**Dipeptidyl Peptidase IV Activity in Patients With ACE-Inhibitor–Associated Angioedema**

Jean Lefebvre, Laine J. Murphey, Tina V. Hartert, Ru Jiao Shan, William H. Simmons, Nancy J. Brown

**Abstract**—Bradykinin and substance P have been implicated as mediators in angiotensin-converting enzyme inhibitor (ACEI)–associated angioedema. Studies investigating the metabolism of bradykinin in sera from patients with a history of ACEI-associated angioedema and controls suggest that there is a defect in a non-ACE, non-kininase I pathway of bradykinin degradation, such as the aminopeptidase P (APP)/dipeptidyl peptidase IV (DPPIV) pathway. This study tested the hypothesis that serum APP or DPPIV activity is decreased in patients with ACEI-associated angioedema. APP and DPPIV activity were measured in sera collected from patients during ACEI-associated angioedema, from patients with a remote history of ACEI-associated angioedema, and from normotensive and untreated hypertensive controls. The effects of acute and chronic ACEI and corticosteroid treatment on serum DPPIV activity were also assessed. DPPIV activity was similar in normotensive volunteers (37.8±6.3 nmol/mL per min), in untreated hypertensive subjects who had been exposed previously to ACEI without angioedema (36.2±4.3 nmol/mL per min), in hypertensive patients with a remote history of angioedema (35.1±8.5 nmol/mL per min), and in chronically ACEI-treated hypertensive subjects (36.1±5.6 nmol/mL per min). DPPIV activity decreased with increasing age (R²=0.10, P=0.016). Subject group significantly affected DPPIV activity (F=6.208, P=0.016) such that DPPIV activity was significantly lower in patients with ACEI-associated angioedema (26.9±4.1 nmol/mL per min) than in normotensive controls, in previously ACEI-exposed untreated hypertensive volunteers, or in ACEI-treated hypertensive volunteers, even after controlling for age. There was no effect of acute ACE inhibition or corticosteroids on DPPIV activity. With respect to APP activity, there was no difference between groups. These results suggest that DPPIV activity is depressed in individuals with hypertension during acute ACEI-associated angioedema. (*Hypertension. 2002;39[part 2]:460-464.*)

**Key Words:** edema ■ angiotensin-converting enzyme inhibitors ■ peptides ■ bradykinin

Angioedema is a rare but potentially life-threatening side effect of angiotensin-converting enzyme inhibitors (ACEI) characterized by swelling of the lips, tongue, mouth, throat, other parts of the face, or, rarely, the hands, feet, or abdominal viscer.a,1 Whereas the risk of ACEI-associated angioedema has been reported to be 0.1 to 0.4% in predominantly white populations, the risk is increased 4– 5-fold in black populations, suggesting that genetic or environmental factors modulate the risk of angioedema.2,4 The risk of angioedema secondary to vasopeptidase inhibitors, which block both ACE and neutral endopeptidase (NEP), may be higher than that associated with ACE inhibition alone.5

Although the exact mechanism of ACEI-associated angioedema is not known, both bradykinin and substance P, substrates of ACE and NEP, have been implicated in the pathogenesis of angioedema.6– 9 For example, activation of the kallikrein-kinin system has been demonstrated in hereditary angioedema.7 In rats and mice, bradykinin and substance P have been shown to contribute to ACEI-induced plasma extravasation.8,9 In human sera, in vitro and in vivo, bradykinin (Figure 1) is rapidly metabolized at its carboxy-terminal end by ACE (kininase II, EC 3.4.15.1).10 Endopeptidases such as NEP also inactivate bradykinin by cleaving the Pro⁰–Phe⁹ bond, whereas carboxypeptidases N and M (kininase I) hydrolyze the carboxy-terminal Phe⁸–Arg⁹ bond to produce des-Arg⁹–bradykinin.10 At the N-terminus, aminopeptidase P (APP) cleaves bradykinin by hydrolyzing the Arg¹–Pro² bond, rendering bradykinin inactive and susceptible to additional cleavage by dipeptidyl peptidase IV (DPPIV).11,12 In humans, systemic bradykinin is rapidly degraded by ACE to bradykinin 1-5, as well as via the APP/DPPIV pathway.13 In the presence of ACE inhibition, the roles of the kininase I and the APP/DPPIV pathway in the degradation of bradykinin are increased.14,15 Thus, APP inhibition potentiates the effect of bradykinin in an ACEI-treated rat model.16

Blais et al17 previously studied the metabolism of bradykinin in the sera of patients with a history of ACEI-associated angioedema. During ACE inhibition, the relative contribution

Received September 22, 2001; first decision November 2, 2001; revision accepted November 13, 2001.

From the Department of Medicine, Division of Clinical Pharmacology (J.L., L.J.M., R.J.S., N.J.B.) and Division of Allergy, Pulmonary and Critical Care Medicine (T.V.H.), Vanderbilt University School of Medicine, Nashville, Tenn.; and Division of Molecular and Cellular Biochemistry (W.H.S.), Loyola University Medical Center, Stritch School of Medicine, Maywood, Ill. Correspondence to Nancy J. Brown, MD, Medicine and Pharmacology, 560 Medical Research Building I, Vanderbilt University Medical Center, Nashville, TN 37232-6602. E-mail nancy.brown@mcmail.vanderbilt.edu. © 2002 American Heart Association, Inc.

*Hypertension* is available at [http://www.hypertensionaha.org](http://www.hypertensionaha.org)
of kininase I to the degradation of bradykinin is increased in these patients compared with controls. This finding suggests the hypothesis that patients with a history of ACEI-associated angioedema have a defect in a non-ACE (kininase II), non-kininase I pathway of bradykinin metabolism, such as the NEP or APP/DPP IV pathway. Blais et al.18 also reported decreased APP activity in sera from ACEI-treated patients who developed hypersensitivity reactions during dialysis but did not measure APP activity in patients with a history of angioedema.

Two clinical observations implicate a defect in DPP IV activity, rather than APP activity, in the cause of ACEI-associated angioedema. First, although the gene encoding APP activity, rather than APP activity, is associated with ACEI-associated angioedema. Second, immunosuppressive therapy. Finally, serum was obtained from 2 patients who had a history of recurrent angioedema, in the absence of ACE inhibition. All subjects gave informed consent, and the protocols were approved by the Vanderbilt Institutional Review Board, in accordance with the Declaration of Helsinki on medical research involving human subjects.

**Methods**

**Patients and Controls**

Blood samples were obtained within 3 days of the onset of angioedema in 6 patients and at least 1 month after the resolution of ACEI-associated angioedema and discontinuation of ACEI in 7 patients (defined as in remission). Twenty-one normotensive and 16 hypertensive volunteers who had previously taken at least 1 dose of an ACEI but who had never experienced angioedema served as controls. The effect of chronic ACE inhibition was also measured in 8 hypertensive subjects after 3-week treatment with ramipril, 10 mg/d. The majority of controls were participating in mechanistic studies of the effects of ACEI ongoing in our laboratory. In 5 of the normotensive and 4 of the hypertensive subjects, DPP IV activity was measured in sera obtained before and 3 hours after administration of 25 mg of captopril and placebo to assess the acute effect of ACE inhibition per se on DPP IV activity. Because many patients with ACEI-associated angioedema are given corticosteroids, the effect of such a therapy on DPP IV activity was assessed in sera obtained from 8 patients with asthma during and 3 months after corticosteroid therapy. Finally, serum was obtained from 2 patients who had a history of recurrent angioedema, in the absence of ACE inhibition. All subjects gave informed consent, and the protocols were approved by the Vanderbilt Institutional Review Board, in accordance with the Declaration of Helsinki on medical research involving human subjects.

**APP Activity**

Serum APP activity was determined by using the internally quenched fluorescent substrate L-lysyl(e-2-aminobenzoyl)-t-tryptophan-L-prolyl-L-prolyl-4-nitroanilide [H-Lys(e-Abz)-Pro-Pro-pNA] (Bachem). Serum samples (8 µL) were placed into disposable 1.5-ml semimicromethod centrifuge tubes with polyethylene caps (Fisher Scientific). The reaction was initiated by the addition of prewarmed substrate solution to a final volume of 800 µL containing 5 µmol/L substrate, 0.5 mmol/L MnCl₂, and 0.1 mmol/L HEPES (pH 8.0). The cuvettes were incubated in a 37°C shaking water bath and then removed at 5 to 7 time points over a period of 3 to 3.5 hours for measurement of fluorescence in an SPF-500C spectrofluorometer (SLM Instruments). The following instrument parameters were used: excitation 320 nm, 4-nm bandwidth; emission 410 nm, 7.5-nm bandwidth; data source, A channel/B channel; high voltage, 765 (A) and 610 (B); gain, 10 (A and B); filter 3. The change in fluorescence as a result of the release of Lys(e-Abz) was linear with time. Rates, determined by linear regression analysis of all points except 0 time, were averaged from quadruplicate samples. The change in fluorescence for complete cleavage of substrate (4 nmol, determined with purified rat APP) was 6.1. Abz-Gly (Bachem), which has 80% of the fluorescence of Lys(e-Abz), and was used as the routine standard. Pooled human serum (CELLect) (ICN Biochemicals) cleaved the substrate at the rate of 167±7 pmol/min per mL. Activity was inhibited 97% by the specific APP inhibitor apstatin (100 µmol/L).

**Statistics**

Data are presented as means±SD. Comparisons of continuous variables among groups were made by 1-way ANOVA followed by Dunnett’s test for multiple comparisons. Correlations were assessed by least-squares linear regression. A 2-sided P value less than 0.05 was accepted as significant.

**Results**

The Table provides subjects’ characteristics. Mean DPP IV activity was 37.8±6.3 nmol/mL per min in the healthy
There was no effect of acute or chronic P/H11005 controls (untreated HTN; □), ACEI-treated hypertensive controls (ACEI-treated HTN; ●), hypertensive patients during ACEI-associated angioedema (acute ACEI AE; ○), hypertensive patients in remission from ACEI-associated angioedema (with ACEI (non-ACEI AE; •), and patients with angioedema not associated with ACEI (non-ACEI AE; □).

volunteers (Figure 2). Mean DPP IV activity in untreated, previously ACEI-exposed hypertensive controls without angioedema was 36.2 ± 4.3 nmol/mL per min. There was no significant effect of hypertension, ethnicity (mean DPP IV activity 35.9 ± 6.4 nmol/mL per min in whites versus 35.3 ± 6.6 nmol/mL per min in blacks), or gender (mean DPP IV activity 36.6 ± 6.9 nmol/mL per min in men versus 34.3 ± 5.7 nmol/mL per min in women) on DPP IV activity. DPP IV activity decreased with increasing age (R² = 0.10, P = 0.016; Figure 3). There was no effect of acute or chronic ACE inhibition on DPP IV activity. DPP IV activity was 42.8 ± 5.6 nmol/mL per min before and 42.9 ± 4.2 nmol/mL per min after placebo and 44.3 ± 6.9 nmol/mL per min before and 41.0 ± 6.9 nmol/mL per min after captopril. There was no significant effect of 3-week treatment with the ACEI ramipril on DPP IV activity (36.1 ± 5.6 nmol/mL per min; P = 0.424 versus untreated hypertensive controls). There was also no significant effect of corticosteroids on serum DPP IV activity in patients with asthma (50.3 ± 17.3 nmol/mL per min during corticosteroids versus 39.8 ± 6.0 nmol/mL per min 3 months after cessation of corticosteroid therapy; P = 0.225). Twenty of the controls had 2 or more blood samples drawn for the DPP IV activity at least 6 days apart; the estimated intraindividual variability with time was 6.0%.

The patients with ACEI-associated angioedema were significantly older than the normotensive volunteers (P = 0.002) but not the untreated hypertensive controls (P = 0.13) or the ACEI-treated hypertensive controls (P = 0.21). Subject group significantly affected DPP IV activity (F = 6.208, P = 0.016) such that DPP IV activity was significantly lower during ACEI-associated angioedema (26.9 ± 4.1 nmol/mL per min; range 24.0 to 34.6 nmol/mL per min; Figure 2) than that measured in normotensive controls, in previously ACEI-exposed untreated hypertensive volunteers, or in ACEI-treated hypertensive volunteers, even after controlling for age. In contrast, DPP IV activity was similar in sera from untreated hypertensive controls and patients in remission from ACEI-associated angioedema (35.1 ± 8.5 nmol/mL per min; P = 0.734). DPP IV was not depressed in 2 patients with a history of angioedema not associated with ACEI use.

Mean APP activity was similar in normotensive volunteers (154.6 ± 113.7 nmol/mL per min), in untreated hypertensive subjects who had been exposed previously to ACEI without angioedema (115.1 ± 101.6 nmol/mL per min), in hypertensive patients with a remote history of angioedema (89.1 ± 65.7 nmol/mL per min), and in chronically ACEI-treated hypertensive subjects (121.3 ± 87.5 nmol/mL per min). The APP activity tended to be higher during ACEI-associated angioedema (188.4 ± 134.2 nmol/mL per min), although this was not statistically different from that in untreated (P = 0.219) and ACEI-treated (P = 0.294) hypertensive controls, normotensives (P = 0.647), or patients with a remote history of angioedema (P = 0.118). The APP activity was not affected by factors such as age, gender, or race.

**Discussion**

Studies investigating the metabolism of bradykinin in sera from patients with a history of ACEI-associated angioedema and controls suggest that there is a defect in a non-ACE, non-kininase I pathway of bradykinin degradation,17 such as the APP/DPP IV pathway. The present study provides evidence that serum DPP IV activity but not APP activity is significantly lower during ACEI-associated angioedema (35.1 ± 8.5 nmol/mL per min; P = 0.734). DPP IV was not depressed in 2 patients with a history of angioedema not associated with ACEI use.

Mean APP activity was similar in normotensive volunteers (154.6 ± 113.7 nmol/mL per min), in untreated hypertensive subjects who had been exposed previously to ACEI without angioedema (115.1 ± 101.6 nmol/mL per min), in hypertensive patients with a remote history of angioedema (89.1 ± 65.7 nmol/mL per min), and in chronically ACEI-treated hypertensive subjects (121.3 ± 87.5 nmol/mL per min). The APP activity tended to be higher during ACEI-associated angioedema (188.4 ± 134.2 nmol/mL per min), although this was not statistically different from that in untreated (P = 0.219) and ACEI-treated (P = 0.294) hypertensive controls, normotensives (P = 0.647), or patients with a remote history of angioedema (P = 0.118). The APP activity was not affected by factors such as age, gender, or race.

**Discussion**

Studies investigating the metabolism of bradykinin in sera from patients with a history of ACEI-associated angioedema and controls suggest that there is a defect in a non-ACE, non-kininase I pathway of bradykinin degradation,17 such as the APP/DPP IV pathway. The present study provides evidence that serum DPP IV activity but not APP activity is decreased in patients during acute ACEI-associated angioedema. This finding implicates substance P as a key player in the pathogenesis of angioedema.

DPP IV is a type II transmembrane protein that cleaves X-Pro or X-Ala from the N-terminus of bioactive peptides such as substance P. In the case of bradykinin, hydrolysis of the N-terminal Arg-Pro bond by APP renders the molecule susceptible to cleavage by DPP IV at the Pro-Gly bond.12 Because APP inactivates bradykinin, however, a defect in the DPP IV-mediated metabolism of bradykinin is not likely to play a role in the pathogenesis of ACEI-associated angio-
DPP IV and ACE-Inhibitor Angioedema

Lefebvre et al

Deficiency syndrome. Among the 6 patients from whom sera were obtained during ACEI-associated angioedema in animals. Bradykinin stimulates substance P release from nerve fibers, leading to an intricate series of events that include enhanced vascular permeability, leakage of plasma protein into the interstitial space, leukocyte adhesion, mast cell and eosinophil degranulation, release of chemotactic factors, activation of cytokines, and induction of matrix metalloproteases. Proteases in the vicinity of releasing sites normally limit the inflammatory effects of substance P by rapid degradation. NEP and ACE play a pivotal role in this respect, as both enzymes are expressed in the postcapillary venular endothelium, an effective site for mediator-induced plasma extravasation and leukocyte adhesion. DPP IV can be assumed to be complementary to ACE and NEP in degrading a number of neuropeptides, such as substance P, and in regulating the activation of various chemokines whose concentration gradient surrounding the site of inflammation controls leukocyte recruitment and proteolytic activity.

Therefore, we postulated that a defect in DPP IV activity plays a critical role in both eliciting an exaggerated neurogenic response during ACE inhibition and rendering an individual susceptible to angioedema.

In the present study, DPP IV activity was significantly decreased in sera obtained from patients during acute ACEI-associated angioedema but not in sera obtained from patients with a remote history of ACEI-associated angioedema who were in remission and off ACEI treatment. For this reason, the possibility that either ACEI treatment itself or the corticosteroid treatment of angioedema could reduce DPP IV activity was considered and excluded. The finding that DPP IV activity was significantly depressed only during acute angioedema suggests an acquired rather than a genetic defect in DPP IV activity in patients predisposed to ACEI-associated angioedema. Alternatively, genetic factors could modulate the effect of environmental factors on DPP IV activity.

DPP IV activity is decreased by immunosuppressive agents such as cyclosporine and in conditions such as acquired immunodeficiency syndrome. Among the 6 patients from whom sera were obtained during ACEI-associated angioedema in the present study, 1 patient was on immunosuppressive therapy for rheumatoid arthritis and a second patient had acquired immunodeficiency syndrome. Among the 7 patients with a history of ACEI-associated angioedema, 2 were heart transplant patients receiving immunosuppressive agents and 1 patient had a history of multiple myeloma.

The risk of ACEI-associated angioedema is 4 to 5 times higher in blacks compared with whites. However, in the present study, there was no effect of ethnicity on DPP IV activity. DPP IV activity did significantly decrease with increasing age, and patients with acute angioedema tended to be older. Although a previous case-control study of 82 patients with ACEI-associated angioedema found no effect of age on the risk of ACEI-associated angioedema, the study was conducted in an older population and may have missed such an association. Additional studies are needed to test the hypothesis that older age is a risk factor for ACEI-associated angioedema.

One limitation of the present study is the small number of patients with ACEI-associated angioedema studied. However, confirmation of the finding that DPP IV activity is decreased during acute ACEI-associated angioedema in a larger patient population would suggest a strategy for identifying patients at increased risk for this rare but potentially life-threatening side effect of ACE and combined ACE/NEP inhibitors. Hence, the identification of environmental factors (eg, concurrent use of immunosuppressive drugs) or genetic polymorphisms that modulate DPP IV activity could provide the tools to predict which patients are at increased risk of ACEI-associated angioedema.

Acknowledgments

This study was supported by National Institutes of Health Grants HL65193, HL04445, GM07569, AI0152, and RR 00095. We thank T. Orawski for the technical support with the APP assay.

References


Dipeptidyl Peptidase IV Activity in Patients With ACE-Inhibitor-Associated Angioedema
Jean Lefebvre, Laine J. Murphey, Tina V. Hartert, Ru Jiao Shan, William H. Simmons and Nancy J. Brown

Hypertension. 2002;39:460-464
doi: 10.1161/hy0202.103054

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/39/2/460

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/