Renal Hemodynamic and Hormonal Responses to the Angiotensin II Antagonist Candesartan

M. Cecilia Lansang, Suzette Y. Osei, Deborah A. Price, Naomi D.L. Fisher, Norman K. Hollenberg

Abstract—The development of very specific blockers for the angiotensin II type 1 (AT1) receptor made it possible to examine the contribution of angiotensin II to normal control mechanisms and disease with a specificity beyond what ACE inhibitors could provide. In the present study, we explored the contribution of angiotensin II to 2 renal mechanisms: renal hemodynamics and the short feedback loop, in which angiotensin II acts as a determinant of renin release. To make that comparison, we studied healthy volunteers in balance on a 10-mmol sodium intake to activate the renin system. Our goal was to compare the relation between the dose of candesartan, an AT1 receptor blocker, and the renal hemodynamic and hormonal responses. A second goal was to ascertain the relation between time after candesartan administration and the peak response. Twelve healthy subjects (mean age 33±2.3 years) in low-sodium balance were administered candesartan in 4-, 8-, 16-, and 32-mg doses. Candesartan produced a dose-related increase in renal plasma flow, with the maximum vasodilator response at 16 mg (142±13 mL·min⁻¹·1.73 m⁻²) occurring during the first 4 hours after the dose. Likewise, candesartan caused a dose-related rise in plasma renin activity, with 32 mg as the dose producing the greatest response at 4 and 24 hours after administration. The peak plasma renin activity achieved in this study (15.3±1.6 ng·L⁻¹·s⁻¹; 55.0±5.6 ng angiotensin 1·mL⁻¹·h⁻¹) was found at the 4- to 8-hour interval after dosing in a subset of subjects (n=5) who received the 16-mg dose 4 hours earlier than the other subjects. On the basis of the difference in the relation between dose and response and the relationship between time after drug administration and response, the determinants of the renal hemodynamic and hormonal response can be said to differ. The remarkable rise in plasma renin activity after candesartan is substantially larger than that in earlier studies with ACE inhibition, providing additional evidence for non–ACE-dependent angiotensin II generation in the kidney. (Hypertension. 2000;36:834-838.)

Key Words: angiotensin II ■ candesartan ■ renal circulation ■ plasma ■ renin

Much of the present understanding of the renin-angiotensin system (RAS) derives from studies that outline the mechanisms for its activation or suppression and the effects of these on renal hemodynamics and renin release. Not only does angiotensin II (Ang II) directly inhibit renin release through a short feedback loop, but ACE inhibitors have been recognized to increase plasma renin activity (PRA) since shortly after these agents became available.¹–⁴ The use of ACE inhibitors to investigate mechanisms, however, has led to ambiguous conclusions; because the substrates on which they act include bradykinin, alternative mechanisms could contribute to an ACE inhibitor–induced response.⁵,⁶ More recently, attention has focused on non–ACE-dependent Ang II generation and the concomitant use of Ang II antagonists as pharmacological probes for dissection of pathophysiology and normal mechanisms.⁷

We noted that the PRA responses to 2 Ang II antagonists, eprosartan and irbesartan, were larger than expected from our experience with ACE inhibitors, but this did not constitute the primary focus of those studies.⁸,⁹ Moreover, the maximum PRA that could be measured from the renin assay was 10.3 ng·L⁻¹·s⁻¹ (37 ng Ang I·mL⁻¹·h⁻¹), and the timing of the samples may not have captured the peak. In the present study, we set out to explore the relation between the dose of an angiotensin II type 1 (AT₁) receptor blocker, candesartan, and the renal hemodynamic and hormonal responses. As a related goal, we have attempted to determine whether the time courses of the renal hemodynamic and hormonal responses were identical.

Methods

Subjects and Protocols
We studied 12 healthy men and women who ranged in age from 19 to 44 years. All were free of cardiovascular, renal, and endocrine diseases, and all were studied during a 9-day hospitalization on a metabolic ward at the General Clinical Research Center at the Brigham and Women’s Hospital, where balance was achieved on a controlled diet.
All subjects were placed on a low-sodium (LS) isocaloric diet that started 2 days before admission and continued throughout the hospitalization, with a daily sodium intake of 10 mmol. Daily dietary potassium (100 mmol) and fluid intake (2500 mL) were constant. Twenty-four-hour urine samples were collected daily and analyzed for sodium and potassium. When LS balance was achieved, usually after 5 to 7 days on the LS diet, the first study was initiated. The protocol was approved by the Human Subjects Committee of the institution, and written informed consent was obtained from each subject.

Each subject participated in 3 experimental days, with each day separated by a rest day. Studies were generally separated by 48 hours and were performed during a 5-hour period in the morning. On the morning of each study day, an intravenous catheter was placed in each arm of each subject: 1 for infusion and the other for blood sampling. The subjects were supine and had been fasting for ≥8 hours. Each study day began with a loading dose and then a 60-minute baseline infusion of p-aminohippurate (PAH; Merck, Sharp & Dohme) and inulin (Inutest polyfructosan; Fresnius Pharma Austria GmbH) before drug administration to determine renal plasma flow (RPF) and glomerular filtration rate (GFR), respectively. This was immediately followed by a constant infusion of PAH and inulin. These methods have been described elsewhere. RPF and GFR determinations were made at baseline and at 45-minute intervals thereafter until 225 minutes (~4 hours), while the subjects were supine. Hormonal measurements were made on blood samples obtained at baseline, at 4 hours, and at 24 hours after drug administration while the subjects were lying supine. To establish the relationship between dose and response, the subjects were administered ascending doses of candesartan (4, 8, 16, and 32 mg) at 8 AM on different days. Five subjects received 3 ascending doses. To assess the influence of time beyond 4 hours on the renal hemodynamic and hormonal response, 7 of the 12 subjects received only 2 ascending doses (8 and 16 mg, or 16 and 32 mg) on the first and third study days; on the second study day, candesartan was administered 4 hours earlier (at 4 AM) and at the same dose as the first day. One subject received only 1 dose of candesartan because he chose to withdraw from the study.

Blood pressure (BP) was recorded during each infusion with an automatic recording device (Dinamap; Critikon) at 5-minute intervals.

**Laboratory Procedures**

Blood samples were collected on ice and spun immediately, and the plasma was frozen until assay. Urinary sodium and serum potassium levels were measured with use of the ion-selective electrode (ISE). PAH and inulin were measured with an autoanalyzer technique. PRA and aldosterone were determined with radioimmunoassay. PR A values that were reported as >10.3 ng·L⁻¹·s⁻¹ (>37 ng·Ang I·mL⁻¹·h⁻¹), the assay maximum, were retested with a 1:5 dilution.

**Statistical Analysis**

Group mean values were calculated with the SEM as the index of dispersion. For renal hemodynamics data, the baseline value taken was the average of the 2 highest consecutive values. The t test and the Mann-Whitney rank sum test were used to compare renal vascular and PRA responses to different candesartan doses.

**Results**

The subject group consisted of 9 men and 3 women with a mean age of 33±2.3 years and a mean body mass index of 23.9±1.5 kg/m². Salt intake restriction produced the anticipated fall in sodium excretion and activation of the RAS (Table).

**Influence of Candesartan on Renal Hemodynamics: Dose-Response Considerations**

Candesartan caused a dose-related increase in RPF (Figure 1). The response to 4 mg (83±23 mL·min⁻¹·1.73 m⁻²) was well below minimum and just above the ED₅₀ value (the dose producing 50% of the peak RPF). A further increase in RPF followed the 8-mg dose (132±26 mL·min⁻¹·1.73 m⁻²; P=0.05 versus 4 mg), and a plateau was reached at the 16- and 32-mg doses (142±13 and 142±31 mL·min⁻¹·1.73 m⁻²; P=0.71 versus 8 mg for both). In contrast, there was no difference between the baseline and peak postdrug GFR values for any of the candesartan doses (4 mg 112±9 and 111±7, 8 mg 113±5 and 115±4, 16 mg 116±5 and 117±4, and 32 ng 123±12 and 124±7 mL·min⁻¹·1.73 m⁻², respectively).

**Time Course of Renal Hemodynamic Response**

RPF did not show an increase over baseline at 48 hours, indicating no residual effect of candesartan on renal hemodynamics at any dose. Without exception, RPF reached a peak in response to each dose during the first 4 hours after candesartan administration. Evaluation of the 7 subjects who received the drug 4 hours earlier, at 4 AM, showed a very good correlation between the change in RPF on the first (0 to 4 hours) and second (4 to 8 hours) study days (r=0.88, P=0.01; Figure 2). By paired t test, the change in RPF did not differ on the 2 days (P=0.36), indicating that the values

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<th>Baseline Characteristics of the Subjects</th>
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<td>PAH clearance, mL·min⁻¹·1.73 m⁻²</td>
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<td>Inulin clearance, mL·min⁻¹·1.73 m⁻²</td>
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Values are mean±SEM.
achieved in the first 4 hours after dosing (first study day) were the same as those achieved 4 to 8 hours after dosing.

**Influence of Candesartan on PRA: Dose-Response Considerations**

Candesartan produced a dose-related increase in PRA that was always manifest at 4 hours (Figures 3A to 3D). At 4 hours, the 4-mg dose increased PRA to $2.1 \pm 0.8$ ng L$^{-1} \cdot$ h$^{-1}$ (7.5$\pm$3.0 ng Ang $I \cdot$ mL$^{-1} \cdot$ h$^{-1}$), the 8- and 16-mg doses caused a larger, although not yet maximal, response ($8.2 \pm 2.6$ and $7.0 \pm 1.1$ ng L$^{-1} \cdot$ s$^{-1}$ respectively), and the 32-mg dose produced the greatest PRA response ($10.2 \pm 1.0$ ng L$^{-1} \cdot$ s$^{-1}$). PRA had not yet peaked at 4 hours, however, in about half of the subjects. At 24 hours, PRA also followed a dose-related increase (4 mg $3.2 \pm 0.6$, 8 mg $4.4 \pm 0.7$, 16 mg $7.3 \pm 0.9$, and 32 mg $10.6 \pm 2.2$ ng L$^{-1} \cdot$ s$^{-1}$). Conversion for PRA is ng Ang $I \cdot$ mL$^{-1} \cdot$ h$^{-1}$ = ng L$^{-1} \cdot$ s$^{-1} \times 3.5997$.

**Time Course of PRA Response**

In the subjects who received 4 mg as the first dose, 48 hours later the PRA was still significantly greater than baseline ($0$ hour $1.0 \pm 0.3$ and 48 hours $1.9 \pm 0.6$ ng L$^{-1} \cdot$ s$^{-1}$; $P=0.05$). Basal PRA before the 32-mg dose was $5.7 \pm 1.9$ ng L$^{-1} \cdot$ s$^{-1}$, reflecting the residual effect of 16 mg candesartan administered 48 hours earlier (Figure 3D).

PRA had not reached peak by 4 hours, in that values were higher 24 hours later in half of the subjects. In the 7 subjects in whom the candesartan dose was administered at 4 AM to examine the interval between 4 and 8 hours after administration, 5 subjects received 16 mg candesartan. In all 5 subjects, the PRA reached a value that exceeded the assay maximum (10.3 ng L$^{-1} \cdot$ s$^{-1}$). With dilution, the values ranged from 10.3 to 19.2 ng L$^{-1} \cdot$ s$^{-1}$ (37.0 to 69.1 ng Ang $I \cdot$ mL$^{-1} \cdot$ h$^{-1}$) and averaged 15.3$\pm$1.6 ng L$^{-1} \cdot$ s$^{-1}$ (55.0$\pm$5.6 ng Ang $I \cdot$ mL$^{-1} \cdot$ h$^{-1}$), the highest value seen in this study (Figure 4).

**BP Levels**

Systolic BP did not change appreciably after drug administration. On the other hand, there was a significant decrease in diastolic BP compared with baseline ($P<0.001$) both at 4 hours after the drug and at the time point that corresponded to the maximal rise in PRA for each subject ($6.0 \pm 0.6$ and $8.6 \pm 1.1$ mm Hg, respectively). However, BP had increased to baseline levels by 24 and 48 hours after dosing when PRA was still increased strikingly. There was no evidence of a relationship between candesartan dose and BP response.

**Natriuresis and Body Weight**

There was a significant natriuresis after drug administration (baseline 24-hour urine sodium $21.7 \pm 2.6$ mmol, postdrug
39.7±6.1 mmol; P=0.002), followed by a return to baseline on the next nonstudy day. There was no dose-response relationship established for natriuresis. Body weight did not change during the interval when candesartan was being administered.

**Aldosterone**

Plasma aldosterone concentration decreased significantly (P<0.001) 4 hours after dosing (324.6±61.0 pmol/L, 11.7±2.2 ng/dL) compared with baseline (729.6±97.1 pmol/L), with the levels returning to baseline by 24 hours (757.3±124.8 pmol/L, P=0.73). Conversion for aldosterone is ng/dL=pmol/L×0.0360. Because potassium excretion levels did not fall, presumably due to dietary supplementation, this return to baseline most likely reflects the normal diurnal pattern of aldosterone secretion rather than aldosterone stimulation by a positive potassium balance.

**Discussion**

We identified a parallel, but different, dose-response relationship for RPF increase and rise in PRA after candesartan administration. Each 4-mg dose induced an unambiguous response that was substantially below the maximum. The definition of the top of a dose-response curve is that a further increase in dose does not induce an increase in response. The 16-mg candesartan dose clearly met that definition for the renal blood supply. For PRA, the 32-mg dose induced an unambiguously greater change, probably reflecting, at least in part a cumulative effect from the previous dosing: the basal values before the 32-mg dose were clearly higher than for the other doses.

Our second goal involved identification of the time course of the response to candesartan cilextil, which is a prodrug. Pharmacokinetic studies have indicated that maximal plasma concentrations of candesartan, the active drug, are not achieved until 4 to 8 hours after drug administration.12 Here, we met another divergence in the renal dynamic and hormonal responses. Renal vasodilatation clearly had achieved a maximum during the first four hours after the administration of candesartan, as the response was not larger in the 4- to 8-hour interval after drug administration, and RPF routinely returned to baseline before the next study, at 48 hours. In the case of PRA, conversely, a substantially larger response occurred in the second 4-hour interval. Indeed, 6 of the 7 subjects who received their second dose at 4 AM on the second study day reached a PRA of 10.3 ng·L⁻¹·s⁻¹ (≥37 ng Ang I·mL⁻¹·h⁻¹), which is the assay maximum. On dilution, their values ranged from 10.3 to 17.9. Another difference between the renal hemodynamic and hormonal response involved duration: Without exception, PRA was increased 48 hours after candesartan administration, when renal hemodynamics had returned to baseline. If candesartan is used as a probe for the study of renin release, further information on the time course of response will be required. In a study in which plasma volume is a crucial element, we had to impose strict limits on phlebotomy, and these limited our sampling for PRA. Because the first sample was taken at 4 hours, our study does not provide data on how quickly candesartan acts on PRA. Because the 32-mg dose was not administered at 4 AM, we do not know whether this dose would have produced an even greater PRA response than 16 mg during the second 4-hour interval.

There was a small, but significant, decrease in diastolic BP. Studies with ACE inhibitors performed by our group and by others have shown similar degrees of BP change using this model, yet the rise in PRA was routinely far smaller than the levels achieved in this study.13–16 Captopril in doses of 10 to 100 mg resulted in a maximum PRA response of 3.3 to 6.9 ng·L⁻¹·s⁻¹.17,18 Enalapril in doses of 2.5 to 20 mg likewise resulted in peak levels of <8.3 ng·L⁻¹·s⁻¹.17,18 Because we have had substantial experience with responses to ACE inhibition, no attempt was made to compare responses to candesartan with responses to ACE inhibition in this study. BP had returned to baseline by 24 hours, so a BP fall could not have contributed to the sustained PRA response. The natriuresis caused by candesartan may have been somewhat larger than that induced by ACE inhibitors but was actually modest and not dose related.17,18 Unfortunately, we do not have data on the temporal relationship of urinary sodium excretion after candesartan administration. The magnitude of the contribution of natriuresis to the substantial rise in PRA induced by candesartan cannot be determined.

What about other agents in its class? Eprosartan, another Ang II antagonist, similarly achieved peak PRA in 4 hours. In contrast, peak PRA with eprosartan on an LS diet was generally achieved in 2.5 hours, with the values returning to baseline by 24 hours, indicating no cumulative effect. The greatest PRA response to eprosartan was seen with the 200-mg dose at 4.5 hours, with a mean value of 8.6±1.9 ng·L⁻¹·s⁻¹. A study on yet another Ang II antagonist, irbesartan, likewise on LS balance, showed a peak PRA response of 9.5±0.3 ng·L⁻¹·s⁻¹. Thus, the PRA responses to both eprosartan and irbesartan were also larger than those seen with ACE inhibition. However, because the PRA assay maximum was 10.3 ng·L⁻¹·s⁻¹; the actual peak values were not determined in all subjects. Azizi et al19,20 likewise reported larger renin responses with candesartan than those seen with ACE inhibition.

The peak renal vasodilator response to candesartan was similar to our previous experience with the Ang II antagonists irbesartan and eprosartan and to the renin inhibitors enalkiren and zankiren, averaging ≈140 to 150 mL·min⁻¹·1.73 m⁻², of which ≈40% probably represents blockade of non-ACE pathways.9,13,21,22 More complete blockade of non–ACE-dependent pathways with Ang II antagonist may explain why the PRA response to candesartan was much larger than that seen with ACE inhibition.

In pathological states, the PRA can reach very high levels. We described a group of patients with severe uncompensated heart failure on a restricted-sodium diet whose mean PRA was 18.1±3.3 ng·L⁻¹·s⁻¹ (range 4.1 to 26.9) in the same renin assay system.23 In patients with unilateral renal artery stenosis on LS balance who were administered captopril, the mean PRA was reported to be 12.2 ng·L⁻¹·s⁻¹.4 Hemorrhage also activates the RAS, but loss of up to 10% of blood volume may not be sufficient to stimulate renin response. The PRA values in our study (highest 19.2 ng·L⁻¹·s⁻¹) certainly overlap those seen in these disease states and are much...
greater than those achieved with ACE inhibition while on sodium restriction.

Why should there be such a difference in the relation between candesartan dose, time, and the magnitude of the renal hemodynamic response peaked within 4 hours, suggesting that conversion of the prodrug to the active moiety and delivery to the intrarenal location of the receptors could be completed within 4 hours. Does the delay in the peak PRA response to 4 to 8 hours, or 24 hours, reflect an inherently sluggish response? The answer is no. Tuck et al\(^ {24}\) showed that the time to a peak rise in PRA in response to the robust stimulus provided by standing in addition to an LS diet reached the peak within 2 hours. The very large and delayed PRA response to block of the AT\(_1\) receptor may well not only reflect the acute stimulus to renin release after the removal of Ang II negative feedback but also be mediated, at least in part, through increased renal renin gene expression,\(^ {25}\) which may also have contributed in the apparent prolonged response to candesartan, which was still evident 48 hours after dosing.

Why the angiotensin-dependent short feedback loop plays such an important role may well reflect phylogenetic development. The renin system evolved when cartilaginous fish began to develop bone >500 million years ago, preceding any contribution of angiotensin to BP and angiotensin-dependent aldosterone release.\(^ {26}\) At that time, renin seemed to act primarily as an intrarenal hormone, contributing to volume regulation through its renal vascular action.\(^ {26}\) In that context, the development of a dominant Ang II–dependent brake on renin release could be seen as a kind of safety valve. This remarkable contribution of angiotensin to the control of renin release has become evident only when the more complete blockade possible with the Ang II antagonists became available.

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References
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