

Letter to the Editor

Hereditary angioedema: Key role for kallikrein and bradykinin in vascular endothelial-cadherin cleavage and edema formation

To the Editor:

Hereditary angioedema (HAE) is characterized by recurrent episodes of edema that can be life-threatening if not treated properly.¹ It is an autosomal-dominant inherited disease caused by C1 inhibitor deficiency as a result of mutations in the

SERPING1 gene. Because of C1 inhibitor deficiency in HAE, the plasma proteolytic cascades are activated during an attack (plasma kallikrein and coagulation factor XIIa) and kinins are generated. Among these, bradykinin has been shown to be the predominant mediator of enhanced vascular permeability in HAE attacks.^{2,3} Microvascular permeability is tightly controlled by a series of complex interactions between junctional proteins.⁴ In the endothelium, adherens junctions are formed by the transmembrane adhesive protein vascular endothelial (VE)-cadherin.⁴ The

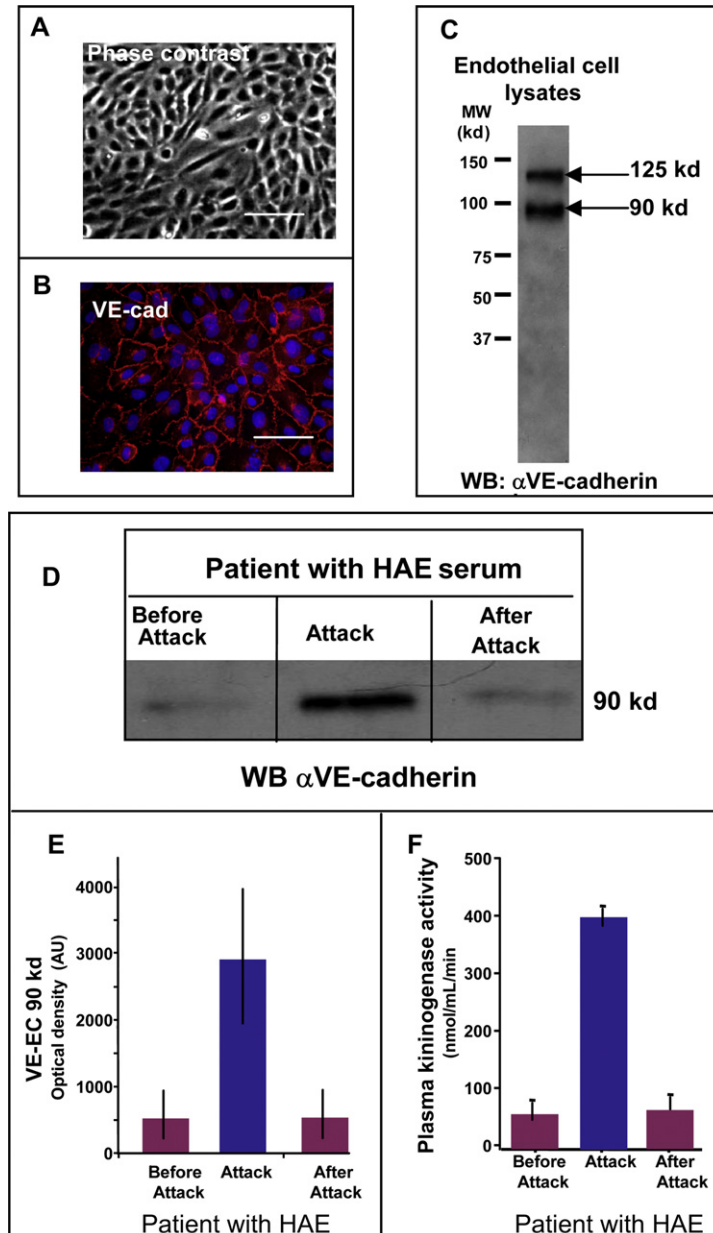


FIG 1. VE-cadherin (VE-cad) extracellular domain in HAE patient sample. **A**, Phase contrast microscopy of cultured endothelial cells. Scale bar: 50 μ m. **B**, VE-cadherin immunostaining. Scale bar: 50 μ m. **C**, VE-cadherin immunoblot on HUVECs. **D**, VE-cadherin immunoblot of HAE patient serum samples out of attack (lane 1), during the attack (lane 2), and after the attack (lane 3). **E**, Densitometric analysis of VE-cadherin immunoblots expressed as absorbance values (mean $\pm$ 2 SD; n = 4). **F**, Plasma kininogenase activity levels (mean $\pm$ 2 SD; n = 4). HUVEC, Human umbilical vascular endothelial cell; MW, molecular weight; WB, Western blot.

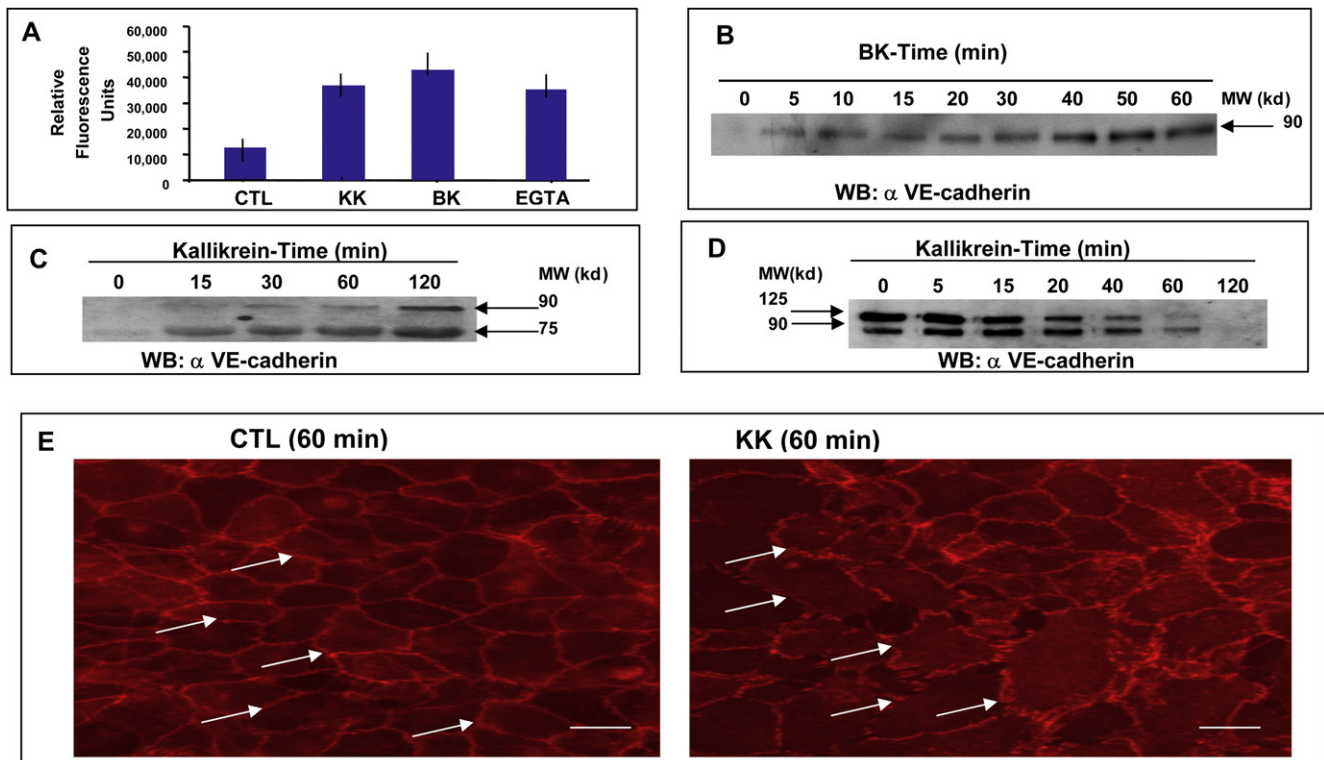


FIG 2. Bradykinin (BK) induced increased endothelial permeability through a process involving VE-cadherin proteolytic cleavage. **A**, *In vitro* vascular permeability assay on endothelial cells treated for 4 hours with BK (10^{-7} mol/L), kallikrein (KK; 10^{-7} mol/L), or EGTA (1 mmol/L; positive control; mean $\pm$ SD; $n = 3$). **B**, VE-cadherin content of conditioned media from unstimulated cells and BK-stimulated HUVECs (10^{-6} mol/L) for 5 to 60 minutes (HUVEC extracts were loaded as positive control; 5 μ g; $n = 3$). **C**, VE-cadherin content of conditioned media from KK (0.6 U)-treated cells. **D**, *In vitro* VE-cadherin (0.5 μ g) cleavage by KK (0.6 U; $n = 3$). **E**, VE-cadherin immunostaining after KK treatment (0.60 U for 60 minutes; $n = 4$). Scale bar: 50 μ m. CTL, Cytotoxic T lymphocyte; MW, molecular weight; WB, Western blot.

integrity of the endothelial cell-cell junction depends on the adhesive function and cell surface expression of VE-cadherin.⁵ Previous studies have demonstrated that disruption of cell-cell junctions depends on VE-cadherin tyrosine phosphorylation or cleavage.⁶ Therefore, we explored whether VE-cadherin was involved in the pathogenesis of HAE.

VE-cadherin is expressed at endothelial cell-cell junctions at the periphery of the cells (Fig 1, A and B). VE-cadherin Western blot analysis (using mouse mAb to the extracellular domain of human VE-cadherin [clone BV9]) of cell lysates allowed determination of the molecular mass of the protein (125 kd) and its extracellular domain (90 kd; Fig 1, C).⁷ VE-cadherin serum level was measured in a 54-year-old woman with HAE type I and a low plasma C1-inhibitor level (12% normal range). The blood samples were taken during an attack and between attacks (1 before and 1 after an episode). The 90-kd VE-cadherin extracellular domain was detected in the patient's serum during the attack (Fig 1, D, lane 2), whereas it was barely detectable before and after the attack (Fig 1, D, lanes 1 and 3). Standard scanning and image intensity analysis performed on 4 independent immunoblots showed a significant increase ($P < .01$; Student *t* test) in the average density of the low-molecular-weight anti-VE-cadherin band observed during the attack compared with baseline (Fig 1, E). The emergence of this split product and its extent are significantly associated ($P < .001$, Spearman test) with the increase in plasma kininogenase activity (64.1 ± 4.6 nmol/mL/min before the attack,

396.9 ± 28.5 during the attack, and 56.6 ± 5.1 after the attack; Fig 1, F).⁸ We have analyzed sera from another patient (30-year-old woman with HAE type I) during 2 different attacks (the leg and the buttock). Western blot analysis showed the same increase of VE-cadherin during each attack (data not shown).

To explore one possible molecular mechanism leading to VE-cadherin cleavage from the endothelium, we investigated the effects of bradykinin and kallikrein in a cell culture permeability assay. By using a fluorescein isothiocyanate-dextran cell permeability assay, we demonstrated that bradykinin (10^{-7} mol/L) and kallikrein (10^{-7} mol/L) increased human umbilical vein endothelial cell (HUVEC) permeability to the same extent as ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA; 1 mmol/L) used as a positive control (Fig 2, A). Release of VE-cadherin from HUVECs was measured after 2 to 4 hours of bradykinin (10^{-7} mol/L) stimulation or 0 to 120 minutes of kallikrein (10^{-7} mol/L) treatment. Bradykinin stimulation induced a time-dependent release of a VE-cadherin fragment (90 kd). These fragments are almost undetectable in untreated cells (Fig 2, B). Kallikrein induced 90-kd and 75-kd VE-cadherin release within 15 minutes, which increased to 120 minutes (Fig 2, C). The 75-kd fragment was not seen in plasma. We hypothesized that when active kallikrein is added to endothelial cells, its protease activity in inducing VE-cadherin cleavage is greater than that of kallikrein in plasma.

VE-cadherin release during protease incubation was faster than after bradykinin stimulation, which is in agreement with a direct proteolytic action of kallikrein on VE-cadherin. In support of this concept, the amino acid sequence of the VE-cadherin extracellular domain contains 2 putative cleavage sites for the protease kallikrein at the peptide bonds R⁴¹¹-T⁴¹² and R⁵⁶⁵-T⁵⁶⁶ (sequence identifier, P03952) that could lead to the observed 75-kd and 90-kd fragments, respectively. We hypothesized that kallikrein could directly cleave the VE-cadherin from the microvasculature during an HAE attack, leading to release of the 90-kd fragment into the blood. To test this hypothesis, we performed an *in vitro* proteolytic assay by using purified human plasma kallikrein and an endothelial cell lysate containing a high amount of VE-cadherin as substrate. The reaction was run for 0 to 120 minutes (37°C). Kallikrein induced a time-dependent decrease in both the 125-kd and 90-kd forms of VE-cadherin (Fig 2, D). In addition, after kallikrein challenge, VE-cadherin immunostaining showed several intercellular openings between adjacent cells (Fig 2, E). These results support the idea that the mediators of HAE attack induce VE-cadherin cleavage that might contribute to increased endothelial cell permeability.

Previous studies have demonstrated that disruption of cell-cell junctions depends on intracellular kinases and/or phosphatases. VE-cadherin tyrosine phosphorylation is a general mechanism involved in weakening of the endothelial cell barrier resulting from cell stimulation by growth factors and inflammatory cytokines.⁵ Bradykinin receptors are G protein-coupled receptors that can activate the intracellular tyrosine kinase pathways. We found that bradykinin induced VE-cadherin tyrosine phosphorylation in HUVECs within 5 to 10 minutes (data not shown), suggesting that a link between VE-cadherin phosphorylation and cleavage cannot be excluded, because the phosphorylation occurs within a few minutes, whereas the truncated form of VE-cadherin was detected in the culture medium after 30 minutes.

In conclusion, the detection of a soluble VE-cadherin in the patient's serum during an HAE attack might have potential clinical interest as a biomarker for the diagnosis. Because we have shown that both bradykinin and kallikrein have a direct effect on permeability, it is possible that a combination of a kallikrein

inhibitor (ecallantide) and a B2 receptor antagonist (icatibant) could be used to treat a severe attack.

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