

Endostatin and Thrombospondin-1 levels are increased in the sera of patients with chronic spontaneous urticaria

Ilaria Puxeddu · Sara Casigliani Rabl ·
Filomena Panza · Federico Pratesi · Valeria Rocchi ·
Isabella Del Corso · Paola Migliorini

Received: 20 May 2013 / Revised: 1 August 2013 / Accepted: 9 August 2013 / Published online: 1 September 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Chronic urticaria (CU) is a common disease characterized by recurrent itchy wheals and/or angioedema for more than 6 weeks. Increased levels of the pro-angiogenic mediator vascular endothelial growth factor (VEGF) have been described in skin disorders, such as chronic urticaria (CU), psoriasis and atopic dermatitis. Up to now, no data on the role of VEGF endogenous inhibitors Endostatin (ES) and Thrombospondin-1 (TSP-1) in CU are available. The aim of our study is to investigate the potential involvement of ES and TSP-1 in patients with chronic spontaneous urticaria (CSU). The levels of ES and TSP-1 were measured in the sera of 106 adult patients with CSU and 98 healthy subjects by enzyme immunoassays. The serum levels of the anti-angiogenic mediators ES and TSP-1 resulted significantly higher in CSU than in control subjects. Analysis of these mediators in CSU sub-groups, defined by the results of the autologous serum skin test (ASST), identified a significant increase of ES and TSP-1 in both ASST-positive and ASST-negative sub-groups as compared to the controls. Levels of ES and TSP-1 do not parallel the disease severity in CSU. Our study suggests that the extracellular matrix (ECM) fragments ES and TSP-1 with anti-angiogenic activity play a potential role in the pathogenesis of CSU but do not parallel disease activity.

Keywords Chronic spontaneous urticaria · Angiostatic mediators · Autologous serum skin test · Endostatin · Thrombospondin-1

Introduction

Chronic urticaria (CU) is a common and disabling disease characterized by recurrent itchy wheals and/or angioedema for more than 6 weeks [24]. These symptoms are the consequence of skin mast cells degranulation with release of histamine and other vasoactive mediators. Chronic spontaneous urticaria (CSU) is frequently characterized by auto-reactivity as intradermal injection of autologous serum causes wheal and flare reaction in 30–60 % of patients [7]. Autoantibodies (anti-IgE, anti-FcεRI, anti-TPO) [1] are detected in only one-third of the cases, suggesting that other circulating mediators released by immune cells and/or structural cells may be involved in the pathogenesis of CSU.

Vascular endothelial growth factor (VEGF) is the main pro-angiogenic mediator with relevant vasoactive properties. An increased VEGF has been observed in the circulation of patients with skin disorders, including CU [21]. This might be responsible for the increase of vascular permeability in the skin, leading to the development of the transitory itchy wheals observed in CU. The functional activity of VEGF is tightly regulated by endogenous anti-angiogenic mediators mainly produced by the degradation of extracellular matrix (ECM) components, such as collagens and fibronectin.

Endostatin (ES), a proteolytic fragment of collagen type XVIII, was first described as a potent inhibitor of angiogenesis [23]. In addition to its well-known activity as down-regulator of VEGF, ES has been recently proposed as a new vasoactive mediator due to its direct effect on endothelial cells [22]. Increased serum levels of ES have been reported in autoimmune skin diseases, such as scleroderma [8] and in skin cancer such as melanoma [12]. Up to now, no data on the role of ES in patients with CSU are available.

I. Puxeddu · S. C. Rabl · F. Panza · F. Pratesi · V. Rocchi ·
I. Del Corso · P. Migliorini (✉)
Department of Clinical and Experimental Medicine, Clinical
Immunology and Allergy Unit, University of Pisa,
Via Roma 67, 56126 Pisa, Italy
e-mail: paola.migliorini@med.unipi.it

Parallel to ES, Thrombospondin-1 (TSP-1), a heparin-binding protein with anti-angiogenic activity stored in the ECM, seems to play a crucial role in several stages of the inflammatory response [13]. Its role in chronic inflammatory diseases is still controversial. In some cases, TSP-1 activates leukocytes, enhances chemotaxis, and accelerates fibrosis. In others, it prevents injury and progressive damage and enhances homeostasis [13]. An increased level of TSP-1 has been detected in autoimmune diseases, such as rheumatoid arthritis where it seems to exert pro-inflammatory effects [15, 19]. In chronic inflammatory skin diseases, such as psoriasis, it is up-regulated in skin biopsies following immunosuppressive therapy with the anti-TNF- α agent Etanercept [3]. Recently, it has been shown that TSP-1 destabilizes contact between endothelial cells due to its direct effect on the cells, suggesting a contribution to skin vasodilation and consequent extravasation in CSU [9].

The aim of our study was to investigate the circulating levels of ES and TSP-1 in patients with CSU, where vasodilation is a crucial step for the development of wheals and flare. In addition, we investigated the potential role of these mediators in the setting of the disease.

Methods

We studied 106 CSU patients (64 female and 42 male; age 46 ± 16 , range 14–90) and 98 sex-matched and age-matched healthy subjects (NHS). CSU was diagnosed according to the EAACI/GA²LEN/EDF guidelines [24]. Demographic and clinical characteristics of the patients are reported in Table 1.

Patients with chronic cholinergic and chronic inducible urticaria as well patients with other concomitant diseases were excluded from the study. All the patients were being treated with non-sedating oral H1-antihistamines (40 % of the patients were treated with desloratadine 5 mg/day, 30 % levocetirizine 5 mg/day, 10 % rupatadine 10 mg/

day, 20 % ebastine 10 mg/day), stopped 5 days before serum samples were taken for ASST and for the detection of ES and TSP-1. No patients received corticosteroids or cyclosporine treatments. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. All the subjects studied gave their informed consent before enrolment. Before starting H1-antihistamine therapy, disease activity was calculated according to the recent EAACI/GA²LEN/EDF guidelines [24], using the urticaria activity score of seven consecutive days (UAS7) and subdivided into mild, moderate and severe urticaria. ASST was performed as previously reported [11].

ES and TSP-1 concentrations were measured in the sera of CSU and in the control group by sandwich enzyme immunoassay (Duo-set, R&D Systems, Inc., Minneapolis, MN, USA), according to manufacturer's instructions, following sera dilution (1/200).

Results were expressed as median and interquartile ranges. The non-parametric Mann–Whitney *U*-test was used to compare data between the patients' group and the NHS. A *P* value <0.05 was considered statistically significant.

Results

According to the UAS classification, urticaria was mild in 36, moderate in 56 and severe in 14 patients. Among the CSU patients, 43 (41 %) were positive and 63 (59 %) negative to ASST. We analyzed the levels of ES in the sera of patients with CSU and in control subjects. As shown in Fig. 1a, the levels of ES were significantly higher in CSU (3.8 ng/ml; range 1.12–5.50) when compared to the NHS (0.76 ng/ml; range 0.43–2.28) ($P < 0.0001$). In CSU patients, no significant difference between the ASST-positive (3.68 ng/ml; range 1.19–5.90) and negative (3.86 ng/ml; range 1.11–5.01) sub-groups has been observed. Interestingly, the CSU patients with the highest concentration of ES were all positive at the ASST. No relation was detected between ES levels and disease severity, according to the UAS7. Parallel to ES, the levels of TSP-1 (Fig. 1b) was significantly increased in the sera of CSU (74.3 ng/ml; range 54.0–92.9) as compared to NHS (51.8 ng/ml; range 38.0–76.3) ($P < 0.0001$), without any significant differences between ASST-negative and positive sub-groups. As observed for ES, the levels of TSP-1 did not parallel the severity of the urticaria.

Discussion

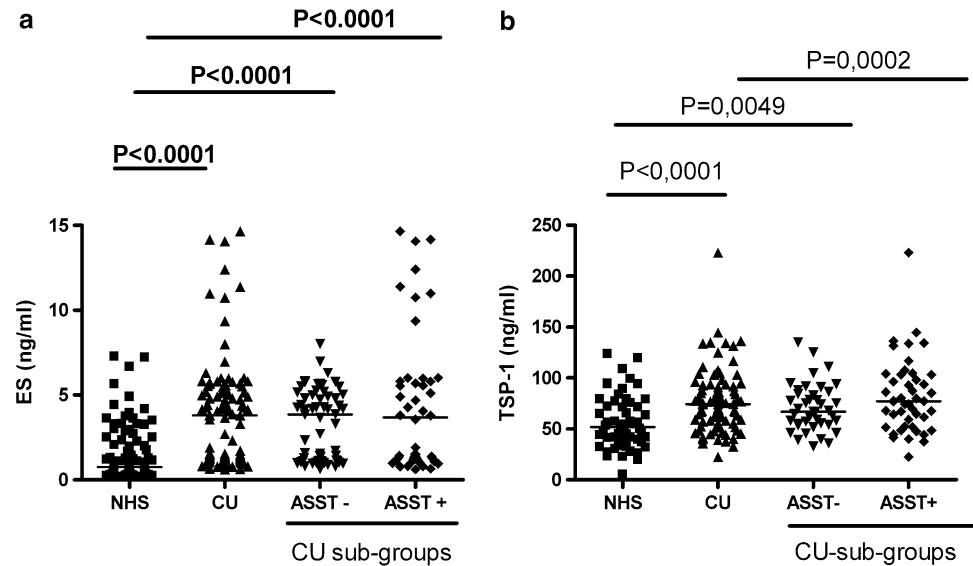
In this study, we showed for the first time that ES and TSP-1, products of ECM degradation, are increased in the sera of CSU patients.

Table 1 Demographic and clinical characteristics of CSU patients

	CSU patients (<i>n</i> = 106)
Age (mean $\pm$ SD) (range)	46 $\pm$ 16 (14–90)
Sex (F/M)	64/42
Disease duration (years) (median and range)	2 (0.5–19)
AE	50/106
Mild	36/106
Moderate	56/106
Severe	14/106

SD standard deviation, F female, M male, AE angioedema

Fig. 1 Levels of ES and TSP-1 in the sera of CSU patients and in the ASST-positive and negative sub-groups. Levels of ES (a) and TSP-1 (b) in the sera of CSU patients and in the ASST-negative (ASST-) and ASST-positive (ASST+) subgroups versus control group (NHS). Data are expressed in ng/ml and lines represent median values. The non-parametric Mann–Whitney *U*-test was used to compare data between the patients' group and the NHS. A *P* value <0.05 was considered statistically significant



Mast cells are the main skin cells involved in the pathogenesis of urticaria. An activation of these cells with consequent degranulation seems to be responsible for vasodilation in the skin [16]. The underlying causes of mast cell activation in CSU are largely unknown and remain to be identified. A sizable subgroup of CSU patients express IgG auto-antibodies to IgE or to the α -subunit of the high affinity IgE receptor, Fc ϵ RI [6, 7], or IgE antibodies against TPO [1]. However, the observation that a large part of CSU has no auto-antibodies suggests that other circulating mediators are potentially involved in the pathogenesis of the disease. This is partially confirmed by several independent studies demonstrating an increase of inflammatory mediators in CSU, such as IL-6 [4], IL-18 [17], IL-17, IL-23, TNF- α [2]. The identification of biomarkers relevant for follow-up and management of the disease remains an important goal. Previously published results on potential biomarkers are often contradictory and suffer a limited correlation with disease activity [14]. This is probably due to the low number of subjects evaluated, different criteria for patient inclusion, different methodologies used and thus difficulties in comparing data [14].

To have more reliable results, we have selected for the present study, a large number of patients with CSU according to the recent EAACI guidelines. In our study, all the patients were evaluated when H1-antihistamine therapy was stopped. The increase of ES and TSP-1 in the sera of CSU patients suggests that these ECM fragments might be involved in the pathogenesis of CSU. The functional activity of ES has been linked for many years to its anti-angiogenic activity by interference with the VEGF/VEGFR binding [18]. However, a vasorelaxation activity of ES on endothelial cells via NO synthesis has been described [22]. These data may suggest that ES might contribute to the

vascular leakage in the skin of patients with CSU, leading to the development of its clinical features, such as wheals and flare formation. ECM fragments are often the results of the proteolytic activity of a wide variety of catalytic enzymes released in the tissues by mast cells such as Cathepsins and metalloproteases (MMPs) [5]. Thus, the increase of ES in CSU might be the result of proteolytic enzymes released from the activated skin mast cells. Following mast cell activation, MMPs are released in the tissue and may induce ES formation from Collagen XVIII, leading to vasorelaxation and amplification of inflammation. Parallel to ES, we have showed that TSP-1 is increased in the sera of CSU patients, suggesting a potential role of this endogenous mediator in CSU. TSP-1 is involved in angiogenesis, cancer and inflammation. It regulates multiple cellular events involved in reparative processes in the skin, including cell adhesion, migration, ECM expression/organization and regulation of growth factor activity [20]. Beside its well-known anti-angiogenic activity, TSP-1 has the property to destabilize endothelial cell contacts. This might contribute to vasodilation in the skin, important step for the development of wheals and flare during urticaria. On the other hand, a role of TSP-1 in resolution of inflammation has been proposed. Under this view, the increased levels of TSP-1 in CSU might represent one of the mechanisms counteracting inflammation.

Comparing the levels of ES and TSP-1 in ASST-positive and ASST-negative sub-groups, no significant differences were detected, suggesting that in CSU an increase in circulating ES and TSP-1 levels might reflect the inflammatory process independently of the involvement of an autoimmune response. Our results are in accordance with our previous study on IL-18 [17] and with the data of Kasperska-Zajac et al. [10] showing no differences in the

plasma levels of IL-6 between ASST-negative and positive sub-groups.

When the potential relevance of ES and TSP-1 in the clinical setting of the disease was investigated, no differences in ES and TSP-1 levels among mild, moderate and severe CSU groups have been detected. These findings indicate that the levels of these ECM fragments are not a potential marker of urticaria activity, but probably an epiphenomenon.

In conclusion, our results suggest the potential involvement of ECM fragments with anti-angiogenic activity in the pathogenesis of CSU. It remains to be understood whether ES and TSP-1 up-regulation is a consequence of the inflammatory reaction or one of the causes of the pathology.

Acknowledgments This work was supported by a project grant from Tuscany Region, Italy (POR: 3242/2009).

Conflict of interest All the authors declare that they have no conflicts of interest.

References

- Altrichter S, Peter HJ, Pisarevskaja D, Metz M, Martus P, Maurer M (2008) IgE mediated auto-allergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PLoS ONE* 6:e14794
- Atwa MA, Emara AS, Youssef N, Bayoumy NM (2013) Serum concentration of IL-17, IL-23 and TNF- α among patients with chronic spontaneous urticaria: association with disease activity and autologous serum skin test. *J Eur Acad Dermatol Venereol*. Mar 4. doi:10.1111/jdv.12124. [Epub ahead of print]
- Avramidis G, Krüger-Krasagakis S, Krasagakis K, Fragiadaki I, Kokolakis G, Tosca A (2010) The role of endothelial cell apoptosis in the effect of etanercept in psoriasis. *Br J Dermatol* 163:928–934
- Dos Santos JC, Azor MH, Nojima VY, Lourenço FD, Prearo E, Maruta CW, Rivitti EA, da Silva Duarte AJ, Sato MN (2008) Increased circulating pro-inflammatory cytokines and imbalanced regulatory T-cell cytokines production in chronic idiopathic urticaria. *Int Immunopharmacol* 8:1433–1440
- Felbor U, Dreier L, Bryant RA, Ploegh HL, Olsen BR, Mothes W (2000) Secreted cathepsin L generates endostatin from collagen XVIII. *EMBO J* 19:1187–1194
- Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschläger M, Kinet JP, Stingl G (1995) Serum IgG auto-antibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 96:2606–2612
- Grattan CE, Francis DM, Hide M, Greaves MW (1991) Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 21:695–704
- Hummers LK, Hall A, Wigley FM, Simons M (2009) Abnormalities in the regulators of angiogenesis in patients with scleroderma. *J Rheumatol* 36:576–582
- Isenberg JS, Ridnour LA, Dimitry J, Frazier WA, Wink DA, Roberts DD (2006) CD47 is necessary for inhibition of nitric oxide-stimulated vascular cell responses by thrombospondin-1. *J Biol Chem* 281:26069–26080
- Kasperska-Zajac A, Sztylec J, Machura E, Jop G (2011) Plasma IL-6 concentration correlates with clinical disease activity and serum C-reactive protein concentration in chronic urticaria patients. *Clin Exp Allergy* 41:1386–1391
- Konstantinou GN, Asero R, Maurer M, Sabroe RA, Schmid-Grendelmeier P, Grattan CE (2009) EAACI/GA(2)LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 64:1256–1268
- Kurschat P, Eming S, Nashan D, Krieg T, Mauch C (2007) Early increase in serum levels of the angiogenesis-inhibitor endostatin and of basic fibroblast growth factor in melanoma patients during disease progression. *Br J Dermatol* 156:653–658
- Lopez-Dee Z, Pidcock K, Gutierrez LS (2011) Thrombospondin-1: multiple paths to inflammation. *Mediators Inflamm* 2011:296069. Epub 2011 Jul 3
- Metz M, Krull C, Maurer M. (2013) Histamine, TNF, C5a, IL-6, -9, -18, -31, -33, TSLP, Neopterin, and VEGF are not elevated in chronic spontaneous urticaria. *J Dermatol Sci*. Mar 21. Pii:S0923-1811(13)00090-X
- Pohlert D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne RW, Wolf G (2009) TGF-beta and fibrosis in different organs-molecular pathway imprints. *Biochim Biophys Acta* 1792:746–756
- Puxeddu I, Piliponsky AM, Bachelet I, Levi-Schaffer F (2003) Mast cells in allergy and beyond. *Int J Biochem Cell Biol* 35:1601–1607
- Puxeddu I, Italiani P, Giungato P, Pratesi F, Panza F, Bartaloni D, Rocchi V, Del Corso I, Boraschi D, Migliorini P (2013) Free IL-18 and IL-33 cytokines in chronic spontaneous urticaria. *Cytokine* 61:741–743
- Ribatti D (2009) Endogenous inhibitors of angiogenesis: a historical review. *Leuk Res* 33:638–644
- Stuhlmüller B, Ungethüm U, Scholze S, Martinez L, Backhaus M, Kraetsch HG, Kinne RW, Burmester GR (2000) Identification of known and novel genes in activated monocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 43:775–790
- Sweetwyne MT, Murphy-Ullrich JE (2012) Thrombospondin1 in tissue repair and fibrosis: TGF- β -dependent and independent mechanisms. *Matrix Biol* 31:178–186
- Tedeschi A, Asero R, Marzano AV, Lorini M, Fanoni D, Berti E, Cugno M (2009) Plasma levels and skin-eosinophil-expression of vascular endothelial growth factor in patients with chronic urticaria. *Allergy* 64:1616–1622
- Wenzel D, Schmidt A, Reimann K, Hescheler J, Pfitzer G, Bloch W, Fleischmann BK (2006) Endostatin, the proteolytic fragment of collagen XVIII, induces vasorelaxation. *Circ Res* 98:1203–1211
- Zatterstrom UK, Felbor U, Fukai N, Olsen BR (2000) Collagen XVIII/endostatin structure and functional role in angiogenesis. *Cell Struct Funct* 25:97–101
- Zuberbier T, Asero R, Bindslev-Jensen C, Walter Canonica G, Church MK, Giménez-Arnau A, Grattan CE, Kapp A, Merk HF, Rogala B, Saini S, Sánchez-Borges M, Schmid-Grendelmeier P, Schünemann H, Staubach P, Vena GA, Wedi B, Maurer M (2009) EAACI/GA2LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy* 64:1417–1426