Pharmacologic Demonstration of the Synergistic Effects of a Combination of the Renin Inhibitor Aliskiren and the AT1 Receptor Antagonist Valsartan on the Angiotensin II–Renin Feedback Interruption

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Abstract. Interrupting the renin-angiotensin system (RAS) with a usual daily dose of a single-site RAS inhibitor does not achieve complete and long-lasting pharmacologic blockade. Hormonal and BP effects were compared for 48 h after administration of single oral doses of 300 mg (high dose) of the renin inhibitor aliskiren (A300) and 160 mg (standard antihypertensive dose) of the AT1 receptor antagonist valsartan (V160) and their combination each at half dose (A150 + V80) in 12 mildly sodium-depleted normotensive individuals. In this double-blind, placebo-controlled, randomized, four-period crossover study, A300 decreased plasma renin activity and angiotensin I and II levels for 48 h, stimulated immunoreactive active renin release more strongly than V160, and decreased urinary aldosterone excretion for a longer duration than V160. In contrast to V160, the A150 + V80 combination did not increase plasma angiotensins. The renin and aldosterone effects of the A150 + V80 combination were similar to those of A300 and greater than those of V160. When plasma drug concentrations were taken into account, the A150 + V80 combination had a synergistic effect on renin release. The A150 + V80 combination lowered BP at least as effectively as either higher dose monotherapy. In conclusion, in mildly sodium-depleted normotensive individuals, the long-lasting effects of aliskiren alone or in combination with valsartan on plasma immunoreactive active renin and urinary aldosterone effects demonstrate strong and prolonged blockade of angiotensin II at the kidney and the adrenal level. Moreover, a renin inhibitor and AT1R antagonist combination may provide synergistic effects on RAS hormone levels.

Numerous experimental and clinical studies have demonstrated that the combination of currently available angiotensin I–converting enzyme (ACE) inhibitors and AT1 receptor (AT1R) antagonists provides additive or synergistic effects on BP lowering (1) and on the prevention of cardiovascular (2) and renal lesions (3). These observations have previously been explained by AT1R blockers inhibiting the effects of non–ACE-dependent angiotensin II (Ang II) production (4,5) or by the bradykinin-NO–related effects of ACE inhibition (6). However, the additive effects of low doses of two different renin angiotensin system (RAS) inhibitors may be better explained by inhibition of the biologic effects of the reactive renin release that is triggered by single-site RAS blockade. The amount of compensatory renin release is proportional to the extent of decrease in the amount of Ang II generated or bound to the AT1R of the renal juxtaglomerular cells. This counterregulation may be overcome by using higher-than-usual or repeated doses of single-site RAS blockers (7,8) or by neutralizing the biologic effects of the counterbalancing rise in active renin by using a combined RAS blockade.

Direct demonstration of the importance of renin in this counterregulatory mechanism has not previously been possible in humans because of the absence of convenient orally available renin inhibitors. Thus, we investigated whether a combination of the orally active potent alkane carboxamide renin inhibitor aliskiren (150 mg) (9,10) and the AT1R antagonist valsartan (80 mg), which inhibit the RAS at the first and last steps, respectively, results in a stronger blockade of the RAS and larger decreases in aldosterone excretion than higher doses of each RAS inhibitor given alone. Synergistic effects of a combination of a renin inhibitor and AT1R blocker on BP have been demonstrated in a preclinical study (11). We have used the same clinical and laboratory methods as those previously used to differentiate the combination of an ACE inhibitor and
AT1R antagonist from each component (12,13). The present study therefore compared, in mildly sodium-depleted normotensive volunteers, the pharmacokinetic–pharmacodynamic relationship on renin release of a single oral dose of a 150-mg aliskiren–80-mg valsartan combination with those of 300 mg of aliskiren and 160 mg of valsartan.

Materials and Methods

Study Design

A double-blind, double-dummy, placebo-controlled, randomized, four-period, crossover study design with 2-wk washout intervals was used. The protocol was approved by the Comité Consultatif de Protection des Personnes se prétendant à des Recherches Biomédicales (Paris-Cochin, France), and the procedures followed were in accordance with the Declaration of Helsinki. After giving informed, written consent, 12 healthy white normotensive male volunteers (aged between 18 and 35 yr) received a single oral dose of 300 mg of aliskiren (A300), 160 mg of valsartan (V160), a combination of these two drugs (each at half dose; A150+V80), or matched placebos. Treatments were allocated according to the Latin square design. Aliskiren (film-coated tablet), valsartan, and placebo were placed in capsules that were identical in appearance. A 300-mg dose of aliskiren was used because this was the highest dose so far tested in early phase II trials in hypertensive patients (10). A 160-mg dose of valsartan was used as this is the standard registered daily dose in several countries and the highest dose in the Valsartan Antihypertensive Long-term Use Evaluation (VALUE) trial (14). In the combination treatment, the dose of each drug was halved to test whether this RAS blockade had a synergistic/additive effect on renin release, aldosterone excretion, and BP lowering.

Study Protocol

Mild sodium depletion, which results in a two- to threefold increase in plasma active renin concentration, was used to amplify the renin response to RAS blockade and to provide optimal conditions to unmask the renin dependence of BP in the normotensive volunteers (12,13). Volunteers were instructed to arrive at the Clinical Investigation Center at 6 p.m. on the evening before each phase of the study (day 0). Mild sodium depletion was induced on day 0 by giving volunteers a single oral dose of 40 mg of furosemide at 9 p.m. and supplying them with a sodium-restricted diet (30 mmol/d) during each 60-h treatment period as described previously (12,13). Volunteers were instructed to arrive at the Clinical Investigation Center at 6 p.m. on the evening before each phase of the study (day 0). Mild sodium depletion was induced on day 0 by giving volunteers a single oral dose of 40 mg of furosemide at 9 p.m. and supplying them with a sodium-restricted diet (30 mmol/d) during each 60-h treatment period as described previously (12,13). Between treatment periods, sodium intake was unrestricted.

At 9 a.m. on the study day (day 1), volunteers received the study drugs, their combination, or matched placebos, according to the randomization schedule, after a 1-h period of rest in a semirecumbent position to allow for equilibration of BP, heart rate, and hormones. Fluid intake was unrestricted on the study days (1500 to 2000 ml/24 h). Volunteers remained in a resting, semirecumbent position for blood sampling and BP measurements. All volunteers were in a fasting state from 12 h before to 6 h after drug administration. Blood was sampled before and at various time points after drug intake for plasma active renin, plasma renin activity (PRA; measured by the trapping assay), plasma Ang I and Ang II, aldosterone, and circulating drug levels. PRA measures the enzymatic activity of active renin in plasma and investigates the inhibitory effect of aliskiren. Plasma active renin is determined using an immunoradiometric assay and measures the number of active renin molecules independent of their enzymatic activity (15). Mean arterial pressure (MAP; mean of 10 measurements performed at 2-min intervals) was determined using an automatic validated BP recorder (Press Mate BP 8800; Colin Co., Komaki-City, Japan). Urine volume and aldosterone extractable at pH 1 were measured for each fractionated urine sample.

Laboratory Methods

The methods and antibodies used for sampling and determining plasma and urine hormones were as described in a previous clinical investigation of renin inhibitors (16,17). Circulating levels of aliskiren and valsartan were determined by liquid chromatography with tandem mass spectrometric detection (LC/MS/MS) (detection limits, 0.5 ng/ml and 20 ng/ml, respectively). The peak plasma concentration (Cmax), time to peak (tmax), the area under the curve up to the last measured time point (AUC0–t), and the AUC extrapolated to infinity (AUC0–∞) were determined for each individual concentration-time profile (A300, A150, V160, and V80) by a noncompartmental method using WinNonlin Pro 4.0 software (Mountain View, CA).

Statistical Analyses

Data were analyzed by ANOVA for a four-by-four crossover design (18). When the F test was significant (P < 0.05), paired comparisons were made between specific treatments, using the Holm procedure (19). Regression was estimated by the least squares method. Stata Statistical Software (Release 7.0; College Station, TX) was used for statistical analysis. Data are expressed as geometric means with 95% confidence intervals (CI) for non-normal data and as means ± 1 SD for normally distributed data. P < 0.05 was considered to be significant.

Results

As expected, the baseline levels of PRA, plasma active renin, Ang I, Ang II, and aldosterone were high in the mildly sodium-depleted volunteers.

Effects of A300, V160, and the A150+V80 Combination on PRA

A300 and V160 had opposing effects on PRA. A300 completely inhibited PRA within 1 h of intake, and this inhibition persisted for 48 h. By contrast, PRA increased considerably within 4 h of V160 intake and was still higher than placebo at 24 and 48 h after dosing (Table 1, Figure 1).

In volunteers who were treated with the A150+V80 combination, PRA was reduced below control values within 1 h of drug administration, in contrast with the increase in PRA observed with V160. PRA returned thereafter to baseline levels 12 h after A150+V80 intake and from this point onward was not different from placebo.

Effects of A300, V160, and the A150+V80 Combination on Plasma Active Renin Concentration

Baseline plasma active renin concentrations were high and significantly correlated with baseline PRA (r = 0.70, P < 0.001, not shown). Placebo intake was associated with an increase in plasma active renin concentration from 42 (95% CI; 33 to 54) to 73 (95% CI; 54 to 98) pg/ml at 48 h; this was due to the effects of sodium restriction. All active drugs given alone or in combination massively increased plasma active renin levels from baseline within 4 h of drug intake, and all had a similar 48-h plasma active renin profile (Table 1, Figure 2). The peak plasma active renin concentration with the A150+V80
combination was of similar magnitude to that observed with A300 alone and significantly higher than after V160 alone. The duration of the increase in plasma active renin concentration was significantly longer for A300 or A150/V80 than for V160, as indicated by the significantly higher 24- and 48-h post-dose plasma active renin concentrations and AUC values (Table 1). The AUC values of plasma active renin concentration for A300 and the A150/V80 combination were similar, but plasma active renin concentration 48 h after A300 intake was significantly higher than that after intake of the A150/V80 combination (Table 1).

Pharmacokinetic/Pharmacodynamic Interactions that Affect Plasma Active Renin Concentration

The tmax of aliskiren occurred earlier than that of valsartan, and the plasma half-life of aliskiren was longer than that of valsartan (Table 2). AUC and Cmax increased overproportionally with respect to the dose administered for both aliskiren and valsartan, with AUC0–24 ratios for A300 to A150 of 4.4 (95% CI, 3.1–6.2) and for V160 to V80 of 2.9 (95% CI, 2.2 to 3.9; Table 2). Between-subject variability in Cmax and AUC was similar for aliskiren and valsartan.

To adjust for the nonproportionality in the increase of the plasma drug concentration to the dose administered, we calculated for each volunteer the ratio of the AUC0–24 for absolute changes in plasma active renin concentration to the AUC0–24 for plasma aliskiren or valsartan concentrations. This analysis defines for each individual a normalized index of active renin release or “renin/pharmacokinetic index” (RPI), expressed in pg active renin/ml per ng drug/ml (20). The RPI calculated for A300 was 7.87 pg/ml per ng/ml (95% CI, 6.21 to 9.97), and the RPI calculated for V160 was 0.34 pg/ml per ng/ml (95% CI, 0.21 to 0.55).

Effects of A300, V160, and the A150+V80 Combination on Plasma Ang I and Ang II Concentrations

Baseline plasma Ang I and Ang II concentrations were strongly correlated with plasma active renin concentrations ($r = 0.91$ and

| Table 1. Plasma renin activity and plasma active renin concentration. |
|------------------------|------------------------|------------------------|
| Parameter              | Baseline               | 4 H Post-Dose           | 24 H Post-Dose           | 48 H Post-Dose           |
| Plasma renin activity  | ng Ang I/ml per h      | ng Ang I/ml per h       | ng Ang I/ml per h       | ng Ang I/ml per h       |
| A300                   | 1.43 (1.14–1.79)       | 0.5 (0.3–1)             | 0.11                   | 0.11                   |
| V160                   | 1.40 (0.93–2)          | 0.06 (0.03–0.13)        | 0.82 (0.54–1.31)        | 3.5 (2.4–4.9)           |
| A150/V80               | 1.36 (0.92–1.7)        | 0.08 (0.04–0.15)        | 0.8 (0.53–1.3)          | 10 (6.2–16.6)           |
| A300                   | 1.50 (1.1–1.9)         | 0.5 (0.3–1)             | 0.11                   | 0.11                   |
| V160                   | 1.40 (0.93–2)          | 0.06 (0.03–0.13)        | 0.82 (0.54–1.31)        | 3.5 (2.4–4.9)           |
| A150/V80               | 1.36 (0.92–1.7)        | 0.08 (0.04–0.15)        | 0.8 (0.53–1.3)          | 10 (6.2–16.6)           |
| Plasma active renin    | pg/ml                  | pg/ml                   | pg/ml                   | pg/ml                   |
| A300                   | 40 (29.5–53)           | 6.5 (4.5–9.9)           | 0.11                   | 0.11                   |
| V160                   | 41 (31.4–56)           | 6.6 (4.5–9.9)           | 0.11                   | 0.11                   |
| A150/V80               | 40 (29.5–53)           | 6.5 (4.5–9.9)           | 0.11                   | 0.11                   |

Data are geometric means (95% confidence interval [CI]). A150+V80, combination of 150 mg of aliskiren with 80 mg of valsartan; A300, 300 mg of aliskiren; V160, 160 mg of valsartan; P, placebo; AUC, area under curve calculated according to the trapezoidal rule; F3,33 (F test by an ANOVA for a four-by-four crossover design).
After treatment with A300, V160, or the A150/V80 combination, plasma Ang I and Ang II profiles followed the same pattern as the PRA profiles (Table 3, Figure 2). The concentrations of Ang I and Ang II increased in parallel in response to V160 for up to 48 h after drug intake. In contrast, A300 significantly decreased plasma Ang I and Ang II concentrations to very low levels, which remained significantly lower than those measured after placebo for >24 h. In volunteers who were given the A150+V80 combination, plasma levels of both Ang I and Ang II remained similar to the values measured after placebo administration, in contrast to the increase in both peptides that was observed with V160 (Table 3, Figure 2).

Effects of A300, V160, and the A150+V80 Combination on Plasma and Urinary Aldosterone Concentrations

Plasma and urinary aldosterone concentrations followed a circadian rhythm. In comparison with placebo, A300, V160, and the A150+V80 combination significantly decreased plasma aldosterone concentrations for 6 h after drug intake; there was no significant difference in effect between the active treatments (58 ± 16 versus 52 ± 22 versus 56 ± 21%, respectively; NS) (Table 4). In parallel, urinary aldosterone concentrations were significantly lower after administration of A300, V160, or the A150+V80 combination compared with placebo, with no significant differences in effect among the active treatments during the 8 h after drug intake (Table 4, Figure 3). Compared with placebo, A300 and the A150+V80 combination suppressed urine aldosterone excretion for up to 24 h after drug intake, whereas the effect of V160 on urinary aldosterone concentration persisted for no more than 12 to 18 h. Both A300 and the A150+V80 combination reduced 24-h cumulative urine aldosterone excretion to a significantly greater extent than V160 (Table 4, Figure 3).

Effects of A300, V160, and the A150+V80 Combination on MAP

Compared with placebo, A300, V160, and the A150+V80 combination significantly decreased MAP within 4 h of drug intake. There were no significant differences between A300, V160, and the lower dose combination in terms of the extent of MAP reduction. Twenty-four hours after drug intake, the difference between the active treatments and placebo was no longer significant in the mildly sodium-depleted normotensive volunteers investigated in this study. In addition, when the decrease in MAP was assessed by the AUC0–24 of MAP decrease, the three active treatments differed from placebo but the difference between treatments in paired comparisons was NS (Table 5).

Safety

No adverse event occurred during the investigation, and aliskiren and valsartan and their combination were well tolerated.

Discussion

The results of the present study show that in mildly sodium-depleted normotensive individuals, the RAS blockade induced by a low dose of an AT1R antagonist can be enhanced by inhibiting renin activity, through co-administration of a potent, orally active, long-lasting renin inhibitor. The enhanced RAS blockade generated by the combined treatment at low doses resulted in greater plasma immunoreactive active renin and urinary aldosterone effects than those of the standard 160-mg dose of valsartan in monotherapy. The renin and aldosterone levels of both Ang I and Ang II remained similar to the values measured after placebo administration, in contrast to the increase in both peptides that was observed with V160 (Table 3, Figure 2).

Figure 2. Time course of plasma active renin concentration (top), plasma angiotensin I (middle), and angiotensin II (bottom) concentrations. — A300; — V160; —, A150+V80; —, placebo. Data are geometric means.

$\text{r} = 0.93, P < 0.001$, respectively, not shown). After treatment with A300, V160, or the A150+V80 combination, plasma Ang I and Ang II profiles followed the same pattern as the PRA profiles (Table 3, Figure 2). The concentrations of Ang I and Ang II increased in parallel in response to V160 for up to 48 h after drug intake. In contrast, A300 significantly decreased plasma Ang I and Ang II concentrations to very low levels, which remained significantly lower than those measured after placebo for >24 h. In volunteers who were given the A150+V80 combination, plasma levels of both Ang I and Ang II remained similar to the values measured after placebo administration, in contrast to the increase in both peptides that was observed with V160 (Table 3, Figure 2).

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The effects of the combination were similar to those of the high 300-mg dose of aliskiren in monotherapy. The A150/V80 combination lowered BP at least as effectively as either higher dose monotherapy.

Comparison of the Hormonal Effects of the Renin Inhibitor and the AT1R Antagonist and Analysis of the RPI

Our results showed that in mildly sodium-depleted normotensive individuals, (1) aliskiren decreases PRA and plasma Ang I and Ang II concentrations in a dose-dependent manner for up to 48 h after intake, confirming that it is a potent, orally active, long-lasting renin inhibitor; and (2) a single oral dose of A300 stimulated greater immunoreactive active renin release from the juxtaglomerular cells of the kidney than the standard daily dose of V160. The potency of aliskiren by comparison with valsartan was assessed according to the plasma levels achieved by each drug, by the calculation of the RPI. The RPI is a pharmacokinetic/pharmacodynamic index that takes into account actual drug exposure rather than the oral dose of the drug administered and has been used previously to characterize two different AT1R antagonists, valsartan and candesartan (20). This correction is especially important in studies in which drug pharmacokinetics are nonlinear, as was the case in the present study. The RPI for A300 was 7.87 pg/ml per ng/ml (95% CI, 6.21 to 9.97), and that for V160 was 0.34 pg/ml per ng/ml (95% CI, 0.21 to 0.55). Thus, according to the RPI, exposure to ~20 times more valsartan than aliskiren is required to trigger the release of one renin molecule in plasma. Finally, the duration of the decrease in urinary aldosterone was longer after A300 treatment than after V160 treatment. However, the decrease in BP was similar for both drugs in these mildly sodium-depleted normotensive individuals.

Combined Blockade of the RAS by Aliskiren and Valsartan

We have compared the effects on hormone and BP levels of a high dose of a renin inhibitor or a standard dose of an AT1R antagonist given alone with those of each of these components administered in combination (at half dose). Under conditions in which the pharmacokinetic parameters of neither drug were modified when the drugs were combined, combination of aliskiren with valsartan prevented the increase in PRA and plasma Ang I and Ang II concentrations that was observed with

Table 2. Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Drugs</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0–48&lt;/sub&gt; (ng·h/ml)</th>
<th>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (ng·h/ml)</th>
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<tr>
<td>Aliskiren 300 mg</td>
<td>306 ± 136</td>
<td>2.17 ± 1.40</td>
<td>25 ± 4</td>
<td>1497 ± 429</td>
<td>1714 ± 497</td>
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<tr>
<td>Aliskiren 150 mg</td>
<td>85 ± 53</td>
<td>2.25 ± 1.55</td>
<td>30 ± 8</td>
<td>398 ± 239</td>
<td>489 ± 295</td>
</tr>
<tr>
<td>Valsartan 160 mg</td>
<td>2396 ± 1247</td>
<td>3.08 ± 1.44</td>
<td>8.33 ± 3.84</td>
<td>17422 ± 7673</td>
<td>17813 ± 7758</td>
</tr>
<tr>
<td>Valsartan 80 mg</td>
<td>749 ± 336</td>
<td>2.67 ± 0.99</td>
<td>5.57 ± 1.69</td>
<td>5716 ± 2724</td>
<td>6032 ± 2663</td>
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</table>

* Data are mean ± SD. C<sub>max</sub>, concentration at peak; t<sub>max</sub>, time to peak; t<sub>1/2</sub>, plasma half-life; AUC<sub>0–48</sub>, area under time curve up to the last measured time-point; AUC<sub>0–∞</sub>, AUC extrapolated to infinity.

Table 3. Plasma angiotensin I and angiotensin II concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (pg/ml)</th>
<th>6 H Post-Dose (pg/ml)</th>
<th>24 H Post-Dose (pg/ml)</th>
<th>48 H Post-Dose (pg/ml)</th>
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<tr>
<td>Plasma angiotensin I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A150 + V80</td>
<td>11 (9–14)</td>
<td>14 (8–24)</td>
<td>44 (37–54)</td>
<td>38 (32–44)</td>
</tr>
<tr>
<td>A300</td>
<td>19 (15–24)</td>
<td>4 (3–5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 (11–17)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 (14–23)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V160</td>
<td>10 (7–13)</td>
<td>150 (92–247)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>97 (70–136)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>55 (46–67)&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td>P</td>
<td>18 (12–25)</td>
<td>20 (15–28)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>34 (25–45)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>25 (19–32)&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
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<tr>
<td>F&lt;sub&gt;3,33&lt;/sub&gt;</td>
<td>0.3</td>
<td>92&lt;sup&gt;e&lt;/sup&gt;</td>
<td>71&lt;sup&gt;e&lt;/sup&gt;</td>
<td>46&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma angiotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A150 + V80</td>
<td>9 (7–11)</td>
<td>10 (6–16)</td>
<td>19 (15–23)</td>
<td>16 (13–19)</td>
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<tr>
<td>A300</td>
<td>12 (10–13)</td>
<td>2.6 (1.7–4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7 (6.4–9.2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9 (7–12)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>V160</td>
<td>7 (5–8)</td>
<td>73 (48–111)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>41 (31–54)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>25 (21–29)&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td>P</td>
<td>10 (7–14)</td>
<td>12 (9–15)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>15 (12–19)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>11 (9–14)&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>F&lt;sub&gt;3,33&lt;/sub&gt;</td>
<td>0.8</td>
<td>91&lt;sup&gt;e&lt;/sup&gt;</td>
<td>67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>41&lt;sup&gt;e&lt;/sup&gt;</td>
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</table>

* Data are geometric means (95% CI).
*<sup>b</sup> P < 0.05 versus A150 + V80.
*<sup>c</sup> P < 0.05 versus A300.
*<sup>d</sup> P < 0.05 versus V160.
*<sup>e</sup> P < 0.001.
valsartan alone. The AUC<sub>0–24</sub> of plasma active renin concentration for the A150/H11001/V80 combination was significantly higher than that achieved with 160 mg of valsartan and similar to that achieved by A300 alone. A300 and the A150/H11001/V80 combination decreased urinary aldosterone excretion for a similar duration, whereas V160 had a shorter effect. A300 and the A150/H11001/V80 combination thus seemed to be equipotent with regard to these two parameters. However, to interpret these results correctly, we must take into account that plasma aliskiren concentration does not increase proportionally to the dose given. The plasma concentrations of aliskiren increased by a factor of 4.4 (95% CI, 3.1 to 6.2), rather than by two, when the dose was doubled from 150 to 300 mg. This observation accounts for the larger-than-expected increase in the amount of renin released in response to A300, because the amount of renin release is directly related to the aliskiren concentration achieved at the level of the juxtaglomerular cells. After taking this nonproportionality into account, we can conclude that there is a synergistic effect on immunoreactive active renin release and on the decrease in urinary aldosterone excretion when these doses of a renin inhibitor (A150) and an AT1R antagonist (V80) are combined. In mildly sodium-depleted normotensive individuals, lower dose combination therapy lowered BP to a similar extent as either high-dose aliskiren monotherapy or standard-dose valsartan monotherapy. This may be due to the limited range of BP variation that can be detected in mildly sodium-depleted normotensive individuals, the large within-subject BP variability, and the low statistical power of the study for this parameter. The BP effects of a combination treatment associating various doses of aliskiren and valsartan need to be investigated in hypertensive patients.

Combined RAS inhibition makes it possible to use lower doses of each component to achieve a more effective and long-lasting RAS blockade (1). We show that, when nonlinearity of drug pharmacokinetics is taken into account, a combination of aliskiren (150 mg) and valsartan (80 mg) provided

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**Table 4.** Plasma and urinary aldosterone concentrations<sup>a</sup>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma aldosterone pg/ml</th>
<th>Urinary aldosterone μg/g</th>
<th>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. Baseline</th>
<th>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. A150+V80</th>
<th>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. A300+V80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>143 (118–177)</td>
<td>39 (23–56)</td>
<td>0.18</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>A150+V80</td>
<td>132 (106–182)</td>
<td>39 (23–56)</td>
<td>0.35</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>A300+V80</td>
<td>133 (107–182)</td>
<td>40 (24–56)</td>
<td>0.35</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>V160</td>
<td>132 (106–182)</td>
<td>40 (24–56)</td>
<td>0.35</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. Baseline</td>
<td>0.18</td>
<td>0.4</td>
<td>0.35</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. A150+V80</td>
<td>0.4</td>
<td>0.4</td>
<td>0.35</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>P&lt;sub&gt;F3,33&lt;/sub&gt; vs. A300+V80</td>
<td>0.35</td>
<td>0.4</td>
<td>0.35</td>
<td>0.35</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are geometric means (95% CI).

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**Figure 3.** Time course of urine aldosterone excretion. □, A300; ▀, V160; ▀, A150+V80; □, placebo. Data are geometric means.

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more effective RAS inhibition than double the dose of the AT1 receptor antagonist (160 mg) or renin inhibitor (300 mg) alone. The question of the influence of dose selection and dosing interval remains critical for all RAS inhibitors and their effects on BP and end-organ protection (21–23). The additive or synergistic effects of such combinations are more evident at low doses (which are the usual dose) than at high doses (22). An additional advantage of synergy or additivity between two antihypertensive drugs, therefore, is that the doses of individual drugs may be reduced to achieve the same therapeutic response. In summary, as with the dual RAS blockade generated by combining an AT1R antagonist and an ACE inhibitor (1), the combination of two pharmacologic agents inhibiting the initial and final steps of the RAS (renin and the AT1 receptor, respectively) minimizes or even overcomes the “escape” that occurs with single-site RAS blockade in an acute setting.

**Potential Impact of the Results**

The long duration of action of aliskiren, alone or in combination with valsartan, on the increase in plasma immunoreactive active renin and the decrease in urinary aldosterone is an interesting finding. It suggests that in both the kidneys and the adrenal glands, Ang II can be efficiently blocked through renin inhibition of aldosterone excretion by aliskiren alone or in combination with valsartan, which may also be due to direct inhibition of the adrenal RAS (31), may be per se potentially beneficial to patients with chronic nephropathies (32).

These investigations of single-dose administration in mildly sodium-depleted normotensive individuals were performed to test the plausibility of a pharmacologic synergy or additivity of two RAS inhibitors acting at different steps of the pathway, renin and AT1 receptors. They have shown that the interruption of the RAS at the level of the Ang II–renin feedback with a low-dose combination of aliskiren and valsartan is similar to a high dose of aliskiren but more marked than with the standard dose of valsartan 160 mg. These results support further clinical studies at different doses of these compounds to investigate the long-term effects of renin inhibitor–AT1R antagonist combinations in various clinical contexts, including hypertension, chronic proteinuric nephropathies, and chronic heart failure. Indeed, results obtained in recent clinical trials suggest enhanced nephroprotective (3) and cardioprotective (2) effects of the dual RAS blockade with ACE inhibitors and AT1 receptor antagonists.

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