Integrating Drug Pharmacokinetics for Phenotyping Individual Renin Response to Angiotensin II Blockade in Humans

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Abstract—Renin release into plasma has been used to investigate the drug dose-dependence of renin-angiotensin system inhibition because it is proportional to the interruption of the permanent negative feedback loop of angiotensin II on renin secretion. We investigated the 24-hour between-subject differences in renin profiles by analyzing the time-dependence of individual renin responses in 16 mildly sodium-depleted normotensive subjects exposed in a 4-period crossover study to single oral doses of 8- and 16-mg (C8 and C16) candesartan cilexetil and 80- and 160-mg (V80 and V160) valsartan. C8 had a similar effect to V160 in terms of the increase in active renin concentration and decrease in blood pressure. C16 had the strongest effect and V80 the weakest effect on renin release. Within- and between-subject variability was more marked for valsartan pharmacokinetics than for candesartan pharmacokinetics and influenced variability in renin response. To eliminate some of the variability caused by the pharmacokinetics of each drug, we corrected the area under time curve of plasma renin levels by that of plasma drug levels to obtain an individual normalized index of renin release or “renin/pharmacokinetic index”. In these experimental conditions, this index was found to be a reproducible individual characteristic affecting renin response, in addition to the pharmacokinetics and pharmacological properties of angiotensin II type-1 receptor antagonists. The pharmacokinetic–pharmacodynamic model of renin release described here could be of value for the identification and investigation of renin release abnormalities in patients with hypertension and for the comparison of renin-angiotensin system blockers. (Hypertension. 2004;43:785-790.)

Key Words: blood pressure ■ renin ■ angiotensin antagonist

Renin release into plasma has been used to investigate the drug dose-dependence of renin-angiotensin system (RAS) inhibition1-3 because the decrease in angiotensin II (Ang II) production at the level of juxtaglomerular cells induced by angiotensin I-converting enzyme (ACE) or renin inhibitors or the displacement of Ang II from Ang II type-1 (AT1) receptors by antagonists interrupts the permanent feedback inhibition of renin release mediated by Ang II.4 The use of renin release as a sensitive plasma biomarker of an acute RAS blockade has several advantages: (1) it can be used for all class of inhibitors, including the ACE inhibitor-AT1 receptor antagonist combination5 and renin inhibitors,6 provided that renin concentration is measured by immunoradiometric assay; (2) the range of variation for this factor is large, at ~2 orders of magnitude; and (3) it is a more precise measurement than blood pressure (BP), another marker used to quantify the intensity of RAS blockade, especially in sodium-depleted subjects.5,6 In contrast to the increase in renin concentration observed after RAS blockade, the magnitude of the decrease in BP may be influenced by a placebo effect and offers a limited range of variation (5 to 15 mm Hg).

We investigated in detail between-subject differences in renin regulation profiles by means of a precise analysis of the time-dependence of individual renin responses in 16 mildly sodium depleted normotensive subjects exposed in strictly controlled conditions to a single oral usual daily dose of 2 well characterized AT1 receptor antagonists, candesartan cilexetil and valsartan. Our results demonstrate that renin responsiveness can be quantified by integrating changes in renin secretion over time with changes in drug plasma levels. We showed for the first time to our knowledge that renin responsiveness is a reproducible individual characteristic, which, like the pharmacokinetic and pharmacological properties of AT1 receptor antagonists, influences renin response.

Methods
A single-dose, double-blind, randomized, 4-way crossover study design was used. Sixteen healthy normotensive white male volunteers aged 18 to 35 years completed the study after giving written informed consent for participation in this protocol, which was approved by the Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales (Paris-Cochin, France). Each

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subject received a single oral dose of 8-mg candesartan cilexetil (C8), 16-mg candesartan cilexetil (C16), 80-mg valsartan (V80), and 160-mg valsartan (V160) on 4 separate occasions, according to a Latin square design. Each treatment period was separated from the previous treatment period by a 10- to 21-day washout interval. The procedures followed were in accordance with institutional guidelines.

Study Protocol
Mild sodium depletion, which increases by 2- to 3-fold plasma active renin, was used to amplify the renin response to AT1 receptor blockade and to provide optimal conditions to unmask the renin-dependency of BP in the normotensive subjects. For each phase, subjects were instructed to arrive at the Clinical Investigation Center at 6:00 PM on the evening before the study (D0). Mild sodium depletion was induced by giving subjects a single oral dose of 40-mg furosemide at 9:00 PM on D0 and supplying them with a sodium-restricted diet (30 mmol/d) during the 36 hours of each treatment period as previously described. Between treatment periods, volunteers were instructed to consume their usual sodium diet. On the study day (D1), after a light caffeine-free and fat-free breakfast at 7:00 AM, subjects were comfortably installed in a semi-recumbent position on their beds. An indwelling cannula was inserted into a brachial vein for blood sampling. At 9:00 AM, after 1 hour of rest in the semi-recumbent position to allow equilibration of BP and hormones, the subjects received a single oral dose of the assigned treatment (C8, C16, V80, or V160) with 50 mL water and remained in the same position for 6 hours. Fluid intake was not restricted on study days, and subjects were given a light sodium-free meal 6 hours and 12 hours after the dose. Systolic and diastolic BP (SBP and DBP, mean of 10 measurements performed at 2-minute intervals) were determined before and 1, 2, 3, 4, 5, 6, 9, 12, 23, and 24 hours after drug intake with a validated automatic BP recorder (Press Mate BP 8800; Colin Co, Komaki-City, Japan). Blood samples were also taken at these time points.

Laboratory Methods
The methods used to determine plasma active renin concentration were as previously described. Plasma candesartan concentration was determined by high-performance liquid chromatography with ultraviolet detection (detection threshold <1 nmol/L). Plasma valsartan concentration was also determined by high-performance liquid chromatography with ultraviolet detection (detection threshold <4 nmol/L).

Statistical Methods
Data were analyzed using crossover-design ANOVA models to test for the effects of treatment, period, sequence, and carryover. If the F test was significant (P<0.05) and there was no period, sequence or carryover effect, paired comparisons were made between specific treatments using Bonferroni correction. The assumptions of ANOVA (homogeneity of variance and normality) were verified for each variable and natural logarithmic transformation was applied where appropriate. Regression was estimated by the least-squares method. Stata Statistical Software (Release 7.0; College Station, Tex) was used for statistical analysis. Data are expressed as mean±1 SD in the tables, unless otherwise specified; P<0.05 was considered significant.

Results
Pharmacokinetics Results
Candesartan cilexetil was not detected in plasma because it is totally converted to candesartan during gastrointestinal absorption. The tmax of candesartan was achieved later than that of valsartan (C16: 3.5±0.8 hours and C8: 3.6±1.0 hours versus V160: 2.6±1.2 hours and V80: 3.0±1.4 hours). Both areas under curve (AUC0-24) and Cmax increased dose-dependently for candesartan and valsartan (Table).

We analyzed between- and within-subject variability in plasma drug concentrations by calculating the ratio of the AUC0-24 for plasma drug concentrations to the dose (AUC0-24/dose ratio). The median AUC0-24/dose ratios (range) were 114 (79; 238) nmol·h−1 for C16, 111 (69; 159) nmol·h−1 for C8, 75 (41; 178) nmol·h−1 for V160, and 87 (58; 278) nmol·h−1 for V80. After log transformation of the ratio, we studied the proportion of the variance in the ANOVA accounted for by within-subject error and that caused by between-subject error. The within-subject coefficient of variation for the AUC0-24/dose ratio was 42.8% for valsartan and 18.6% for candesartan, whereas the between-subject coefficient of variation was 40.8% for valsartan and 22.5% for candesartan. This indicates that both the within- and between-subject variability in the ratio of the AUC0-24 for plasma drug levels to dose was higher for valsartan than for candesartan.

Effects of Candesartan Cilexetil and Valsartan on Plasma Active Renin Concentrations and on SBP and DBP
As expected, both active treatments increased plasma active renin levels significantly and dose-dependently from their respective baselines. The time to peak for plasma active renin concentration was significantly shorter after valsartan treatment than after treatment with the prodrug candesartan cilexetil (V160: 4.7±2.5 hours and V80: 5.4±2.5 hours versus C16: 6.9±2.0 hours and C8: 7.6±2.0 hours). Peak plasma active renin concentrations were ranked as follows, in ascending order: V80, C8, V160, and C16. However, the differences between drugs and between doses were not significant (Table). Twelve and 24 hours after drug intake, the highest plasma active renin concentrations were achieved with C16, and the lowest with V80 (Table). The area under the absolute changes in plasma active renin concentration against time curve from 0 to 24 hours (ie, corrected by the baseline active renin value; AUC0-24) for C16 was significantly higher than that for C8, V160 and V80. AUC0-24 for plasma active renin concentration did not differ significantly between C8, V160, and V80. For similar peak values, a significantly higher AUC0-24 indicates a longer duration of renin stimulation. As for drug plasma concentration, we analyzed the within-subject variability in plasma active renin concentration and found that the within-subject coefficient of variation for the AUC0-24 of changes in plasma active renin was larger for valsartan (59%) than for candesartan (48%).

No significant differences between drugs or doses were observed in terms of the decrease in SBP or DBP, for peak effect, or for AUC0-24 (Table).

Analysis of the Individual Renin Release and Decrease in BP Induced by Candesartan Cilexetil and Valsartan
We first checked that there was a significant log-linear correlation between the AUC0-24 for absolute changes in plasma active renin concentration and the AUC0-24 for plasma drug concentrations (C16: r=0.62, P<0.01; C8: r=0.55, P=0.03; V160: r=0.73, P<0.001; V80: r=0.51, P=0.04, not shown). We then calculated the ratio of the AUC0-24 for absolute changes in plasma active renin concentration to that
for plasma drug concentrations for each subject to eliminate the variability caused by the drug pharmacokinetics. This ratio defines an individual normalized index of active renin release or "renin/pharmacokinetic index" (RPI) expressed in pg active renin/mL per nmol drug/mL. The correction of renin levels by drug plasma levels in the RPI reduced the within-subject variability: the within-subject coefficient of variation for the RPI was reduced from 59% to 40% for valsartan compared with the noncorrected AUC_{0-24} of plasma active renin.

A highly significant log-linear correlation was observed between RPI_{C16} and RPI_{C8} (r=0.89, P<0.001, Figure) and between RPI_{V160} and RPI_{V80} (r=0.64, P<0.01, Figure), although the values obtained were more scattered for valsartan. These results, especially those for candesartan, indicate that the amount of renin released for a given plasma concentration of an AT1 receptor antagonist differs considerably between individuals but appears to be a characteristic of each individual.

We then averaged RPI_{C16} and RPI_{C8} for each individual, and RPI_{V160} and RPI_{V80}, to analyze the relationship between the RPIs to each drug, independently of the dose administered. We observed a highly significant log-linear correlation between mean RPI_{candesartan} and mean RPI_{valsartan} (r=0.93, P<0.001, Figure). Thus, the amount of renin released per nmol of AT1 receptor antagonist is independent of the antagonist administered to a given subject and dependent on an intrinsic characteristic of Ang II–renin feedback in that subject.
There was no correlation between the AUC\(_{0-24}\) for absolute changes in SBP or DBP and the AUC\(_{0-24}\) for plasma drug concentrations (not shown). Therefore, individual BP responsiveness to these doses of \(AT_1\) receptor blockers cannot be studied using this approach.

**Discussion**

The juxtaglomerular cells are differentiated vascular smooth muscle cells that share common structures and functions with vascular smooth muscle cells and react to the calcium-mediated effects of \(AT_1\) receptor activation by synthesizing and releasing renin.\(^{10}\) Renin release into plasma can therefore be considered a biomarker of any acute change in the equilibrium between Ang II produced in the vicinity of the juxtaglomerular cells and membrane \(AT_1\) receptors.

Our data confirm that the dose-dependent response of renin release to \(AT_1\) receptor blockade covers a larger range of variation than that can be investigated by monitoring BP decrease. Indeed, the BP-lowering effect was already maximal at peak with the lowest doses of valsartan (V80) and candesartan (C8) used. In contrast, a quantification of the RAS interruption far above the renin-dependent decrease in BP was detected by the increase in immunoreactive active plasma renin concentration that occurred rapidly, and dose-dependently, after initial exposure to an \(AT_1\) receptor antagonist.

BP, plasma Ang II concentration, plasma active renin concentration measurements, and neutralization of BP response to challenges with exogenous Ang I or Ang II\(^{11}\) coupled with an in vitro Ang II radioreceptor binding assay quantifying the displacement of Ang II by RAS blockers\(^{12}\) can be used to assess the intensity and duration of a RAS blockade. Even in controlled conditions, it is difficult to demonstrate small changes in BP in sodium-depleted normotensive subjects, especially 24 hours after drug intake, as shown in this experiment and in a previous study.\(^{6}\) Although, plasma Ang II concentration increases in response to \(AT_1\) receptor blockade, it is difficult to measure and it is directly correlated with plasma renin concentration.\(^{6}\) The increase in plasma active renin concentration is the most straightforward marker for monitoring time-dependent RAS blockade after intake of the drug. An increase in plasma immunoreactive renin concentration reflects RAS blockade regardless of the RAS blocker used and is proportional to the interruption of the permanent calcium-mediated suppression of renin release and synthesis by Ang II bound to its receptors at the juxtaglomerular cells in the kidney. Variation in the increase in plasma active renin concentrations reflects the intensity and the duration of Ang II neutralization effects after drug intake. This marker has been shown to parallel strictly the intensity of RAS blockade, as assessed by in vitro Ang II receptor binding assays,\(^{13}\) which is as effective for assessment of the degree of \(AT_1\) receptor blockade as in vivo assessment by exogenous Ang II injection.\(^{14}\)

**General Results**

We used the AUC\(_{0-24}\) of plasma active renin as a marker of intensity and duration of \(AT_1\) receptor blockade in this investigation to analyze the overall pharmacodynamic response to the 2 \(AT_1\) receptor antagonists administered at 2 different doses. In this well-defined model of mild sodium depletion, this parameter is directly related to the dose and the dosing interval and allowed us to compare doses and drugs. It is analyzed at the group level, and we have used a crossover design to reduce the between-subjects variability of the overall pharmacodynamic response.

Using the AUC\(_{0-24}\) of absolute changes in plasma active renin, a first global conclusion to be drawn from this study is that 8-mg dose of candesartan is equivalent to 160-mg dose of valsartan in terms of renin concentration increase, ie, blockade of the RAS. The most potent effect on renin release, especially 24 hours after intake, was obtained with 16-mg candesartan, whereas the least potent effect was obtained with 80-mg valsartan. The persistence of high renin levels 24 hours after the administration of 16-mg candesartan, in the presence of low plasma levels of the drug, represents a longer duration of action and is consistent with the reported tight binding and slower dissociation rate of candesartan from \(AT_1\) receptors\(^{15}\) observed in 2 different ex vivo/in vitro radioligand binding assays.\(^{13}\) These results are consistent with those obtained in previous studies in healthy volunteers.\(^{13,16}\) However, as pointed out by Maillard et al,\(^{11}\) differences in the efficacy of \(AT_1\) receptor antagonists in hypertension and congestive heart failure depend heavily on the choice of dose and the dosing interval: differences between 2 \(AT_1\) receptor antagonists may disappear when dosing is properly selected.\(^{13}\)

The same trends were observed for BP decrease, although the difference between drugs and doses did not achieve statistical significance.

**Analysis of Between- and Within-Subject Variability in the Pharmacokinetics of the Drugs and in the Pharmacodynamic Response**

The pharmacokinetic parameters derived from our data were consistent with previous findings for valsartan and candesartan cilexetil.\(^{9}\) The assessment of between- and within-subject variability in the AUC\(_{0-24}\) for plasma levels of both drugs is particularly interesting, because these 2 types of variability contribute to the variability of the pharmacodynamic response. In circumstances in which the 2 drugs were administered after a light breakfast, which is known to reduce the bioavailability of valsartan,\(^{9}\) both the within- and between-subjects variability of the valsartan pharmacokinetics were more marked than those of candesartan. This pharmacokinetic variability in turn affected the variability of the renin response, which was more marked after valsartan treatment.

Using a model-independent method, the AUC\(_{0-24}\) for the plot of absolute changes in renin concentration against time was related to the corresponding AUC\(_{0-24}\) for the plot of drug concentration over time: the 2 parameters were found to be log-linearly related, demonstrating the relationship between drug pharmacokinetic variability and variability in the pharmacodynamic response. Variability in the pharmacokinetics of a drug implies less predictable changes in pharmacodynamic effects when it is used, whereas a more precise titration for each subject or patient is possible for drugs with less variable pharmacokinetics.\(^{17}\)
RPI as a Tool to Analyze Individual Renin Release

Because renin release is directly linked to blockade of the AT1 receptor, the finding of a direct and close correlation between drug plasma levels and renin release made it possible to calculate an index of renin responsiveness, the RPI, in the controlled conditions of this experiment performed in healthy subjects. Such calculations cannot be performed for the decrease in BP, which is dependent on many other regulatory and counter-regulatory systems.

In the sodium-depleted normal volunteers of similar age and body weight studied here, the variability of the renin response depended on variability in the drug concentration signal at the level of the juxtaglomerular cells, which depends on the pharmacokinetics of the drug, and on the inhibition constant of each drug at the receptor level. To determine more precisely the individual response to AT1 receptor blockade, we corrected plasma renin levels by plasma drug levels in the RPI and thus reduced part of the variability caused by the pharmacokinetic properties of each drug. This correction allowed to compare for a given individual the renin release induced by RAS blockade, independently of the dose and/or the type of the AT1 receptor antagonist used, and thus defines an individual normalized index of active renin release. This index made it possible to demonstrate for the first time that renin responsiveness to a stimulus induced by RAS blockade was highly reproducible in a given subject on 4 different occasions, even using different drugs and different doses. Environmental factors, mainly age and exposure to dietary sodium, and genetic factors probably play a role in individual RPI determinism and deserve further study. There is probably a genetic component to renin responsiveness, because white and black subjects differ in renin concentrations, even in normotensive subjects. Renin levels have been shown to be heritable in sodium-loading and depletion studies performed in monozygotic and dizygotic twins and relatives of patients with essential hypertension. Finally, in a large multicenter study, the analysis of 175 hypertensive white subjects from 86 families (71 pairs, 15 trios) showed plasma renin concentrations to be highly correlated between hypertensive siblings on high- and low-salt diets after adjustment for age, sex, and natriuresis. However, the genes involved in this familial resemblance have yet to be identified.

Perspectives

The pharmacokinetic–pharmacodynamic analysis based on AUC0–24 of plasma immunoreactive renin reflects RAS blockade regardless of the RAS blocker used and could be therefore applied to ACE inhibitors, renin inhibitors, and combined blockade of the RAS. It provides new possibilities for exploring the intensity and duration of RAS blockade, for defining the equipotency at the level of the juxtaglomerular cells of different methods of RAS inhibition, and for interpreting the influence of the daily dose of RAS blockers on the results of primary and secondary prevention studies.

The principal interest of the RPI is to characterize individual renin release to RAS blockade independently of the variability in the pharmacological stimulus. Therefore, the pharmacokinetic–pharmacodynamic model of individual renin release based on the RPI described here is potentially useful for identifying and investigating renin release abnormalities in patients with hypertension.

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