared the changes of sVE-cadherin serum levels in patients with CSU before and after H_1 antihistamine treatment. Furthermore, the effects of histamine on sVE-cadherin release by HMEC-1 cells were determined. The inhibition effects of H_1 and H_2 antihistamines on sVE-cadherin release, VE-cadherin phosphorylation, and VE-cadherin cleavage were evaluated in histamine-treated HMEC-1 cells in this study.

Methods

Ethics Statement

This human research was approved by the ethics committee of Chengdu Second People's Hospital (Chengdu, China; number 2015007). All patients were Chinese and gave written informed consent.

Patients and Control Cohorts

Fifty-four patients with CSU, 28 patients with atopic dermatitis (AD), and 42 age- and sex-matched healthy controls were enrolled in this study. CSU was confirmed and diagnosed by Chengdu Second People's Hospital. In addition, 42 healthy controls were recruited among healthy subjects who were free of other diseases. A detailed history and physical examination were conducted for each patient and the basic demographic information from each group is listed in Table 1. The severity of CSU was evaluated according to weekly and daily Urticaria Activity Scores (UAS7 and UAS).[9] The autologous serum skin test (ASST) was performed in each patient with CSU.[11] The disease severity in patients with AD was determined by the Scoring of Atopic Dermatitis index.[10] All patients and healthy subjects were not treated 2 weeks before this study. Of these, 21 patients with CSU were treated with oral H_1 antihistamine (cetirizine 10 mg once daily or fexofenadine 60 mg twice daily) at the licensed dose for 2 weeks and were not taking any other medications. Patients with CSU who received antihistamines were selected randomly. None of the healthy subjects in the study were taking any medications. Serum samples from these patients before and after treatment were obtained.

Assay for sVE-Cadherin Levels

Levels of sVE-cadherin in serum and cultured supernatants were detected with commercially available enzyme-linked immunosorbent assay kits (catalog number DCADV0; R&D Systems Inc, Minneapolis, Minnesota) according to the manufacturer's instruction.

Cell Culture

The HMEC-1 cells were obtained from the Centers for Disease Control and Prevention (Atlanta, Georgia). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, New York) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), and 100 U/mL of penicillin, 100 U/mL of streptomycin, and 2 mmol/L of L-glutamine (Gibco). In this study, HMEC-1 cells were seeded in plates at 1×10^5 cells/cm^2. After a 48-hour incubation, HMEC-1 cells were treated with 100 μmol/L of histamine at different time points or histamine (100 μmol/L for 20 minutes) plus different antihistamines.

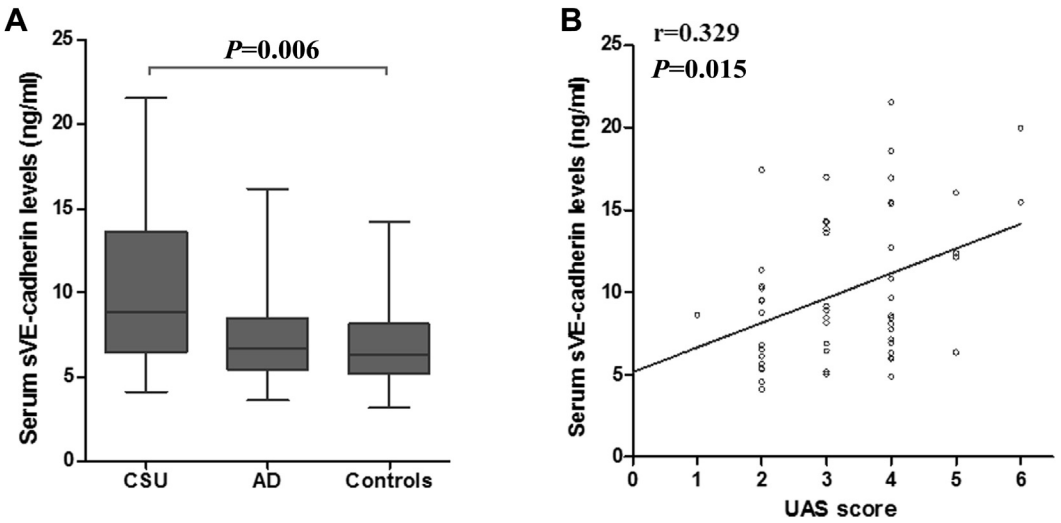

Figure 1. Increased serum levels of sVE-cadherin in patients with CSU. Serum sVE-cadherin levels in patients with CSU, patients with AD, and controls were measured by enzyme-linked immunosorbent assay. (A) Patients with CSU had significantly higher serum sVE-cadherin levels compared with patients with AD and healthy controls. Data are presented as median ± interquartile range. *P* values are based on the Mann-Whitney U test. (B) A positive correlation was found between sVE-cadherin serum levels and UASs in patients with CSU by Spearman tests. AD, atopic dermatitis; CSU, chronic spontaneous urticaria; sVE, soluble vascular endothelial; UAS, Urticaria Activity Score.