Renal and Metabolic Clearance of N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (AcSDKP) During Angiotensin-Converting Enzyme Inhibition in Humans

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Abstract—We investigated the contributions of angiotensin-converting enzyme (ACE) and glomerular filtration to creating the new metabolic balance of the hemoregulatory peptide N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) that occurs during acute and chronic ACE inhibition in healthy subjects. We also studied the effect of chronic renal failure on the plasma concentration of AcSDKP during long-term ACE inhibitor (ACEI) treatment or in its absence. In healthy subjects, a single oral dose of 50 mg captopril (n=32) and a 7-day administration of 50 mg captopril BID (n=10) resulted in a respective 42-fold (range, 18- to 265-fold) and 34-fold (range, 24-fold to 45-fold) increase in the ratio of urinary AcSDKP to creatinine accompanied by a 4-fold (range, 2- to 6.8-fold) and 4.8-fold (range, 2.6- to 11.8-fold) increase in plasma AcSDKP levels. Changes in plasma AcSDKP and in vitro ACE activity over time showed an intermittent reactivation of ACE between each captopril dose. In subjects with chronic renal failure (creatinine clearance, 60 mL/min per 1.73 m²), plasma AcSDKP levels were 22 times higher (95% confidence interval, 15 to 33) in the ACEI group (n=35) than the control group (n=23); in subjects with normal renal function, they were only 4.1 times higher (95% confidence interval, 3.2 to 5.3) in the ACEI group (n=19) than the non-ACEI group (n=21). Renal failure itself led to a slight increase in plasma AcSDKP concentration. In conclusion, intermittent reactivation of ACE between doses of an ACEI is the major mechanism accounting for the lack of major AcSDKP accumulation during chronic ACE inhibition in subjects with normal renal function. (Hypertension. 1999;33:879-886.)

Key Words: oligopeptides ■ metabolism ■ peptidyl-dipeptidase A ■ angiotensin-converting enzyme inhibitor ■ kidney failure

Angiotensin I–converting enzyme (ACE) (kininase II, dipeptidyl carboxypeptidase I, EC 3.4.15.1) displays activity toward a broad range of substrates, at least in vitro,1 and has two homologous N-terminal and C-terminal active domains.2 N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), a negative regulator of hematopoiesis isolated from bone marrow,3,4 is a natural substrate preferentially hydrolyzed by the N-terminal active site of ACE.5,6 AcSDKP hydrolysis is blocked by ACE inhibitors (ACEIs) such as captopril and lisinopril in vitro5,6 and in vivo.7,8 After a single administration to normal subjects or during long-term treatment in hypertensive patients, an ACEI increases plasma AcSDKP levels by 5-fold to 6-fold compared with control subjects or patients.7,8 The consistently high concentration of plasma AcSDKP during chronic ACE inhibition suggests that AcSDKP clearance occurs by an alternative route, probably via the kidney,9 taking into account the low molecular weight of the tetrapeptide (MW 487). The long-term consequences of such an accumulation of an inhibitor of hematopoietic stem cell proliferation in plasma and perhaps in tissue are unknown. However, a single dose of lisinopril significantly decreases the proportion of cycling hematopoietic primitive cells in irradiated mice.10

In this study, we investigated the mechanisms involved in the maintenance of high plasma AcSDKP concentrations when plasma, and probably tissue, hydrolysis of AcSDKP by ACE is inhibited by single and repeated oral doses of captopril in healthy subjects. We also investigated the effects of chronic renal failure on the plasma concentration of AcSDKP in patients during long-term ACEI treatment or in its absence.

Methods

Single Oral Dose and Multiple Oral Dose Studies in Healthy Subjects

Single Oral Dose Open Study
Thirty-two normotensive male volunteers aged 18 to 35 years were instructed to arrive at the Broussais Clinical Investigation Center at 6 PM on the evening before the study (day 0); they remained at the center for 36 hours. On the study day (day 1), after 1 hour of rest in

Received July 6, 1998; first decision July 27, 1998; revision accepted November 5, 1998.
From the Centre d’Investigations Cliniques (M.A., J.-L.R., J.M.), Hôpital Broussais, INSERM et Assistance Publique des Hôpitaux de Paris; CEA (E.E.), Service de Pharmacologie et d’Immunologie, Gif-sur-Yvette; and Institut de Chimie des Substances Naturelles (J.W.-B., V.G.), Centre National de la Recherche Scientifique, Gif-sur-Yvette, France.
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a semirecumbent position to allow equilibration of hormones, volunteers were given a single oral dose of 50 mg captopril with 25 mL water at 9 AM. Blood was sampled before and 1, 2, 4, 6, 10, and 24 hours after the dose for determination of plasma AcSDKP, angiotensin I (Ang I), Ang II, and ACE activity. Before oral dosing, subjects voided their bladder to complete a 12-hour urine collection for AcSDKP and creatinine determinations. Two further 12-hour urine collections were completed after drug intake.

**Multiple Oral Dose Study**

Ten normotensive male volunteers were given 50 mg captopril twice daily for 7 days, and 2 were given a matched placebo. Volunteers were instructed to arrive at the center at 8 AM each day from the first (day 1) to the seventh (day 7) study day and were given their morning oral dose of 50 mg captopril or placebo with 25 mL water at 9 AM. They were asked to take the second daily dose at around 9 PM at least 2 hours after dinner. On the seventh day, they were instructed to return to the center at 6 PM and were given their last 50 mg captopril dose at 9 PM. They remained at the center for 60 hours. Blood was sampled each day at 9 AM before captopril intake and on days 1 through 6 for determination of plasma AcSDKP, in vitro hydrolysis of [3H]AcSDKP, ACE activity, Ang I, and Ang II. A spot urine sample was also collected each day for determination of AcSDKP and creatinine. A pharmacodynamic study of 50 mg captopril BID administration with blood samplings before and 1, 2, 4, 6, 12, and 24 hours after the morning dose was undertaken for 2 subjects on day 4. From the evening of day 7 to day 11, blood was sampled immediately before and 12, 18, 24, 36, 42, 48, 60, and 84 hours after the last dose of captopril. Seven urine collections were made after the last dose for determination AcSDKP and creatinine (0 to 12 hours, 12 to 18 hours, 18 to 24 hours, 24 to 36 hours, 42 to 48 hours, and 48 to 60 hours). A spot urine sample was collected 84 hours after the last dose. Two separate phases were analyzed: the first, “on treatment,” was from day 1 to day 8 at 9 AM (the first 12 hours after the last evening dose of captopril); the second, “off treatment,” was from day 8 to day 11.

Volunteers gave written informed consent to receive treatment with 50 mg captopril or a matched placebo. The protocol was approved by the Comité Consultatif de Protection des Personnes se prélant à des Recherches Biomédicales (Paris-Cochin, France).

**Study in Patients With Chronic Renal Failure**

Fifty-eight consecutive patients with chronic renal failure (creatinine clearance <60 mL/min per 1.73 m²) were recruited for the study during an outpatient visit or a hospital admission. Thirty-three were regularly undergoing hemodialysis. Patients were defined as being treated with an ACEI if this class of drug was prescribed for more than 1 month (ACEI group, n = 35). The age- and sex-matched control group (non-ACEI group, n = 23) consisted of patients with chronic renal failure who had never been treated with an ACEI or for whom ACEI treatment had been withdrawn more than 1 month before inclusion in the study. Another control group consisted of 40 hypertensive patients with normal renal function ( creatinine clearance ≥60 mL/min per 1.73 m²; ACEI group, n = 19; non-ACEI group, n = 21) selected from the group of hypertensive patients previously reported.8

In both ACEI groups, with impaired or normal renal function, blood for plasma AcSDKP determination was taken at various times from 2 to 48 hours after the last dose. For all groups, blood samples were collected from patients in the sitting or lying position for at least 5 minutes. For hemodialysis patients, blood was sampled from the arterial line at the start and end of the dialysis session. Plasma AcSDKP determinations were made blind to the treatment.

**Laboratory Methods**

**Single Oral Dose and Multiple Oral Dose Studies in Healthy Subjects**

For each hormone determination, subjects were placed in a semirecumbent position 1 hour before blood sampling. Heparinized tubes were used to collect blood for determination plasma AcSDKP and in vitro ACE activity. For plasma angiotensin radioimmunoassays, blood samples (10 mL) were collected rapidly (within 10 seconds) into prechilled EDTA-K2 evacuated tubes, and 0.5 mL of an inhibitor mixture containing 62.5 mmol/L EDTA, 100 μmol/L of the renin inhibitor remikiren, and 100 μmol/L enalaprilat was immediately added to prevent the generation and degradation of angiotensins in vitro.9 Blood samples were immediately centrifuged at 3500 rpm at 4°C and stored at −80°C until assay.

Plasma and urinary AcSDKP concentrations were determined by a competitive enzyme immunoassay10 previously used for pharmacokinetic studies in humans11 with a detection limit of 0.2 pmol/mL. Captopril (10⁻⁴ mol/L) was immediately added to the blood samples for measurement of plasma AcSDKP. These samples were then instantaneously centrifuged at 2500 rpm at 4°C and stored at −20°C until assay. Spot urine samples were immediately frozen at −20°C until assay. Six- or 12-hour urine collections were kept at 4°C during the collection period, and 10-mL urine samples were then taken after homogenization and stored at −20°C. Nonanchored ACE is present in small amounts in urine,10 so we checked the stability of exogenous AcSDKP (200 mmol/L) in fresh urine samples over 24 hours at 4°, 20°, and 37°C. No significant degradation was detected.

**Evaluation of In Vitro and In Vivo ACE Activity by 4 Techniques**

Plasma ACE activity was quantified by a spectrophotometric method (using the synthetic substrate Hip-His-Leu) according to Cushman and Cheung,12 by an antibody-trapping assay according to Nussberger et al (using Ang I as substrate),13 by using [3H]AcSDKP as substrate,14 and by the ratio of plasma Ang II to Ang I. For the purposes of comparability, the results are expressed as percentage of change from baseline ACE activity (expressed as 100% according to the technique used) in the figures.

The kinetics of AcSDKP hydrolysis were investigated by adding 15 μL AcSDKP solution containing 1 μCi of the radiolabeled peptide [3H]AcSDKP (85 Ci/mmol), specifically tritiated at the lysyl residue, to 450 μL plasma (final concentration, 4×10⁻⁴ mol/L). After 2 hours of incubation at 37°C, samples (30 μL) were taken, and 10-μL carrier solution (2 mg/mL) (AcSDKP [Ipsen-Biotech]; Lys-Pro and Lys [ICSN-CNRs]) was immediately added and the mixture frozen on dry ice. All samples were analyzed in duplicate by high-voltage electrophoresis and scintillation counting as previously described.3 The radioactivity specifically associated with [3H]AcSDKP and its degradation products was then quantified. The relative changes in the residual radioactivity associated with [3H]AcSDKP after 2 hours of incubation represent the in vitro inhibition of plasma ACE activity using [3H]AcSDKP as substrate (expressed as a percentage). ACE induction (defined here as an increase in plasma ACE activity) was evaluated after captopril was stopped: The plasma samples of 2 subjects were dialyzed at 4°C in a Slide-A-Lyser 10 000 MWV cassette (Pierce) for 24 hours against 10⁻³ mol/L EDTA in 50 mmol/L NaCl and then against 50 mmol/L NaCl alone. ZnCl₂ (final concentration, 10⁻³ mol/L) was added to the samples before the kinetics of AcSDKP hydrolysis (ACE activity using [3H]AcSDKP as substrate) were studied.

For plasma angiotensin measurements, 2 mL plasma was extracted by solid-phase extraction chromatography with a vacuum extraction device on phenylsilica silica columns (Bondelut PH, Analytichem) according to Nussberger et al,15 and the dried extracts containing angiotensins were diluted in 500 μL Tris-HCl buffer (0.1 mol/L, pH 7.5) containing 2 g/L bovine serum albumin. Recovery of angiotensins was 98.5 ± 3.5%. For the Ang I radioimmunoassay, we used a polyclonal antibody that cross-reacts 100% with des-[Asp] Ang I and <0.25% with Ang II, Ang III, and their later analogues.16 For the Ang II radioimmunoassay, we used a monoclonal antibody (gift from D. Simon and B. Pau, Sanofi, Montpellier, France) that cross-reacts 190% with des-[Asp] Ang II, 154% with Ang-(3–8), 51% with Ang-(4–8), 0.1% with Ang II, 2% with Ang-(1–9), and 1% with Ang-(2–10).17 No cross-reactivity was detected with the angiotensin peptides lacking phenylalanine in the carboxy terminus. In both standard curves, 0.5 pg/tube can be detected, and 50% displacement of the tracer is achieved at 5 and 10 pg/tube for Ang I and Ang II.
respectively. The within- and between-assay coefficients of variation were 6% and 13%, respectively. The relative changes in the ratio of plasma Ang II to Ang I represent endothelial and plasma ACE activity in vivo.

**Binding of AcSDKP to Plasma Proteins**

AcSDKP binding to plasma proteins was determined by ultrafiltration using the commercially available MPS-1 apparatus (Amicon Corp). Human plasma (500 μL) was mixed with 10 μL tritiated AcSDKP (2×10^6 cpm) and 25 μL AcSDKP at various final concentrations (2 to 200 nmol/L). The mixture was incubated for 1 hour at 37°C. The MPS-1 devices loaded with samples were centrifuged at 1500g for 10 minutes at 37°C. Filtrate and initial solutions were counted by a liquid scintillation counter (12-14 Rackbeta, LKB, Wallac). The percentage of free peptide was determined from the ratio of its concentrations in the ultrafiltrate and initial solutions. We checked that AcSDKP was not absorbed to the reservoir of the MPS-1 apparatus by replacing the plasma with water. The degradation of AcSDKP by plasma ACE was prevented by adding captopril to a final concentration of 0.1 μmol/L.

**Pharmacokinetic Parameters**

In the single dose study, the area under the curve (AUC) of concentration versus time (0 to 24 hours) was calculated according to the trapezoidal rule for plasma AcSDKP. Before captopril intake, apparent renal AcSDKP clearance (CLR) was calculated according to CLR=U0/P, where U0 is the total amount of AcSDKP excreted in the 12-hour urine collection, and P is plasma AcSDKP concentration at time zero. Apparent renal AcSDKP clearance after captopril intake was calculated by CLR=U0/AUC0–24, where U0 is the total amount of AcSDKP excreted in the 24-hour urine collection after dosing. In the multiple dose study, apparent renal AcSDKP clearance at steady state (between days 7 and 8) was calculated by CLR=U0/P, where U0 is the total amount of AcSDKP excreted in the 12-hour urine collection after the last captopril dose, and P is the mean of 2 consecutive plasma AcSDKP concentrations determined immediately before and 24 hours after the last dose on day 7.

**Statistical Methods**

In the single dose study, plasma AcSDKP concentrations at baseline, peak, and 24 hours after treatment were compared using paired t tests. During the "on treatment" period of the multiple dose study, plasma concentrations were analyzed by a one-way ANOVA for repeated measurements. The effects of renal failure and ACEi treatment between groups was studied using two-way ANOVA. When a significant interaction was present, the effect of each factor (ACEi/renal function status) was studied within each group. The effects of hemodialysis on plasma AcSDKP concentration was studied using a one-way ANOVA for repeated measurements. The effects of renal failure and ACEi treatment after administration of a single oral dose of 50 mg captopril to 32 healthy subjects. Middle, ACE inhibition measured in vitro by the Cushman assay (Hip-His-Leu as substrate, ○) and estimated in vivo using the Ang II/Ang I ratio (●) after administration of a single oral dose of 50 mg captopril to 32 healthy subjects. Bottom, Evolution of urinary AcSDKP/creatinine ratio after administration of a single oral dose of 50 mg captopril to 32 healthy subjects (mean±SEM).

**Results**

**Single Oral Dose Study**

**Effects of Captopril on Plasma and Urinary AcSDKP**

No placebo was included in this study because it has been shown that plasma AcSDKP levels and ACE activity are stable over 24 hours.7 Plasma AcSDKP levels increased significantly from 0.57±0.18 pmol/mL to a peak value of 3.65±0.88 pmol/mL (Figure 1, P<0.0001), decreasing monoexponentially toward a 24-hour value that was significantly higher than baseline (1.05±0.31 pmol/mL, P<0.001, Figure 1). Before captopril administration, AcSDKP was excreted in the urine in small amounts (urinary AcSDKP/creatinine ratio: 0.5±0.2 nmol/mmol), and apparent CLR was low (10±6 mL/min). After captopril intake, there was a 42-fold (18- to 265-fold) increase of the AcSDKP/creatinine ratio in the first urine collection period and a 9-fold (3.6- to 30-fold) rise in CLR (82±28 mL/min). In the second 12-hour urine collection after captopril administration, the amounts of AcSDKP excreted were approximately decreased by half compared with the first 12-hour urine collection postdose (Figure 1).

**Effects of Captopril on In Vitro and In Vivo Plasma ACE Activity**

In vitro plasma ACE activity 1 hour after captopril intake measured by Cushman’s assay decreased rapidly from 33±9 to 8±4 mU/mL (77±13% reduction from baseline). It began to increase 2 hours after the dose, returning to its baseline levels 24 hours after treatment (Figure 1). One hour after
captopril intake, the plasma Ang II/Ang I ratio decreased from 0.60±0.26 to 0.01±0.01 (98±2% reduction from baseline), and it remained low (0.35±0.18) for 24 hours postdose (63±37% of baseline, Figure 1).

**Multiple Dose Study**
Plasma AcSDKP and in vitro ACE activity were stable over the 11 days of the investigation in the 2 placebo-treated subjects (data not shown).

**Evolution of In Vitro and In Vivo ACE Activity From Day 1 to Day 8 (On Treatment)**
Twelve hours after the second captopril dose, ACE activity using [3H]AcSDKP as substrate was 64±8% lower than baseline, and ACE inhibition remained at the same level from day 1 to day 8 (F5,35=18, P<0.0001; Table 1 and Figure 2). The time course evolution of in vivo ACE activity assessed by changes in the Ang II/Ang I ratio was superimposed on to the in vitro ACE activity assessed by the hydrolysis of [3H]AcSDKP (Table 1 and Figure 2). When Ang I was used as substrate, ACE activity was reduced by 39±8% on day 2 and by 49±4% on day 8 (Table 1 and Figure 2).

Plasma ACE activity assessed by the Cushman assay fell slightly on day 1 and returned to its baseline level from day 2 onward (Table 1 and Figure 2). During each 12-hour dosing interval, in vitro plasma ACE activity using [3H]AcSDKP or Hip-His-Leu as substrate varied between minimum and maximum values (see Figure 2 inset showing the concentration-time curves of the 2 subjects for whom a complete pharma-codynamic study was carried out on day 4 between 2 doses of captopril).

**Evolution of Plasma AcSDKP and Urinary AcSDKP/Creatinine Ratio From Day 1 to Day 8 (On Treatment)**
Before captopril administration, small amounts of AcSDKP were excreted in urine (AcSDKP/creatinine ratio: 0.9±0.4 mmol/mmol). Mirroring ACE inhibition assessed by the in vitro hydrolysis of [3H]AcSDKP, both plasma AcSDKP and urinary AcSDKP/creatinine ratio reached a plateau on day 1 and remained constant from day 1 to day 8 (F5,35=17, P<0.0001; Figure 3). The mean urinary AcSDKP/creatinine ratio increased by 34-fold (range, 24- to 45-fold) from baseline. Parallel to the time course evolution of ACE activity, plasma AcSDKP concentrations varied between a maximum and minimum level between doses of captopril (Figure 3 inset).

**Evolution of In Vitro and In Vivo ACE Activity From Day 8 to Day 11 (Off Treatment)**
ACE activity measured by in vitro hydrolysis of [3H]AcSDKP returned to pretreatment levels on day 11 when captopril was...
stopped on day 7. A similar pattern was observed for the Ang II/Ang I ratio and ACE activity using Ang I as substrate (Table 1 and Figure 2). Again, there was a marked difference between the time course evolution of plasma ACE activity as measured by the Cushman assay and those determined by the other three techniques. Plasma ACE activity as measured by the Cushman assay increased linearly from day 8 to day 11, to a level that was statistically higher than its pretreatment level (day 11 versus day 1: 46 ± 11 versus 35 ± 9 mU/mL, P < 0.001; Table 1 and Figure 2). An increase in ACE activity measured as in vitro hydrolysis of [3 H]AcSDKP was detected after dialysis of the plasma samples (Figure 4).

Evolution of Plasma AcSDKP, Urinary AcSDKP/creatinine ratio, and Urinary Amounts of AcSDKP Excreted From Day 8 to Day 11 (Off Treatment)

Plasma AcSDKP levels and urinary AcSDKP/creatinine ratio decreased monoexponentially from day 8 to day 11 to final values significantly higher than baseline values (plasma AcSDKP on day 11 versus baseline: 1.74 ± 1.27 versus 1.22 ± 1.00 pmol/mL, P < 0.05; final AcSDKP/creatinine ratio versus baseline: 3.9 ± 2.7 versus 0.9 ± 0.4 nmol/mmol, P < 0.0001; Figure 3). Apparent CLₘ for the first 12-hour urine collection after the last evening dose of captopril was similar to that calculated in the single oral dose study (86 ± 36 versus 82 ± 28 mL/min, respectively). The ratio of AcSDKP CLₘ to endogenous creatinine clearance determined at the same time was 0.69 ± 0.27. The amount of AcSDKP excreted in urine at steady state was 236 ± 73 nmol/12 h, which corresponds to the amount measured in the first 12-hour urine collection after the last dose of captopril.

Effects of ACEI Treatment and Renal Failure on Plasma AcSDKP Concentrations

In each ACEI group, various types and daily doses of ACEIs were administered when blood samples were taken (not shown). Geometric mean plasma AcSDKP concentrations in patients with chronic renal failure were 22 times (95% confidence interval [CI], 15 to 33) higher in the ACEI group than the non-ACEI group for all time intervals between blood sampling and ACEI intake (Table 2). The ranges of plasma AcSDKP concentrations for patients with renal failure in the ACEI and non-ACEI groups did not overlap (Table 2). The highest plasma AcSDKP levels were observed in ACEI-treated hemodialysis patients (n = 16; geometric mean, 41 [13.3 to 192] pmol/mL; not shown). For patients with normal renal function, plasma AcSDKP concentrations were 4.1 times (95% CI, 3.2 to 5.3) higher for the ACEI group than the non-ACEI group (Table 2). In the absence of ACEI treatment, plasma AcSDKP concentrations were slightly higher for patients with renal failure than for patients with normal renal function (Table 2). Plasma AcSDKP concentration decreased significantly after hemodialysis, falling from 41 (13.3 to 192) pmol/mL to 13.5 (4.4 to 30.9) pmol/mL in the ACEI group (n = 16) and from 1.4 (0.6 to 3.4) pmol/mL to 0.77 (0.36 to 2.1) pmol/mL in the non-ACEI group (n = 17; F₃,₆₂ = 93.5, P < 0.0001). At the end of dialysis, plasma AcSDKP concentration was 17 times (95% CI, 11.8 to 25.7) higher in the ACEI group than the non-ACEI group.
AcSDKP Clearance During ACE Inhibition in Humans

### Binding of AcSDKP to Plasma Proteins

The binding of AcSDKP to human plasma proteins in vitro was found to be <1%.

### Discussion

Long-term administration of ACEIs to patients with normal renal function does not result in a larger accumulation of AcSDKP in plasma than a single dose of 50 mg captopril given to healthy subjects. This shows that a new equilibrium is set up between production and elimination, and our data suggest that AcSDKP clearance by the kidney is probably partly responsible for preventing this accumulation.

We found that small amounts of AcSDKP were excreted physiologically in urine and that the amount excreted in urine greatly increased when ACE was inhibited. The increase in both plasma and urinary AcSDKP levels was due to the inhibition of peptide hydrolysis, as shown by the very similar patterns of change over time for plasma AcSDKP concentration, urinary AcSDKP/creatinine ratio, and ACE activity using [3 H]AcSDKP as substrate in the captopril multiple dose study. Comte et al reported that 15 days’ treatment of healthy subjects with 20 mg/d enalapril resulted in a 4.8 times higher plasma AcSDKP concentration but only a 4 times higher 24-hour AcSDKP excretion in urine. However, the Comte study was not designed to analyze the mechanism involved in the new balance in AcSDKP metabolism or to analyze the kinetics of changes in AcSDKP excretion in urine.

The apparent renal clearance of the endogenous peptide when ACE is not inhibited is physiologically very low (10±6 mL/min), accounting for about 1% to 2% of the total clearance, which was estimated to be 1300±240 mL/min during a 12-hour infusion of the exogenous peptide to healthy subjects. The total amount of AcSDKP excreted in urine in the absence of ACE inhibition is theoretically the result of the glomerular filtration of its intact free plasma fraction, which is then degraded by ACE in the brush border of tubular cells. Apparent renal clearance of AcSDKP increased from 10±6 to 82±28 mL/min after administration of a single dose of captopril, a value that is similar to the one calculated at steady state in the multiple dose experiment (86±36 mL/min). The increase in urinary AcSDKP concentration during acute and chronic ACE inhibition is due to at least 2 possible mechanisms: First, since there was <1% binding of AcSDKP to human plasma protein, the amount of intact free plasma AcSDKP is very high and undergoes extensive glomerular filtration; second, tubular ACE is inhibited by captopril filtered by the glomerulus. The similar patterns of change in plasma AcSDKP concentration and urinary AcSDKP/creatinine ratio during both the “on treatment” and “off treatment” periods suggest that both mechanisms operate at the same time, proportionally to the amount of ACEI active in plasma and at the surface of endothelial and tubular cells.

The ratio of apparent renal clearance of AcSDKP to endogenous creatinine clearance during chronic ACE inhibition was 69±27%. Thus, the lower level of clearance of AcSDKP than of creatinine shows that AcSDKP is probably not secreted into urine either directly from plasma or as a result of local synthesis. AcSDKP entirely undergoes glomerular filtration and is presumably not reabsorbed, because it is mostly ionized at the pH of urine; therefore, the lower apparent renal clearance of AcSDKP may be due to the persistence of a significant level of degradation of the peptide in tissues or urine by ACE for some time at least, suggesting that ACE is not completely and permanently inhibited by 50 mg captopril BID. At the end of each dosing interval, captopril began to dissociate from the N- and C-terminal active sites of plasma, endothelial, and tubular ACE. At the same time as the dissociation of captopril, the N-terminal active site of ACE began to hydrolyze AcSDKP in the presence of high plasma, interstitial, and urinary concentrations of the peptide, which were in continuous competition with the falling levels of the inhibitor (see Figures 2 and 3 insets). The intermittent reactivation of ACE is shown by the persistence of only 64% reduction in ACE activity using [3 H]AcSDKP as substrate at the end of each captopril dose interval. Therefore, the contribution of apparent renal clearance to total clearance of the peptide is permanently changed over time when captopril is submitted to its own pharmacokinetic processes (absorption, distribution, metabolism, and elimination).

The exact contributions of the 2 mechanisms, renal clearance and intermittent reactivation of ACE, is best appreciated by studying patients with chronic renal failure. We found that there was a much higher level of accumulation of AcSDKP in plasma during chronic ACE inhibition in patients with chronic renal failure than in patients with normal renal function. AcSDKP levels were high in patients with normal renal function treated with an ACEI but only moderately so
because ACE was reactivated intermittently between administrations of the ACEIs, when plasma concentrations of the drug were decreasing. No such intermittent reactivation of ACE occurred during end-stage renal failure because of the accumulation of the ACEI in plasma and tissues.19 As AcSDKP degradation was almost permanently blocked and the renal route of elimination of the tetrapeptide was impaired, there was a major accumulation of AcSDKP in plasma. Whether these high endogenous plasma concentrations of AcSDKP are associated with high concentrations of the peptide in tissues is not known. However, the plasma concentrations of AcSDKP in ACEI-treated patients with chronic renal failure were much higher than those determined during a 12-hour continuous infusion of 62.5 μg/kg of the synthetic AcSDKP peptide.13 Lower doses of exogenous AcSDKP (2.5 to 12.5 μg/kg) have been shown to be active in preventing the myelotoxic effects of anticancer chemotherapy (cytarabine and ifosfamide) in cancer patients.20

**Urinary AcSDKP as a Marker of ACE Inhibition**

The major increase in AcSDKP concentration during chronic ACE inhibition (34 times higher) means that AcSDKP determined in urine is a very sensitive marker of ACE inhibition in vivo, as previously demonstrated for AcSDKP determined in plasma.8 However, urinary AcSDKP is easier to use than plasma AcSDKP for detecting patients’ noncompliance with ACE inhibitor treatment. It requires only a random spot urine sample and integrates all the fluctuations in AcSDKP plasma level resulting from intermittent reactivation of ACE at the end of each dose interval of the ACEI. It is less prone to false-negative results because the increase in excretion in urine is much higher (34 times higher) than the increase in plasma concentration (4.8 times higher) during chronic ACE inhibition. AcSDKP determination in urine during ACE inhibition is the unique parameter that gives an information about the inhibition of ACE in the brush border of tubular cells.

**Determination by Four Techniques of Plasma ACE Activity During Chronic ACE Inhibition With 50 mg Captopril BID**

Both in vitro assays using the natural substrates [3H]AcSDKP and Ang I demonstrated the same pattern of in vivo ACE inhibition as assessed by the Ang II/Ang I ratio. These methods provided accurate results, whereas the Cushman assay (using the synthetic substrate Hip-His-Leu) did not. However, of the four techniques, only the Cushman assay detected an increase of ACE activity when captopril was stopped, contrasting with the progressive return of ACE activity to its pretreatment levels on day 11 measured by the other techniques (Figure 2). The increase in plasma ACE activity, which is one of the markers of ACE induction, is a known consequence of long-term administration of ACEIs.21,22 The increased levels of ACE activity may be explained by the dissociation of captopril by dilution in the Cushman assay. In addition, the elimination of captopril bound to ACE active sites by dialysis of plasma samples also demonstrated this induction when assessed by the hydrolysis of [3H]AcSDKP. However, this difference may also be due to captopril dissociating more rapidly from the C- than the N-terminal active site, because it binds to the N-domain with a higher affinity at both low and high chloride concentrations.23 Alternatively, it may be because AcSDKP and Ang I are physiological substrates for ACE, whereas Hip-His-Leu is not. AcSDKP has a low Km (41 μmol/L), similar to that for Ang I (16 μmol/L), whereas that of Hip-His-Leu is much higher (1540 μmol/L).2,6

In conclusion, excretion of AcSDKP in urine is higher when its hydrolysis by ACE is inhibited by captopril during single and repeated administration. It can therefore be used as a marker of in vivo ACE inhibition, as for high plasma levels. However, the intermittent reactivation of ACE between doses is probably the major mechanism preventing a major AcSDKP accumulation during chronic ACE inhibition in patients with normal renal function. The large-scale accumulation of AcSDKP in plasma during chronic inhibition of AcSDKP hydrolysis by an ACEI, which is much greater when renal function is impaired, indicates that no counter-regulatory mechanisms exist to lower plasma AcSDKP concentrations to normal levels. The large amounts of AcSDKP produced each day in vivo are very rapidly cleared from the organism by 2 very effective mechanisms: enzymatic cleavage of the Asp-Lys peptide bound by ACE and glomerular filtration.

**Acknowledgments**

This work was supported by a grant from INSERM. The authors wish to thank the nursing staff of the Clinical Investigation Center at the Broussais Hospital who carried out the protocol and the clinical staff of the Nephrology Department at the Broussais Hospital and at the AURA. We thank Dr Thanh-Tam Guenye for angiotensin determinations.

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_Hypertension_. 1999;33:879-886
doi: 10.1161/01.HYP.33.3.879

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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