Effect of Contrasted Sodium Diets on the Pharmacokinetics and Pharmacodynamic Effects of Renin–Angiotensin System Blockers

Michel Azizi, Anne Blanchard, Beny Charbit, Grégoire Wuerzner, Séverine Peyrard, Eric Ezan, Christian Funck-Brentano, Joël Ménard

Abstract—Dietary sodium, the main determinant of the pharmacodynamic response to renin–angiotensin system blockade, influences the pharmacokinetics of various cardiovascular drugs. We compared the effect of contrasted sodium diets on the pharmacokinetics of single oral doses of 8 mg candesartan cilexetil, 160 mg valsartan, 10 mg ramipril, and 50 mg atenolol administered to 64 (16 per group) normotensive male subjects randomly assigned to sodium depletion (SD) or sodium repletion (SR) in a crossover study. Pharmacodynamic response was assessed as the increase in plasma renin concentration for renin–angiotensin system blockers and electrocardiographic changes in PR interval duration for atenolol. The area under the curve (AUC) for plasma candesartan and atenolol concentrations was significantly lower for SR than for SD (respective ratios of AUC_{SR/SD} 0.74; [90% CI, 0.66–0.82] and 0.69 [90% CI, 0.54–0.88], respectively), indicating a lack of bioequivalence between SR and SD. SR did not affect the pharmacokinetics of valsartan or ramipril. The increase in plasma renin concentration with the 3 renin–angiotensin system blockers was 10 times lower during the SR than the SD period. In the multiple regression analysis, the AUC_{SR/SD} of plasma drug concentration explained <1% and 21% of the variance of the AUC_{0–24} of delta plasma renin concentration for candesartan (P = 0.8882/P = 0.0368) during the SR and SD periods, respectively. The atenolol-induced lengthening of PR interval was fully reversed by SR. Thus, sodium balance modulates the pharmacokinetics of candesartan cilexetil and atenolol, with measurable effects on the selected pharmacodynamic end points. (Hypertension. 2013;61:1239-1245.) * Online Data Supplement

Key Words: atenolol ■ candesartan ■ pharmacodynamics ■ pharmacokinetics ■ ramipril ■ sodium ■ valsartan

There is considerable between-subject variability in the hemodynamic, cardiac, and renal responses to renin–angiotensin system (RAS) blockers, and the contribution of the RAS to blood pressure (BP) control and other vascular, cardiac, and renal functions varies greatly between diseases and individuals.1 Age, genetic factors, ethnicity, and dietary sodium intake, in particular, are the major determinants of the pharmacodynamic effects of RAS blockers.1 Indeed, the hemodynamic, cardiac, and nephroprotective effects of RAS blockers are enhanced by the combination of these drugs with a low-sodium diet or diuretics because of RAS activation.1,4

A pharmacokinetic interaction with the sodium content of the diet has been reported for drugs that do not interfere with the RAS.5,7 We therefore compared the effect of contrasted sodium diets on the pharmacokinetics of single oral doses of various RAS blockers administered to normotensive healthy male subjects: an angiotensin-converting enzyme inhibitor (ramipril) and 2 different angiotensin II receptor blockers (candesartan cilexetil and valsartan). We selected 2 different angiotensin II receptor blockers to investigate whether in a same class, drugs with different physicochemical properties and pharmacokinetic profiles could be affected differently by the sodium content of the diet. To further understand the effects of sodium balance on the pharmacokinetics of these cardiovascular drugs, we also evaluated the effects of dietary sodium on atenolol, a selective β1-adrenergic blocker, that is absorbed passively through the intestinal wall8 and is excreted unchanged by the kidney after glomerular filtration.9 Thus, any change in urinary excretion of atenolol with the sodium content of the diet should directly reflect a change in the net intestinal absorption of the drug in healthy subjects with

Received February 9, 2013; first decision April 1, 2013; revision accepted April 1, 2013.
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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.113.01196/-/DC1.
This trial is registered at ClinicalTrials.gov with trial identifier NCT00310778 (http://www.clinicaltrials.gov/ct/show/NCT00310778).
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Hypertension is available at http://hyper.ahajournals.org
DOI: 10.1161/HYPERTENSIONAHA.113.01196
normal renal function. The pharmacodynamic consequences of dietary sodium–induced changes in the pharmacokinetics of these drugs were assessed by determining the drug-induced increase in plasma renin concentration (PRC) for the angiotensin-converting enzyme inhibitor and the angiotensin II receptor blockers resulting from interruption of the angiotensin II negative feedback loop on renin release\textsuperscript{10}; and the drug-induced changes in PR interval duration and heart rate (HR) on ECG recordings for atenolol.\textsuperscript{6} We found that sodium status modulated the pharmacokinetics of candesartan and atenolol, with measurable but mild effects on the selected pharmacodynamic end points.

**Methods**

**Study Design**

We performed an open-label, randomized, single-dose, 4-panel (candesartan cilexetil, valsartan, ramipril, and atenolol), 2-period (sodium repletion [SR] versus sodium depletion [SD]), crossover study.\textsuperscript{11,12} After informed written consent had been obtained, 64 healthy white normotensive male volunteers (aged 18–35 years) were randomly assigned to SR or SD. In each of these 2 sodium loading conditions, they received a single oral dose of 8 mg candesartan cilexetil (n=16), 160 mg valsartan (n=16), 10 mg ramipril (n=16), or 50 mg atenolol (n=16). These doses are the standard recommended doses for these compounds. The protocol was approved by the Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales (Paris Cochin-Tardieu, France).

**Experimental Protocol**

To induce a mild SD, subjects were given a single oral dose of 40 mg furosemide on the evening before the study day (9:00 pm on day 1) and were provided with a sodium-restricted diet (NaCl <30 mmol/d) during a 60-hour hospitalization period.\textsuperscript{11,12} To induce SR, subjects were given slow release sodium tablets (6 g/d) for 9 days (from day −5 to day 3) and were instructed to eat high-sodium foods preferentially. The effect was considered demonstrated if the 90% confidence intervals (CI) for changes in PRC were within the 0.80 to 1.25 range.

**Safety Parameters**

BP was recorded with an automatic validated BP recorder (Press Mate BP 8800; Colin, Komaki-City, Japan).

**Pharmacokinetic Parameters**

**Drug Assays**

The plasma and urinary concentrations of each compound were measured by liquid chromatography tandem mass spectrometry\textsuperscript{13-16} (see online-only Data Supplement for quantification limits). Candesartan cilexetil and ramipril are prodrugs that are converted into the active metabolites candesartan and ramiprilat, respectively.

**Data Analysis**

We determined peak plasma concentration (C\textsubscript{max}), time to C\textsubscript{max} (t\textsubscript{max}), the plasma terminal half-life (t\textsubscript{1/2}), the area under the curve up to the last measured time point (AUC\textsubscript{0-24}), and the area under the curve extrapolated to infinity (AUC\textsubscript{0-∞}) for each individual concentration–time profile, by a noncompartmental method, with WinNonlin Pro 4.0 software (Pharsight, Mountain View, CA). Bioequivalence was considered to be demonstrated if the 90% confidence intervals (CI) of the ratios for AUC\textsubscript{0-24} and the C\textsubscript{max} between the SR and SD periods were within the 0.80 to 1.25 range.

**Pharmacodynamic Parameters**

**Renin Assay**

PRC was determined blind to the randomization sequence, by immunoradiometric assay (Cisbio International, F91192 Gif-sur-Yvette, France), as previously described.\textsuperscript{11,12}

**ECG Measurements**

In the atenolol group, 10-second digital ECGs were recorded at rest with a Cardioplug digital ECG recorder (Cardionics Inc, Brussels, Belgium), before and at various times up to 24 hours after drug intake. We used HR, PR interval values, and PR interval prolongation (absolute change from baseline, ΔPR; see online-only Data Supplement for details) to compare the pharmacodynamic effects of atenolol in the sodium-depleted and sodium-replete states.

**Statistical Analysis**

Data were analyzed by ANOVA for a 2-by-2 crossover design. The model included treatment, sequence, and period as fixed effects and subject nested within sequence as the random effect. No carryover or sequence effects were detected for any of the results. We therefore report only the effects of SR versus SD status and treatment.

Regression was estimated by the least-squares method, with log-transformed variables. A multiple regression analysis based on both linear mixed and fixed-effect model approaches was used to estimate the proportion of the variance of AUC\textsubscript{0-24} for changes in PRC (AUC\textsubscript{0-24} PRC, log-transformed) explained by basal PRC and the AUC\textsubscript{0-24} of plasma drug concentration. SAS software V9.1 (SAS Institute Inc, Cary, NC) was used for statistical analysis. Data are expressed as geometric means with 95% CI for non-normal data and as means±SD for normally distributed data. Changes from baseline are expressed as median (interquartile range). P values <0.05 were considered significant.

**Results**

We observed the expected differences in body weight and plasma protein, creatinine and renin concentrations, and hematocrit between the 2 sodium diets (Table S1 in the online-only Data Supplement).

**Effects of the Contrasted Sodium Balance States on Study Drug Pharmacokinetics**

The contrasted sodium balance states influenced the pharmacokinetics of the 4 study drugs in different ways (Table 1 and Figure 1).

**Atenolol and Candesartan Pharmacokinetics**

The C\textsubscript{max}, AUC\textsubscript{0-24}, and AUC\textsubscript{0-∞} for atenolol were ≈30% lower during the SR period than during the SD period (P<0.0001 and the t\textsubscript{max} occurred earlier (P=0.03). The 48-hour cumulative urinary excretion of atenolol was 5.3 mg (interquartile range, 3.0–9.6) lower during SR than during SD (P=0.0002; Table 1). Sodium status had no significant effect on t\textsubscript{1/2} for atenolol in plasma. Bioequivalence was not achieved for the SR and SD study periods for atenolol, as shown by the ratios of AUC\textsubscript{0-24} (0.75; [90% CI, 0.69–0.81]) and the C\textsubscript{max} between the SR and SD periods were within the 0.80 to 1.25 range.

The C\textsubscript{max}, AUC\textsubscript{0-48}, and AUC\textsubscript{0-∞} for candesartan were ≈30% lower during the SR period than during the SD period.
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But the tmax did not change. The 48-hour cumulative urine excretion of candesartan was slightly lower during SR than during SD, but the difference between the 2 periods was not statistically significant (Table 1). Sodium status had no significant effect on t1/2 for candesartan in plasma. Bioequivalence was not achieved for the SR and SD study periods for candesartan, as shown by the ratios of AUC0–∞ (0.74; [90% CI, 0.66–0.82]) and the ratios of Cmax (0.69; [90% CI, 0.54–0.88]).

Valsartan and Ramiprilat Pharmacokinetics

By contrast, sodium status had no significant effect on the kinetics of valsartan or ramiprilat (Table 1 and Figure 1). Bioequivalence was achieved for ramipril (ratio of AUC0–∞ for ramiprilat: 0.98; [90% CI, 0.89–1.06] and ratio of Cmax: 1.02; [90% CI, 0.86–1.21]). Bioequivalence was not achieved for valsartan (ratio of AUC0–∞: 1.10; [90% CI, 0.85–1.41] and ratio of Cmax: 1.06; [90% CI, 0.79–1.43]), because of a high level of within-subject variability in valsartan plasma concentrations between the 2 study periods.

PRC for Candesartan Cilexetil, Valsartan, and Ramipril

Baseline PRC was significantly lower after SR than after SD (Table 2). As expected, candesartan cilexetil, valsartan, and ramipril increased PRC during the SR period, but this increase was 10 times greater during the SD period (Table 2 and Figure

Table 1.  Pharmacokinetic Parameters Measured During Sodium Depletion and Repletion

<table>
<thead>
<tr>
<th>Drug</th>
<th>tmax, h</th>
<th>Cmax, ng/mL</th>
<th>t1/2, h</th>
<th>AUC0–48, ng·h/mL</th>
<th>AUC0–∞, ng·h/mL</th>
<th>48-Hour Cumulative Urinary Excretion, mg/48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol (n=16)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sodium repletion</td>
<td>2.09±0.90*</td>
<td>283±81*</td>
<td>10±3</td>
<td>2356±496†</td>
<td>2428±473†</td>
<td>24.1 [20.5; 27.9]†</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>2.95±1.40</td>
<td>350±87</td>
<td>10±2</td>
<td>3153±564</td>
<td>3235±557</td>
<td>30.4 [27.9; 34.5]†</td>
</tr>
<tr>
<td>Candesartan (n=16)</td>
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</tr>
<tr>
<td>Sodium repletion</td>
<td>4.19±1.72</td>
<td>45±25*</td>
<td>14±4</td>
<td>481±222†</td>
<td>512±230†</td>
<td>0.27 [0.20; 0.42]†</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>4.63±2.03</td>
<td>62±31</td>
<td>12±3</td>
<td>640±237</td>
<td>672±245</td>
<td>0.33 [0.26; 0.45]†</td>
</tr>
<tr>
<td>Valsartan (n=16)</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Sodium repletion</td>
<td>2.81±1.33</td>
<td>2431±1583</td>
<td>9±3</td>
<td>17205±9803</td>
<td>17480±9886</td>
<td>8.9 [7.2; 11.6]†</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>2.69±1.08</td>
<td>2018±777</td>
<td>9±3</td>
<td>14727±5330</td>
<td>14932±5353</td>
<td>8.1 [6.3; 11.1]†</td>
</tr>
<tr>
<td>Ramiprilat (n=16)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Sodium repletion</td>
<td>1.75±0.45</td>
<td>27±23</td>
<td>32±13</td>
<td>167±80</td>
<td>215±94</td>
<td>1.0 [0.7; 1.1]†</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>2.00±0.89</td>
<td>24±21</td>
<td>32±8</td>
<td>162±60</td>
<td>211±64</td>
<td>0.9 [0.8; 1.2]†</td>
</tr>
</tbody>
</table>

Data are means±SD or medians (interquartile range). AUC0–48 indicates area under the curve from time 0 to the last time point; AUC0–∞, area under the curve extrapolated to infinity; Cmax, peak plasma concentration; t1/2, plasma terminal half-life; and tmax, time to peak concentration.

*P<0.05, †P<0.001 for sodium repletion vs sodium depletion.

Figure 1. Time course of changes in plasma drug concentration after a single oral dose of 8 mg candesartan cilexetil, 50 mg atenolol, 160 mg valsartan, and 10 mg ramipril in sodium-replete (closed circles) and sodium-depleted (open circles) conditions, in healthy normotensive subjects (data are mean±SD, n=16 subjects per group).
S1 in the online-only Data Supplement). The AUC\(_{0-24}\) of ∆PRC was strongly and significantly correlated with baseline PRC (Figure 2).

**Pharmacokinetic–Pharmacodynamic Correlations**

During the SR period, the AUC\(_{0-24}\) ∆PRC was not correlated with the corresponding AUC\(_{0-24}\) of plasma drug concentration, regardless of the RAS blocker considered (not shown). In the multivariate regression analysis, the AUC\(_{0-24}\) of plasma drug concentration during the SR period did not explain the variance of the AUC\(_{0-24}\) ∆PRC for candesartan (<1%; \(P=0.8882\)), ramipril (6%; \(P=0.2988\)), or valsartan (1%; \(P=0.4312\)), when baseline PRC was taken into account. By contrast, during the SD period, the AUC\(_{0-24}\) ∆PRC was significantly correlated with the AUC\(_{0-24}\) of plasma drug concentration for candesartan (\(r=0.50\); \(P=0.049\), not shown) and ramiprilat (\(r=0.53\); \(P=0.2988\)), or valsartan (1%; \(P=0.4312\)), when baseline PRC was taken into account. In contrast, during the SD period, the AUC\(_{0-24}\) ∆PRC was significantly correlated with the AUC\(_{0-24}\) of plasma drug concentration for candesartan (\(r=0.50\); \(P=0.049\), not shown) and ramiprilat (\(r=0.53\); \(P=0.037\), not shown), but not for valsartan (\(r=0.28\); \(P=0.48\), not shown). In multivariate regression analysis, the AUC\(_{0-24}\) of plasma drug concentration during the SD period explained 21% of the variance of the AUC\(_{0-24}\) ∆PRC for candesartan (\(P=0.0368\)), but only 9% of the variance for ramiprilat (\(P=0.0189\)) and 6% for valsartan (\(P=0.1712\)), when baseline PRC was taken into account.

**Table 2. Plasma Renin Concentration Measured After Renin–Angiotensin System Blocker Intake, in Conditions of Sodium Depletion and Repletion**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Baseline PRC, pg/mL</th>
<th>Peak PRC, pg/mL</th>
<th>AUC(_{0-24}) ∆PRC, pg·h/mL</th>
<th>AUC(_{0-48}) ∆PRC, pg·h/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan cilexetyl (8 mg, n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium repletion</td>
<td>15.7 (12.6–19.4)*</td>
<td>48 (37–61)*</td>
<td>320 (199–513)*</td>
<td>735 (473–1141)*</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>30.7 (24.2–38.9)</td>
<td>315 (242–409)</td>
<td>370 (231–485)</td>
<td>770 (578–923)</td>
</tr>
<tr>
<td>Valsartan (100 mg, n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium repletion</td>
<td>12.7 (9.9–16.3)*</td>
<td>81 (54–120)*</td>
<td>762 (496–1172)*</td>
<td>963 (446–2076)*</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>30.7 (24.2–38.9)</td>
<td>468 (322–680)</td>
<td>5587 (3700–4836)</td>
<td>9319 (6162–14092)</td>
</tr>
<tr>
<td>Ramipril (10 mg, n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium repletion</td>
<td>16.0 (15.0–23.0)*</td>
<td>77 (50–118)*</td>
<td>478 (275–833)*</td>
<td>679 (382–1208)*</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>42.5 (34.1–52.9)</td>
<td>420 (297–594)</td>
<td>3855 (2881–5158)</td>
<td>6762 (5227–8749)</td>
</tr>
</tbody>
</table>

Data are geometric means (95% CI). AUC indicates area under the curve; and PRC, plasma renin concentration.

*\(P<0.001\) for sodium repletion vs sodium depletion.

**PR Interval and HR for Atenolol**

Baseline PR interval was significantly longer during SR than during SD (181±27 ms versus 173±25 ms; \(P=0.014\)). The time course of changes in baseline-adjusted PR interval (APR) after atenolol intake differed significantly between the SR and SD periods (\(P=0.0065\); Figure 3). During the SR period, the PR interval did not change for up to 4 hours after atenolol intake, following its circadian rhythm thereafter. During the SD period, the PR interval increased significantly, reaching a plateau at about +10 ms above baseline from 1 to 4 hours after drug intake (Figure 3). It decreased thereafter, reaching baseline values 9 hours after drug intake and subsequently remaining stable.

Baseline HR was significantly lower during the SR period than during the SD period (57±7 versus 61±10 bpm, respectively; \(P=0.04\)). As expected, 50 mg atenolol significantly decreased HR for up to 3 hours after intake: the peak decrease in HR was −7 [−3 to −13] bpm during the SR period (\(P<0.001\)) and −8 [−6 to −15] bpm during the SD period (\(P<0.001\); Figure 3). The atenolol-induced decrease in HR was not influenced by sodium status (\(P=0.47\); Figure 3). HR returned to baseline 4 hours after intake and followed its circadian rhythm thereafter.

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**Figure 2.** Correlation between the area under the curve (AUC\(_{0-24}\)) of absolute changes in plasma renin concentration (PRC; AUC\(_{0-24}\) ∆PRC, log-transformed) and baseline PRC (log-transformed) in the 3 groups of normotensive subjects receiving a renin–angiotensin system (RAS) blocker (sodium repletion [SR]: closed circles and sodium depletion [SD]: open circles). Insets show the \(r\) and the \(P\) values (n=16 subjects per group).
Safety Parameters
There was no safety issue during the study (see BP and plasma creatinine safety data in the online-only Data Supplement).

Discussion
We found that a high-sodium diet was associated with a $\approx 30\%$ decrease in exposure to a single oral dose of 8 mg candesartan cilexetil or of 50 mg atenolol in healthy male subjects, as shown by the AUCs and $C_{\text{max}}$ values obtained, which were lower than those for SD conditions. No such decrease was observed with ramipril or valsartan. Bioequivalence between the SR and SD states was not met for candesartan cilexetil or atenolol. The plasma half-life of the tested drugs was not modified by dietary sodium intake, suggesting that dietary salt affects the presystemic disposition of candesartan cilexetil and atenolol, rather than their elimination by the liver or the kidney. This conclusion is supported by the lower levels of atenolol recovery in urine during SR. These results are consistent with previous findings showing that a high-sodium diet results in lower plasma concentrations of orally administered verapamil and quinidine than a low-sodium diet. This study extends these findings to some of the drugs interfering directly with the RAS, for which the interaction with dietary sodium intake is critical and provides new insight in the putative mechanisms involved in dietary salt–dependent changes in drug absorption.

Pharmacokinetic Consequences of Differences in Dietary Sodium Intake
Atenolol has a bioavailability of $\approx 60\%$, and it is often used as a marker of flux-mediated paracellular transport in intestinal cellular cultures. The $\approx 30\%$ lower plasma atenolol AUCs in SR than in SD conditions were associated with a shorter $t_{\text{max}}$ and a proportional decrease in 48-hour urinary excretion of atenolol. Atenolol clearance is exclusively renal, so decreases in urinary excretion reflect a decrease in the net intestinal absorption of the drug in these healthy subjects with normal renal function. The differences in passive atenolol transport across the intestinal epithelium may result from differences in transcellular intestinal sodium and glucose reabsorption, attributable to differences in sympathetic nervous system activity during SR/SD. Indeed, SD was associated with signs of sympathetic nervous system activation, including a significant increase in HR and shortening of the PR interval at baseline. Other mechanisms may include changes in the pH of the upper intestinal tract, resulting in changes in the ionization of atenolol, or dietary salt–induced changes in transmucosal water flux.

Candesartan cilexetil is an inactive racemic ester prodrug that is rapidly and completely converted to its active metabolite, candesartan, by presystemic ester hydrolysis of the ester side chain in the gastrointestinal tract. The 30% to 40% lower AUC$_{\text{ss}}$ of candesartan observed in conditions of SR suggest that other mechanisms are involved in this pharmacokinetic interaction because the absorption process for this drug is different from that for atenolol. In the Caco-2 cell model, candesartan cilexetil not only is absorbed by passive diffusion across the membrane but also is a substrate of the energy-dependent intestinal efflux transporter P-glycoprotein (Pg-P) and a Pg-P inhibitor. The absorption of candesartan cilexetil is enhanced by Pg-P inhibitors, such as cyclosporine A and verapamil. We suggest that the main mechanism involved in the decrease in candesartan bioavailability with SR may be the modulation of P-g P activity or expression by SR/SD state, as suggested for verapamil and quinidine, which are both Pg-P and cytochrome P 3A4 (CYP3A4) substrates. A high-sodium diet decreases the plasma concentration of oral quinidine by $\approx 20\%$ and that of oral verapamil by $\approx 60\%$. Interestingly, in 2 separate experiments, we had already observed a similar phenomenon with the direct renin inhibitor aliskiren, which is also a Pg-P substrate.

The precise mechanism responsible for the pharmacokinetic interaction with dietary sodium remains hypothetical and is probably multifactorial, including salt-induced changes to sympathetic gut function or changes to the activity or expression of drug transporters (eg, Pg-P) or CYP3A4 in the intestinal mucosa. Along this hypothesis, Eap et al have shown the impact of salt intake on the gene expression of drugs transporters in humans.

By contrast, dietary sodium content did not influence the bioavailability of valsartan or ramipril, which are not substrates of Pg-P or CYP3A4. The availability of oral valsartan, a readily active carboxylic acid derivative, was not significantly affected by dietary sodium intake, even though the 2 diets could not be considered bioequivalent because of the high within-subject variability of valsartan pharmacokinetics, consistent with previous findings.

Ramipril was the only drug for which bioequivalence was strictly achieved between the 2 diets. It is a monoester carboxylic prodrug with a bioavailability of 50% to 60% extensively and rapidly metabolized to its active diacid derivative, ramiprilat, mainly in the liver. The mechanism by which ramipril and other angiotensin-converting enzyme inhibitors are transported through the gastrointestinal tract remains a matter of debate. Whatever the mechanism, the final conversion of ramipril into its active metabolite is not influenced by dietary salt intake.

Figure 3. Changes in PR interval ($\Delta$PR; left) and in heart rate (right) after a single oral dose of 50 mg atenolol in sodium-replete (closed circles) and sodium-depleted (open circles) conditions, in healthy normotensive subjects (data are means±SD, n=16 subjects per group). HR indicates heart rate.
Pharmacodynamic Consequences of Changes in Drug Exposure With Sodium Depletion

We studied the increase in PRC to analyze the dependence on dietary sodium intake of the pharmacodynamic effects of the 3 RAS blockers and the PR interval for atenolol.

An increase in PRC is the most straightforward and sensitive marker for monitoring time-dependent RAS blockade after drug intake attributable to the interruption of the angiotensin II negative feedback loop on renin release. As expected, SD resulted in a much larger RAS blocker--induced increase in PRC than SR, mostly for pharmacodynamic reasons and also partly for pharmacokinetic reasons. First, the main determinant of the increase in PRC induced by RAS blockers is the basal activity of the RAS in response to dietary sodium intake, as shown by the strong correlations between the AUC\textsubscript{0-24} ΔPRC and basal PRC. Second, the AUC\textsubscript{0-24} ΔPRC was significantly correlated with the corresponding AUC\textsubscript{0-24} of 2 among 3 RAS blockers in the SD state, demonstrating the relationship between drug pharmacokinetic variability and variability in the pharmacodynamic response. The close correlation between plasma drug concentration and renin release in the SD state indicates a higher plasma candesartan concentration contributed to the 21% increase in PRC. No such relationship was observed in conditions of SR, in which the basal activity of the RAS is blunted and the bioavailability of candesartan is lower. Finally, the moderately larger decrease in BP (not shown) may also have per se participated to the larger increase in renin release after the intake of each RAS blocker during SD.

The ~30% decrease in exposure to atenolol with SR also has pharmacodynamic consequences, highlighting the influence of sodium balance. SR was associated with a complete reversal of the effect of atenolol on the lengthening of the PR interval observed during SD, consistent with reported findings for verapamil. The atenolol-induced decrease in HR was not influenced by SR/SD, but this pharmacodynamic marker is less sensitive than changes in PR interval.

Study Limitations

The principal limitations of this study were the population studied (normotensive healthy subjects), the short treatment period (single oral dosing), and the experimental SR/SD conditions. Indeed, these investigations of single-dose administration in normotensive individuals were performed to rapidly show differences in the pharmacokinetics of various RAS blockers with SD and SR as previously observed for aliskiren and other compounds not interfering with the RAS (verapamil and quinidine). We thus used similar experimental conditions, including fasting, however, less extreme, than those used to report for the first time the impact of salt intake on the pharmacokinetics of verapamil and quinidine. Indeed, verapamil or quinidine disposition was assessed after 7 days of a low-salt diet (10 mEq/d) and then after 7 days of a high-salt diet (400 mEq/d) in these studies.

The use of these experimental conditions in our study allowed proving the concept for the first time for candesartan cilexetil and atenolol. We did not assess the impact of food intake or of standard Western sodium intake of =12 g/d, which also may influence the results. However, even though most adult populations have mean sodium intakes >100 mmol/d, still for many, mean intakes are >200 mmol/d (reaching up to 272 mmol sodium/d in China). In populations with such high sodium intake, our findings may have a clinical impact which deserves to be further evaluated.

Perspectives

In strict experimental conditions, we found that contrasting sodium states affected the pharmacokinetics of 2 of the 4 cardiovascular drugs tested and had a direct, but modest, influence on their pharmacodynamic effects. We suggest that these effects may result from the modulation of Pg-P activity or expression by dietary salt, but further investigations of the mechanisms involved are required.

The next question to be addressed is whether these effects will be observed and have pharmacodynamic and clinically meaningful consequences during repeated dosing in patients with hypertension, especially resistant hypertension, or in conditions associated with altered sodium balance. Pharmacokinetic studies are therefore required in conditions associated with physiological (salt diet), pathological changes in sodium balance, with different RAS blockers, after multiple dosing at steady state. Our results provide a possible methodology to perform such studies.

Sources of Funding

This study was funded by a national grant from the Program Hospitalier de Recherche Clinique Régional (AOR06052) and from the National Research Agency (ANR-05-COD). The sponsor was the Assistance Publique des Hôpitaux de Paris. The study was also supported by the Institut National de la Santé et de la Recherche Médicale and the Assistance Publique - Hôpitaux de Paris at the Clinical Investigation Center of Hôpital Européen Georges Pompidou (CIC-9201) and the CIC of Paris-Est Pitié-Salpêtrière University Hospitals (CIC-9304).

Disclosures

Michel Azizi has received consulting fees or grants from Novartis, Sanofi, Actelion, Bayer, Medtronic, Vessix Vascular, and Cordis. Joël Menard has received consulting fees and grants from Novartis. The other authors report no conflicts.

References

The effect of contrasted sodium diets on the pharmacokinetics of single oral doses of various renin-angiotensin system blockers were compared in healthy male subjects.

What Is Relevant?

- A high-sodium diet decreases the bioavailability of candesartan cilexetil and atenolol.
Effect of Contrasted Sodium Diets on the Pharmacokinetics and Pharmacodynamic Effects of Renin–Angiotensin System Blockers
Michel Azizi, Anne Blanchard, Beny Charbit, Grégoire Wuerzner, Séverine Peyrard, Eric Ezan, Christian Funck-Brentano and Joël Ménard

Hypertension. 2013;61:1239-1245; originally published online April 22, 2013;
doi: 10.1161/HYPERTENSIONAHA.113.01196

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Supplemental material

Effect of contrasted sodium diets on the pharmacokinetics and pharmacodynamic effects of renin angiotensin system blockers

Michel Azizi, Anne Blanchard, Beny Charbit, Grégoire Wuerzner, Séverine Peyrard, Eric Ezan, Christian Funck-Brentano, Joël Ménard
Supplemental Methods

Pharmacokinetic parameters

Drug assays

The plasma and urinary concentrations of each compound were measured by liquid chromatography tandem mass spectrometry. Candesartan cilexetil and ramipril are prodrugs that are converted into the active metabolites candesartan and ramiprilat, respectively. The quantification limit was 5 ng/ml for valsartan, 1 ng/ml for candesartan, 0.5 ng/ml for ramiprilat and 1 ng/ml for atenolol. Drug determinations were performed blind to the randomization sequence (sodium depletion vs. sodium repletion).

Data analysis

We determined peak plasma concentration (Cmax), time to Cmax (tmax), the plasma terminal half-life (t1/2), the area under the curve up to the last measured time point (AUC0-48) and the area under the curve extrapolated to infinity (AUC0-∞) for each individual concentration-time profile, by a non compartmental method, with WinNonlin Pro 4.0 software (Pharsight, Mountain View, California). Bioequivalence was considered to be demonstrated if the 90% confidence intervals (CI) of the ratios for AUC0-∞ and the Cmax between the sodium repletion and sodium depletion periods were within the 0.80 to 1.25 range.

Pharmacodynamic parameters

Renin assay

PRC was determined blind to the randomization sequence, by immunoradiometric assay (Cisbio International, F91192 Gif-sur-Yvette, France), as previously described.

ECG measurements

In the atenolol group, 10-second digital electrocardiograms (ECGs) were recorded at rest with a Cardioplug digital ECG recorder (Cardionics Inc., Brussels, Belgium), before and at various times up to 24 h after drug intake. All digital recordings were analyzed at the end of the study blind to the randomization sequence. Heart rate (HR) was determined by averaging RR intervals during 10 seconds recording. For the PR interval measurements (mean of 5 measurements), the same chest lead, with the largest P-wave amplitude, was selected in a given subject for each study period. We used HR, PR interval values and PR interval prolongation (absolute change from baseline, ∆PR) to compare the pharmacodynamic effects of atenolol in the sodium-depleted and sodium-replete states.

Supplementary results

Safety parameters

Baseline systolic BP (SBP) was similar in all groups and was not influenced by dietary sodium status (not shown). A mild decrease in SBP was observed with all drugs, which was moderately larger during the sodium depletion than the sodium repletion period (not shown). In the absence of a placebo period, this effect may be related to a time effect. Baseline creatinine clearance was not influenced by dietary sodium status and none of the drugs had an impact on creatinine clearance, in either set of sodium conditions (not shown).
Supplementary Table S1: Influence of the two contrasted sodium diets on body weight, hematocrit and plasma protein, creatinine and renin concentrations in healthy normotensive subjects

<table>
<thead>
<tr>
<th>Drug</th>
<th>Body weight kg</th>
<th>Plasma proteins g/L</th>
<th>Hematocrit %</th>
<th>Plasma creatinine µmol/L</th>
<th>PRC pg/mL</th>
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<tbody>
<tr>
<td><strong>Atenolol (n=16)</strong></td>
<td></td>
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<tr>
<td>Sodium repletion</td>
<td>75.6 ± 8.8†</td>
<td>67.0 ± 3.6‡</td>
<td>42.7 ± 2.7*</td>
<td>81.6 ± 7.7†</td>
<td>13.0 (9.2; 18.3‡)</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>74.3 ± 8.6</td>
<td>74.7 ± 3.7</td>
<td>44.7 ± 3.5</td>
<td>87.6 ± 9.2</td>
<td>35.4 (26.7; 47.0)</td>
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<td><strong>Candesartan cilexetyl (n=16)</strong></td>
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<tr>
<td>Sodium repletion</td>
<td>71.3 ± 8.8†</td>
<td>63.9 ± 3.9‡</td>
<td>40.8 ± 1.8‡</td>
<td>77.9 ± 9.3‡</td>
<td>15.7 (12.6; 19.4)‡</td>
</tr>
<tr>
<td>Sodium depletion</td>
<td>70.4 ± 9.0</td>
<td>69.6 ± 3.4</td>
<td>43.2 ± 1.6</td>
<td>82.7 ± 9.2</td>
<td>30.7 (24.2; 38.9)</td>
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<tr>
<td><strong>Valsartan (n=16)</strong></td>
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<tr>
<td>Sodium repletion</td>
<td>73.4 ± 6.1†</td>
<td>64.8 ± 3.1‡</td>
<td>40.9 ± 2.1†</td>
<td>76.1 ± 7.6†</td>
<td>12.7 (9.9; 16.3)‡</td>
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<tr>
<td>Sodium depletion</td>
<td>72.3 ± 6.1</td>
<td>71.6 ± 5.1</td>
<td>43.3 ± 2.8</td>
<td>80.7 ± 9.6</td>
<td>30.7 (24.2; 38.9)</td>
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<tr>
<td><strong>Ramipril (n=16)</strong></td>
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<tr>
<td>Sodium repletion</td>
<td>72.1 ± 8.7‡</td>
<td>64.4 ± 3.7‡</td>
<td>41.2 ± 2.0‡</td>
<td>74.8 ± 7.2‡</td>
<td>18.6 (15.0; 23.0)‡</td>
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<tr>
<td>Sodium depletion</td>
<td>70.5 ± 8.4</td>
<td>71.6 ± 4.3</td>
<td>44.7 ± 1.9</td>
<td>82.0 ± 7.5</td>
<td>42.5 (34.12; 52.9)</td>
</tr>
</tbody>
</table>

PRC: plasma renin concentration. Data are means ± SD or geometric means (95% CI).

*p<0.05, † p<0.01, ‡ p<0.001 for sodium repletion vs sodium depletion.
Supplementary Figure S1: Time course of changes in plasma renin concentration (PRC) after a single oral dose of 8 mg candesartan cilexetil, 160 mg valsartan and 10 mg ramipril in sodium-replete (closed circles) and sodium-depleted (open circles) conditions, in healthy normotensive subjects (data are geometric mean, 95% confidence interval).