Endothelial cell activation during edematous attacks of hereditary angioedema types I and II

Erika Kajdácsi, MSc, Péter K. Jani, MSc, Dorottya Csuka, PhD, Lilian Ágnes Varga, PhD, Zoltán Prohászka, MD, DSc, Henriette Farkas, MD, DSc, and László Cervenak, PhD *Budapest, Hungary*

Background: Hereditary angioedema (HAE) caused by C1-inhibitor (C1-INH) deficiency (HAE–C1-INH) is a potentially life-threatening rare disease caused by the decreased activity of C1-INH. Lack of C1-INH leads to overproduction of bradykinin, a potent vasoactive peptide. Although angioedema is induced by bradykinin, the function and activation of endothelial cells (ECs), the targets of bradykinin, have not yet been studied during HAE attacks.
Objective: We studied whether EC function is altered during HAE attacks in comparison with attack-free intervals.
Methods: Forty-six consecutive samples obtained during attacks from 18 patients with HAE–C1-INH were compared with inter-attack samples of the same patients. The patients' sera were tested for von Willebrand factor (VWF) antigen, VWF collagen-binding activity, soluble E-selectin, and endothelin-1 levels by using ELISA and BRAHMS Kryptor technologies.
Results: Levels of all 4 EC markers (VWF antigen, VWF collagen-binding activity, soluble E-selectin, and endothelin-1) were significantly increased during HAE attacks. Their increases were even more obvious in the subgroup of patients without any pre-existing risk factors for endothelial dysfunction.
Conclusion: In this study we demonstrated that ECs are activated during HAE attacks. Our results might suggest the need for revising the knowledge on the pathogenesis of HAE–C1-INH and for reconsidering the role of ECs as a possible novel therapeutic target in patients with this disease.
(J Allergy Clin Immunol 2014;133:1686-91.)

Key words: *Hereditary angioedema, C1-inhibitor deficiency, attack, endothelial cells, activation, von Willebrand factor, endothelin-1, soluble E-selectin, clinical study*

Abbreviations used

BMI:	Body mass index
C1-INH:	C1-inhibitor
C4:	Complement factor 4
CRP:	C-reactive protein
EC:	Endothelial cell
ET-1:	Endothelin-1
HAE:	Hereditary angioedema
HAE–C1-INH:	Hereditary angioedema caused by C1-INH deficiency
sE-selectin:	Soluble E-selectin
WPB:	Weibel-Palade body
VWF:	von Willebrand factor
VWF:Ag:	von Willebrand factor antigen
VWF:CBA:	von Willebrand factor collagen-binding activity

Hereditary angioedema (HAE) caused by C1-INH deficiency (HAE–C1-INH) is a rare disease of autosomal dominant inheritance. It is characterized by recurrent subcutaneous, submucosal, or both types of edematous swelling without wheals and pruritus. According to our current knowledge, increased endothelial permeability underlies the symptoms caused by excess of bradykinin as a result of the insufficient activity of the C1-inhibitor (C1-INH). C1-INH normally inhibits kallikrein, a serine protease, which cleaves bradykinin from the high-molecular-weight kininogen.[1]

Bradykinin is a potent vasodilator; it enhances vascular permeability and causes nonvascular smooth muscle contraction. Endothelial cells (ECs) have 2 types of bradykinin receptors: bradykinin receptor type 2 is expressed constitutively on the cell surface, and bradykinin receptor type 1 is induced by cytokines and other proinflammatory factors.[2,3] In spite of the unquestionable role of bradykinin, the variability in the location and severity of HAE attacks suggests that additional factors might also have their role in pathophysiology. Interestingly, EC function has been barely investigated in patients with HAE[4] and, according to our best knowledge, has not been studied at all during HAE attacks.

ECs regulate several physiologic processes, of which the maintenance of vascular integrity and barrier function are among the most important.[5,6] Endothelial permeability is regulated by multiple factors (eg, thrombin, histamine, reactive oxygen species, vascular endothelial growth factor, TNF-α, and LPS) in addition to bradykinin by using a wide variety of signaling pathways.[7] An increase in endothelial permeability is one of the signs of EC activation. Assessing the *in vivo* functions of ECs is difficult because they do not form a compact, well-localized organ. In addition to studying flow-mediated dilation of the brachial artery, the evaluation of specific endothelial plasma markers is the most frequently used technique. The combination of such markers (eg, von Willebrand factor [VWF], soluble E-selectin [sE-selectin], and endothelin-1 [ET-1]) might properly reflect the activation state of ECs.[4]

VWF has 2 major functions: it mediates the aggregation and adherence of platelets to the extracellular matrix, and it is the carrier molecule for Factor VIII. Only ECs and megakaryocytes produce VWF, but in the absence of substantial thrombosis, most

From the 3rd Department of Internal Medicine, Semmelweis University.
Supported by the National Scientific Research Fund (grant no. OTKA K 100886, to H.F.). L.C. was granted the "Bolyai János" Research Fellowship (BO/00218/10/8).
Disclosure of potential conflict of interest: All authors have received research and travel support from the National Scientific Research Fund.
Received for publication February 26, 2013; revised December 20, 2013; accepted for publication December 24, 2013.
Available online February 9, 2014.
Corresponding author: László Cervenak, PhD, 3rd Department of Internal Medicine, Semmelweis University, Kútvölgyi út 4, Budapest, H-1125 Hungary. E-mail: cela@kut.sote.hu.
0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.1072

of the VWF is released by ECs, and thus VWF is frequently used as the marker of endothelial activation or dysfunction.[8,9] VWF can be secreted constitutively or in a regulated way on induction by chemical (eg, histamine, bradykinin, or thrombin) or physical (shear stress) stimuli. von Willebrand factor collagen-binding activity (VWF:CBA) increases proportionately with the size of the multimeric structure. The total concentration of von Willebrand factor antigen (VWF:Ag) and VWF:CBA together reflects the quantity and function of VWF molecules, as well as the source of their secretion.[10,11] Several enzymes can cleave the VWF multimers to smaller fragments (eg, the a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [ADAMTS13]). This process leaves the antigenic properties of VWF intact; however, the collagen-binding ability of the latter decreases substantially. An increased VWF:CBA value indicates EC activation or damage or reduced cleavage.[10,12]

E-selectin initializes the migration of leukocytes. It is expressed only on the surfaces of activated ECs.[13] Because of enzymatic cleavage, E-selectin is released into the circulation as sE-selectin, but the enzyme involved in the cleavage is yet unknown.[14] Because resting ECs do not express measurable amounts of E-selectin, it is evident that an excessive increase in serum level occurs only when the expression and cleavage of E-selectin are both induced simultaneously. The loss of cell membrane integrity (necrosis) can also cause increased sE-selectin levels.[14]

ET-1 is a 21-amino-acid peptide that maintains and regulates vascular tone. ET-1 is one of the most potent vasoconstrictors acting on the ET-A receptors of vascular smooth muscle cells. It can have a vasodilatory effect when it activates ET-B receptors on the ECs.[15] It is produced predominantly by ECs, and therefore ET-1 can be used as an endothelium-specific marker.[16] ECs constitutively release ET-1 through their basolateral membrane to maintain basal vascular tone. In addition, there is also a regulated secretion triggered by external stimuli, such as hypoxia, thrombin, and shear stress.[15,17,18] Concerning the dual secretory pathway, ET-1 resembles VWF, and previously, both have been supposed to localize in Weibel-Palade bodies (WPBs). Recently, however, VWF has been shown to expel other proteins (including ET-1) from mature WPBs, and thus the secretion of these EC products might be separately regulated.[11,19,20]

Although ECs are known to play a role in HAE, we could not identify any publications on a population-based study evaluating endothelial function in patients with HAE. Recently, we have reported normal endothelial function during inter-attack periods in symptom-free patients with HAE, as evidenced by 4 EC markers: VWF:Ag, VWF:CBA, ET-1, and sE-selectin.[4] However, on the basis of the pivotal role of ECs in the regulation of vascular permeability, it is reasonable to assume that EC activation might contribute to the development of HAE attacks. Therefore our aim was to clarify whether EC activation could be detected by determining these markers during HAE attacks. We also aimed to compare the functional pattern of EC markers during subsequent attacks in the same patients.

METHODS

Diagnosis of HAE–C1-INH

HAE–C1-INH types I and II were diagnosed according to Bowen et al.[21] No patients with HAE type III were recruited in our study.

Patients

We implemented the study at the 3rd Department of Internal Medicine, Semmelweis University, in observance of the World Medical Association Declaration of Helsinki and according to a protocol approved beforehand by the competent research ethics committee.

Eighteen consecutive patients with HAE (5 men and 13 women) from 15 families were enrolled. All had been followed up at the Hungarian HAE Centre between 2008 and 2010 and have been treated for HAE attacks during this period. We analyzed 1 sample from attack-free periods (inter-attack sample) and at least 1 obtained during an attack (during-attack sample) from all patients. We gave 14 patients diagnoses of HAE I and 4 patients diagnoses of HAE II. Because the concentration of the C1-INH antigen might be normal or even increased in patients with HAE type II, this parameter of patients with HAE type II was excluded from analysis.

Seven patients with HAE received danazol regularly, an additional 2 of the 18 patients administered it on demand, and a further 9 patients did not receive this agent at all. (The 2 patients who received danazol on demand were excluded from both the danazol-treated group and from the group not treated with danazol.) Three of the 18 patients with HAE had mild cardiovascular abnormalities (high blood pressure, tachyarrhythmia, or both), an additional 2 patients had type 2 diabetes mellitus, and 1 patient had Crohn disease (inactive at the time of sampling).

Patients with HAE who were smoking during the study are included in the current smokers subgroup. Former smokers, who had given up smoking more than a year before their blood samples were collected, and never smokers were pooled to form the nonsmokers subgroup.

Sample collection and handling

After an overnight fast of at least 6 hours, blood sampling was done during inter-attack periods. The during-attack status of a sample was determined by a specialist medical professional by evaluating the symptoms and complaints of the patients, according to criteria for edematous attacks described in the consensus document.[21]

Aliquots of serum and plasma were stored at −70°C until laboratory analysis.

Measurement of laboratory parameters

Plasma C-terminal-pro-endothelin-1 (CT-proET-1) levels, which are indicative of ET-1, were measured by using the BRAHMS Kryptor fluorescent sandwich immunoassay technology (BRAHMS GmbH, Hennigsdorf, Germany), as described by Papassotiriou et al.[22] Levels of sE-selectin, VWF:Ag, and VWF:CBA were measured by means of ELISA, as described by Czúcz et al,[4] with a minor modification of VWF:CBA determination: only collagen III (from Sigma Chemical, St Louis, Mo) was used for coating. To calculate VWF:CBA percentages, we used a serial dilution of mixed normal human sera (from 63 healthy control subjects) to obtain a standard curve. One hundred percent indicates the same OD value of a given sample as the OD values of similarly diluted normal human sera. Levels of complement factor 4 (C4) and C1-INH functional activity were measured according to the method of Kelemen et al.[23] The C1-INH antigen concentration was measured by using an in-house ELISA with goat anti-human C1-INH antibodies (purchased from Quidel, San Diego, Calif).

Standard laboratory parameters were measured with a Roche Integra 800 analyzer (Roche, Mannheim, Germany; clinical chemistry, C-reactive protein [CRP]).

The normal range was defined according to the literature (body mass index [BMI]) or the instructions of the manufacturer (CRP, C4, and C1-INH functional activity), or it was calculated as the 2.5th and 97.5th percentiles of values from 112 healthy control subjects (C1-INH antigen concentration, VWF:Ag, VWF:CBA, sE-selectin, and ET-1).

Statistical analysis

For descriptive purposes, the measured values are expressed as medians and 25th to 75th percentiles or as numbers and percentages because most of