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Endogenous Bradykinin and BK1-5 during ACE Inhibitor-Associated Angioedema

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Capsule Summary

Bradykinin concentrations and the ratio of bradykinin to its stable metabolite BK1-5 (RPPGF) were significantly increased in patients presenting with angiotensin-converting enzyme (ACE) inhibitor-associated angioedema compared to ACE inhibitor-treated controls. Cleavage of high molecular weight kininogen was not increased in patients with ACE inhibitor-associated angioedema, indicating that increased bradykinin concentrations result from decreased degradation.

Keywords

angioedema; bradykinin; angiotensin-converting enzyme inhibitor

Abbreviations

ACE (angiotensin-converting enzyme)

BK1-5 (bradykinin 1-5)

HMWK (high molecular weight kininogen)

cHMWK (cleaved high molecular weight kininogen)

NEP (neprilysin)

To the Editor:

Angiotensin-converting enzyme (ACE) inhibitor-associated angioedema is presumed to result from bradykinin. Whereas hereditary angioedema results from increased bradykinin production, ACE inhibitor-associated angioedema could result from decreased degradation of vasoactive ACE substrates. Bradykinin B2 receptor antagonism decreases the duration of attacks of angioedema in patients with C1 inhibitor deficiency.¹ Clinical trials provide conflicting results about the efficacy of the B2 receptor antagonist icatibant in ACE inhibitor-associated angioedema, however.²⁻⁴

The lack of effect of bradykinin B2 receptor antagonism in two of three trials in ACE inhibitor-associated angioedema²⁻⁴ could suggest that bradykinin does not cause ACE inhibitor-associated angioedema, that substance P or other ACE substrates contribute to angioedema during B2 receptor blockade, or that patients with ACE inhibitor-associated angioedema present for medical attention too late for B2 receptor blockade to be effective. In the study that reported benefit of B2 receptor blockade the mean time to treatment was 6 hours, whereas in the two negative studies it was 7.8 and 10 hours. Nussberger and colleagues reported that circulating bradykinin concentrations are increased in patients with ACE inhibitor-associated angioedema and decrease after withdrawal of the ACE inhibitor, but did not include ACE inhibitor-treated controls without angioedema.⁵ To determine whether bradykinin is increased in ACE inhibitor-associated angioedema we measured bradykinin in blood from patients with acute ACE inhibitor-associated angioedema and ACE inhibitor-treated controls. We also measured bradykinin 1-5 (BK1-5 or RPPGF), the stable product of sequential degradation of bradykinin by ACE and neprilysin (NEP). To assess whether the release of bradykinin from high molecular weight kininogen (HMWK) by plasma kallikrein is increased, we measured cleaved HMWK.

We analyzed samples from 41 patients who presented to Vanderbilt University Medical Center with acute ACE inhibitor-associated angioedema. Twenty-seven participated in placebo-controlled randomized clinical trials of icatibant (NCT00517582 and NCT01574248).³ Samples were collected at presentation, an average of 14.0 ± 9.5 hours after the initial onset of symptoms, and, in fifteen patients, after resolution of symptoms (“convalescent” samples). Patients were defined as having ACE inhibitor-associated angioedema if they had swelling of the lips, pharynx or face while taking an ACE inhibitor and had never had swelling in the absence of ACE inhibitor.³ Patients with hereditary angioedema were excluded. Cases with angioedema of the bowel alone were excluded to avoid delayed diagnosis. Patients who presented with features of hypersensitivity, pruritus or urticaria were excluded. Patients who had recurrent angioedema after discontinuation of ACE inhibitor were excluded.

Samples were obtained from 19 controls who had taken an ACE inhibitor for at least one year and never had signs or symptoms of angioedema. Controls were group-matched to cases by race and gender and, to the extent possible, age.

All studies were approved by the Vanderbilt Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent.

For bradykinin and BK1-5, blood was collected into polypropylene tubes containing chilled ethanol to denature kallikrein and kininases. After 30 min at 4°C , samples were centrifuged and supernatant was stored at -80°C . For cleaved HMWK, samples were collected in citrate-containing polypropylene tubes on ice, centrifuged within 20 minutes, and plasma supernatant was stored at -80°C . Bradykinin and BK1-5 were measured by UPLC-ESI-MS/MS

using a modification of a previously published assay for BK1-5.⁶ Methods appear in the supplement.

Serum ACE activity was measured using a commercial kinetic assay (LabCorp, Knoxville, TN). NEP activity was measured but not detected in plasma using a fluorometric assay (Sensolyte®, AnaSpec, Fremont, CA).

A recombinant human antibody (M004-B04) specific for cHMWK was discovered using phage display and an ELISA was developed to quantify cHMWK in human plasma (supplemental methods).

Data are presented as N or means $\pm$ standard deviation in supplemental Table 1, and as individual values with median $\pm$ interquartile range in Figures. For results below the level of detection (D), D/2 was used in analyses. Comparisons between groups were made using a Pearson test or Wilcoxon test. Acute and convalescent samples were compared using the Wilcoxon Signed-rank Test. A two-sided p-value <0.05 was considered significant.

Supplemental **Table 1** provides characteristics of patients with ACE inhibitor-associated angioedema and ACE inhibitor-treated controls. Serum ACE activity was not significantly different between cases and controls. ACE inhibitor use was discontinued in all patients. In ten patients with angioedema ACE activity was measured at presentation and after resolution of symptoms, and there was a trend toward an increase in ACE activity from 15.1 ± 11.8 to 25.8 ± 12.8 U/L over time ($p=0.08$).

Bradykinin concentrations were significantly higher in patients with ACE inhibitor-associated angioedema compared to ACE inhibitor-treated controls (**Figure 1**) and similar to those reported previously during acute attacks in patients with C1 inhibitor deficiency and during

ACE inhibitor-associated angioedema.⁵ BK1-5 concentrations were not different between the groups. As a result, the ratio of bradykinin to BK1-5 was increased in the blood of patients with ACE inhibitor-associated angioedema compared to controls. There was no difference in total kinins (bradykinin plus BK1-5) in cases and controls. There were no differences in bradykinin, BK1-5, or the molar ratio of bradykinin to BK1-5 among racial groups, between men and women, according to smoking history, or between diabetics and non-diabetics.

In 15 patients, samples were collected after resolution of symptoms; the median time to collection was 48 hours (range 16 to 2160 hours). Bradykinin concentrations did not differ significantly in acute and convalescent samples. There was no effect of icatibant on convalescent BK concentrations ($p=1.0$ versus placebo). BK1-5 concentrations were significantly higher in convalescent samples, consistent with discontinuation of ACE inhibition (**Figure 2** and supplement).

We measured cleaved HMWK in six controls, in 13 patients at presentation with angioedema, and in convalescent samples obtained from 11 of the latter. There were no significant differences in cleaved HMWK concentrations in plasma from patients with ACE inhibitor-associated angioedema and ACE inhibitor-treated controls (**supplemental fig1**).

We found for the first time that bradykinin and the ratio of bradykinin to its cleavage product by ACE and NEP, BK1-5, are elevated in patients presenting with acute ACE inhibitor-associated angioedema compared to ACE inhibitor-treated controls. The finding that bradykinin but not BK1-5 was increased in patients with angioedema compared to ACE inhibitor-treated controls, while ACE activity was not significantly different, suggests that degradation of bradykinin to BK1-5 via non-ACE pathways is impaired in patients with ACE inhibitor-associated angioedema. NEP also cleaves bradykinin to BK1-5 and a polymorphism in the gene

encoding NEP has been associated with ACE inhibitor-associated angioedema.⁷ In addition, use of the NEP inhibitor/angiotensin receptor blocker sacubitril/valsartan is associated with angioedema. Unfortunately, we were not able to measure NEP activity in plasma in the present study.

Unlike in hereditary angioedema,⁸ there was no evidence for increased bradykinin production by cleavage of HMWK by plasma kallikrein. This validates the hypothesis that excess bradykinin results from decreased degradation in ACE inhibitor-associated angioedema, and also explains the observation that the plasma kallikrein inhibition is ineffective in treatment of ACE inhibitor-associated angioedema.⁹

These data support the understanding that bradykinin contributes to ACE inhibitor-associated angioedema and suggest that the lack of efficacy of B2 receptor antagonism in most trials in ACE inhibitor-associated angioedema reflects late presentation or involvement of additional vasoactive peptides.

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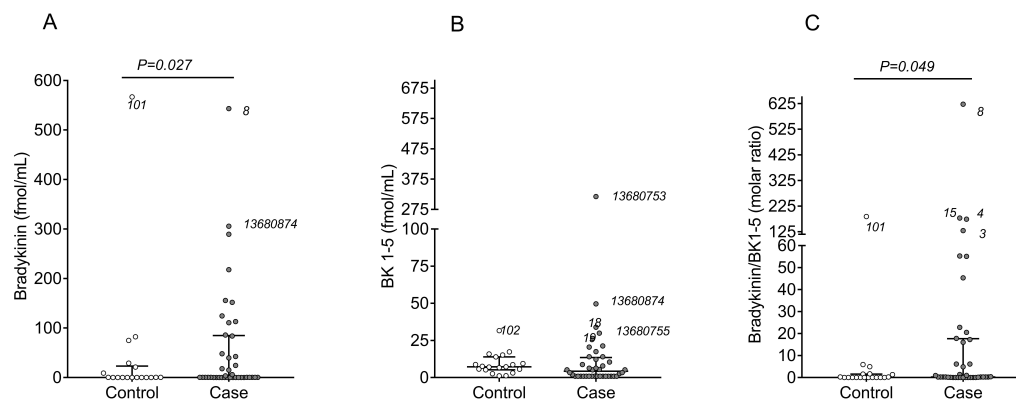
References

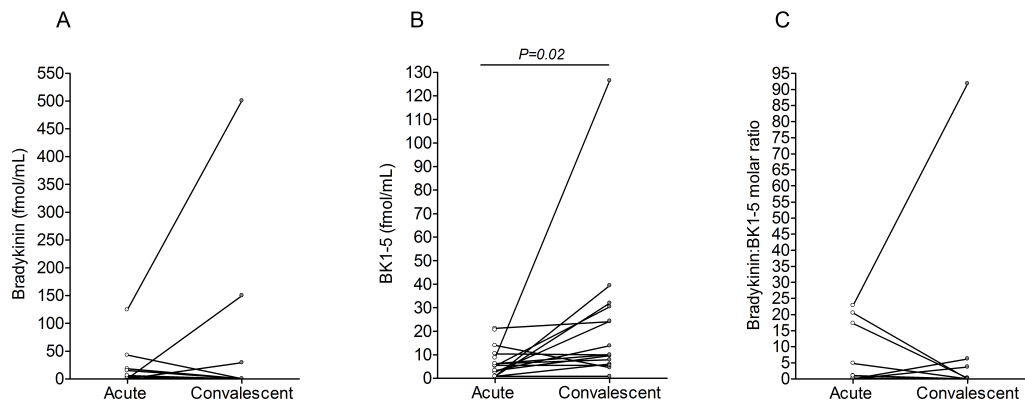
1. Cicardi M, Banerji A, Bracho F, Malbran A, Rosenkranz B, Riedl M, et al. Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema. *N Engl J Med*. 2010;363:532-41.
2. Bas M, Greve J, Stelter K, Havel M, Strassen U, Rotter N, et al. A randomized trial of icatibant in ACE-inhibitor-induced angioedema. *N Engl J Med*. 2015;372:418-25.
3. Straka BT, Ramirez CE, Byrd JB, Stone E, Woodard-Grice A, Nian H, et al. Effect of bradykinin receptor antagonism on ACE inhibitor-associated angioedema. *J Allergy Clin Immunol*. 2017;140:242-248 e2.
4. Sinert R, Levy P, Bernstein JA, Body R, Sivilotti MLA, Moellman J, et al. Randomized Trial of Icatibant for Angiotensin-Converting Enzyme Inhibitor-Induced Upper Airway Angioedema. *J Allergy Clin Immunol Pract*. 2017;5:1402-1409 e3.
5. Nussberger J, Cugno M and Cicardi M. Bradykinin-mediated angioedema. *N Engl J Med*. 2002;347:621-2.
6. Murphey LJ, Hachey DL, Vaughan DE, Brown NJ and Morrow JD. Quantification of BK1-5, the stable bradykinin plasma metabolite in humans, by a highly accurate liquid-chromatographic tandem mass spectrometric assay. *Anal Biochem*. 2001;292:87-93.
7. Pare G, Kubo M, Byrd JB, McCarty CA, Woodard-Grice A, Teo KK, et al. Genetic variants associated with angiotensin-converting enzyme inhibitor-associated angioedema. *Pharmacogenet Genomics*. 2013;23:470-8.
8. Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M and Cicardi M. High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency. *Clin Exp Allergy*. 2014;44:1503-14.

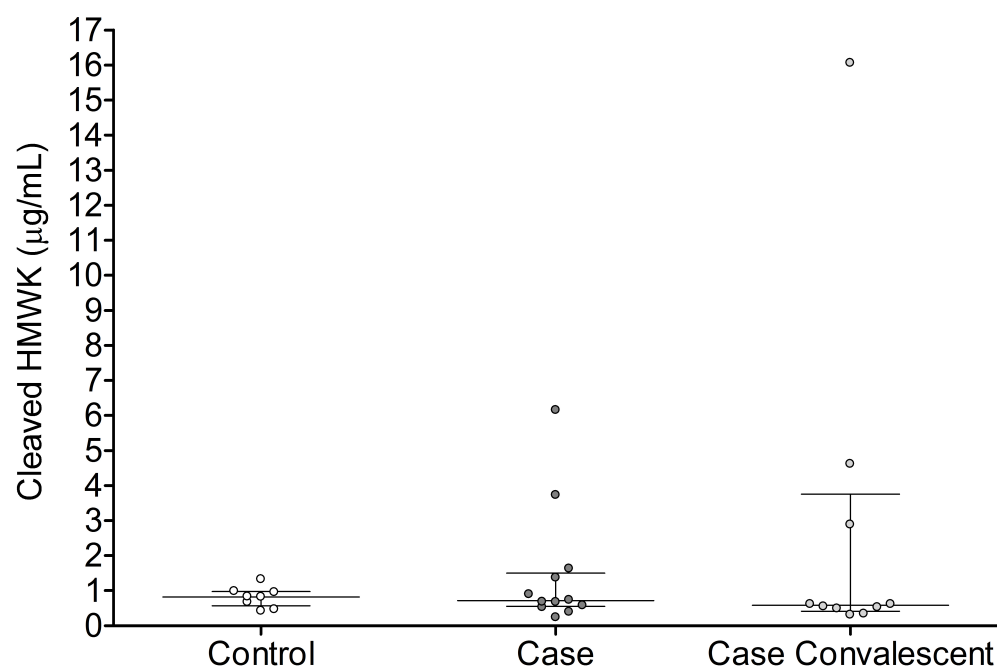
9. Lewis LM, Graffeo C, Crosley P, Klausner HA, Clark CL, Frank A, et al. Ecallantide for the acute treatment of angiotensin-converting enzyme inhibitor-induced angioedema: a multicenter, randomized, controlled trial. *Ann Emerg Med.* 2015;65:204-13.

Figure 1: Concentrations of bradykinin and its metabolite bradykinin 1-5 (BK1-5) in patients presenting with angiotensin-converting enzyme (ACE) inhibitor-associated angioedema (case) and in ACE inhibitor-treated controls. Plots show individual data and the median with interquartile range. ID numbers are provided for subjects with a value greater than two standard deviations above the mean for the controls.

Figure 2: Concentrations of bradykinin and its metabolite bradykinin 1-5 (BK1-5) in blood samples collected during angiotensin converting enzyme (ACE) inhibitor-associated angioedema (acute) and after resolutions of symptoms (convalescent)







Supplement to Letter to the Editor, “Endogenous Bradykinin and BK1-5 during ACE Inhibitor-Associated Angioedema”**Methods***Bradykinin and BK1-5 analysis by UPLC-ESI-MS/MS*

Briefly, a mixture of peptides containing stable isotope-labeled arginine ($^{13}\text{C}_6\text{H}_{14}^{15}\text{N}_4\text{O}_2$, New England Peptide) (2.0 ng bradykinin and 0.5 ng BK1-5 in 15 μL ethanol) as internal standards and 30 μL of 50mM dithiotreitol (Sigma-Aldrich) were added to the ethanolic supernatant. The resulting solution was evaporated in a 40°C water bath under a stream of nitrogen. The residue was then re-constituted in 1.0 mL of 10mM potassium phosphate buffer pH 7.0 and applied to a 3mL, 60 mg Nexus cartridge (Agilent Technologies) preconditioned with 3.0 mL methanol, 6.0 mL water and 6.0 mL 10 mM phosphate buffer. The cartridge was washed with 5 mL water. Peptides were then eluted with 1.5 mL methanol containing 1% 1N HCl and the eluate was evaporated at 40°C under a stream of nitrogen.

The residue was re-dissolved in 1 mL of 1.0% (v/v) trifluoroacetic acid (TFA)/water solution and applied to a 1 mL, 100 mg Hypersep C18 cartridge (Thermo Scientific) preconditioned with 3.0 mL acetonitrile and 5.0 mL 1% (v/v) TFA/water. The sample was then eluted with 1.5 mL of 60% (v/v) acetonitrile and 1% (v/v) TFA/water into polypropylene tubes after the cartridge was washed with 3.0 mL 5% (v/v) TFA/water. The eluate was evaporated at 40°C under a stream of nitrogen. The final residue was stored at -80°C until analysis by ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS).

The residue was dissolved in 30 μ L of 30% (v/v) acetonitrile and 1.0% (v/v) TFA/water solution. The samples were centrifuged at 14,000 g for 5 min to remove any pellet. Samples were then resolved by UPLC (Waters) at 40°C by an Acquity BEH octadecylsilane (C18) column (1.7 μ m, 1.0 x 100 mm) (Waters) using a solvent mixture of acetonitrile/water/formic acid (94.9/5/0.1 (v/v/v) to 69.9/30/0.1 (v/v/v)) at a flow rate of 0.14 ml/min with a 7 min linear gradient. The resolved peptides were delivered to a mass spectrometer (TSQ Vantage, Thermo Scientific) equipped with a heated ESI probe running at a positive SRM mode: spray voltage 3500v, vaporizer temperature 297°C, sheath gas pressure 40 psi, capillary temperature 325°C. The observed product ions produced by sample were: m/z 70 originating from m/z 531 (bradykinin) and m/z 70 from m/z 536 ($[^{13}\text{C}, ^{15}\text{N}]$ bradykinin), m/z 408 originating from m/z 287 (BK(1-5)) and m/z 418 from m/z 292 ($[^{13}\text{C}, ^{15}\text{N}]$ BK(1-5)). Peak ratios of sample to internal standard were used for quantification. The limit of detection for BK1-5 is 1 pg and for bradykinin 0.5 pg.

Measurement of cleaved high molecular weight kininogen (cHMWK)

A recombinant human antibody (M004-B04) specific for cHMWK was discovered using phage display and an ELISA was developed to quantify cHMWK in human plasma by screening against both cHMWK and intact HMWK (both from Enzyme Research Laboratories, South Bend, IN) (presented in abstracts at the European Academy of Allergy and Clinical Immunology in 2016 and 2017). The antibody appears to be highly specific for cHMWK and was validated by correlation with a HMWK Western blot assay with fluorescent detection (Licor) in patients with C1 inhibitor deficiency.⁽¹⁾ The Fab fragment of M004-B04 was coated on 96-well plates (Nunc Maxisorp) overnight in PBS prior to being washed and blocked with 2% BSA Buffer. Samples, standards, and QCs diluted in 0.1% BSA buffer were added to the plate and after a subsequent incubation, plate bound cHMWK was detected by adding pooled mouse anti-HMWK

monoclonal antibodies (Clones 11H05 and 13B12). The plate was washed and a secondary goat anti-mouse HRP is added to the plate, followed by tetramethylbenziden (TMB) substrate, stopping with phosphoric acid and the OD measured at 450nm with subtraction at 630 nm.

Plasma samples from patients with hereditary angioedema due to C1 inhibitor deficiency have elevated cHMWK measured using the cHMWK ELISA used in this study, as well as by HMWK Western blot, LC-MS/MS, or an ELISA based on a different cHMWK-specific antibody.⁽¹⁻⁴⁾

Results

	Cases (41)	Controls (19)
Race, Black: White: Multi	29: 11: 1	10: 9: 0
Gender, Female: Male	27:14	12:7
Age, years	58.9±12.2	64.3±10.5
Diabetes, Yes: No	18:23	6:13
Smoker, Never: Former: Current	13:17:11	11:4:4
ACE activity, U/L	15.6±11.2 (N=38)	20.7±14.6 (N=9)

Table 1: Baseline characteristics of patients with ACE inhibitor-associated angioedema and controls

Data are presented as mean ± standard deviation of the mean or as N.

	Lowest Tertile	Middle Tertile	Highest Tertile
Race, Black: White: Multi	8:4:1	11:4:0	10:3:0
Gender, Female: Male	9:4	9:6	9:4
Age, years	55.1±13.8	59.1±10.5	62.6±12.1
Diabetes, Yes: No	7:6	8:7	6:5
Smoker, Never: Former: Current	6:5:2	2:6:7	5:6:2
ACE activity, U/L	22.2±15.7	11.3±6.9	15.2±7.9

Table 2: Baseline characteristics of patients with ACE inhibitor-associated angioedema grouped by tertile of bradykinin

Data are presented as mean ± standard deviation of the mean or as N.

ACE inhibitor	BK acute (fmol/mL)	BK convalescent (fmol/mL)	BK1-5 acute (fmol/mL)	BK1-5 convalescent (fmol/mL)	Collection time convalescent (hours)
lisinopril	0.24	0.24	0.87	31.92	24
benazapril	42.44	0.24	8.73	126.4	48
lisinopril	0.24	0.24	5.24	30.36	24
enalapril	17.92	0.24	0.87	6.09	21
lisinopril	15.09	0.24	0.87	0.87	48
quinapril	0.24	0.24	10.48	9.93	16
enalapril	0.24	0.24	0.87	39.39	48
enalapril	124.5	500.28	5.45	5.45	408
lisinopril	0.11	29.2	6.29	7.96	384
lisinopril	0.31	149.7	21.28	24.13	2160
lisinopril	0.31	0.31	2.65	24.27	384
lisinopril	0.31	0.31	3.31	13.81	24
lisinopril	3.38	0.11	3.35	9.64	240
lisinopril	0.31	0.31	14.01	4.59	192
enalapril	5.93	0.11	6.29	10.06	1056

Table 3: Individual data for 15 patients with angiotensin-converting enzyme (ACE) inhibitor-associated angioedema who had samples collected during acute symptoms and after resolution of symptoms. BK indicates bradykinin, BK1-5

References

- (1) Banerji A, Busse P, Shennak M, Lumry W, Davis-Lorton M, Wedner HJ et al. Inhibiting Plasma Kallikrein for Hereditary Angioedema Prophylaxis. *N Engl J Med* 2017; 376(8):717-28.
- (2) Zhang G, Sexton DJ, Faucette RR, Qiu Y, Wu J. 2D-LC-MS/MS to measure cleaved high-molecular-weight kininogen in human plasma as a biomarker for C1-INH-HAE. *Bioanalysis* 2017; 9(19):1477-91.
- (3) Hofman ZLM, de MS, Suffritti C, Zanichelli A, van DC, Sebastian SAE et al. Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema. *J Allergy Clin Immunol* 2017; 140(6):1700-3.
- (4) Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M, Cicardi M. High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency. *Clin Exp Allergy* 2014; 44(12):1503-14.

Figure E1: Cleaved high molecular weight kininogen (HMWK) measured in plasma from angiotensin-converting enzyme (ACE)-inhibitor treated controls without angioedema, patients during acute presentation with ACE inhibitor-associated angioedema (case), and after resolution of angioedema (case convalescent).