Effects of Sacubitril/Valsartan (LCZ696) on Natriuresis, Diuresis, Blood Pressures, and NT-proBNP in Salt-Sensitive Hypertension

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Abstract—Salt-sensitive hypertension (SSH) is characterized by impaired sodium excretion and subnormal vasodilatory response to salt loading. Sacubitril/valsartan (LCZ696) was hypothesized to increase natriuresis and diuresis and result in superior blood pressure control compared with valsartan in Asian patients with SSH. In this randomized, double-blind, crossover study, 72 patients with SSH received sacubitril/valsartan 400 mg and valsartan 320 mg once daily for 4 weeks each. SSH was diagnosed if the mean arterial pressure increased by ≥10% when patients switched from low (50 mmol/d) to high (320 mmol/d) sodium diet. The primary outcome was cumulative 6- and 24-hour sodium excretion after first dose administration. Compared with valsartan, sacubitril/valsartan was associated with a significant increase in natriuresis (adjusted treatment difference: 24.5 mmol/6 hours, 50.3 mmol/24 hours, both P<0.001) and diuresis (adjusted treatment difference: 291.2 mL/6 hours, P<0.001; 356.4 mL/24 hours, P=0.002) on day 1, but not on day 28, and greater reductions in office and ambulatory blood pressure on day 28. Despite morning dosing of both drugs, ambulatory blood pressure reductions were more pronounced at nighttime than at daytime or the 24-hour average. Compared with valsartan, sacubitril/valsartan significantly reduced N-terminal pro B-type natriuretic peptide levels on day 28 (adjusted treatment difference: −20%; P=0.001). Sacubitril/valsartan and valsartan were safe and well tolerated with no significant changes in body weight or serum sodium and potassium levels with either treatments. In conclusion, sacubitril/valsartan compared with valsartan was associated with short-term increases in natriuresis and diuresis, superior office and ambulatory blood pressure control, and significantly reduced N-terminal pro B-type natriuretic peptide levels in Asian patients with SSH.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01681576.

Clinical Trials

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alt-sensitive hypertension (SSH) is characterized by an increase in blood pressure (BP) in response to sodium load and is associated with an increased risk of cardiovascular events.1–3 Owing to high dietary salt intake and genetic predisposition, salt sensitivity of BP is of particular clinical relevance in Asian populations.4–6 Considering its pathophysiology, thiazide-type diuretics and hypokalemia caused by thiazide-type diuretics often contribute to morbidity and mortality, if left unrecognized. Hence, antihypertensive agents that improve urinary sodium excretion without altering electrolyte concentrations would be a better therapeutic option for patients with SSH. Among multiple pathophysiological mechanisms, a relative deficiency of atrial natriuretic peptide (ANP) is considered to result in sodium retention and a suboptimal vasodilatory response to sodium loading observed in patients with SSH.12–15 The renin–angiotensin–aldosterone system (RAAS) also plays a role in the pathogenesis of SSH by modulating renal sodium reabsorption.16 Therefore, enhancing the activity of natriuretic peptides along with inhibition of the RAAS may be an effective,
pathogenesis-oriented, and safe alternative to improve BP control in patients with SSH.17

Sacubitril/valsartan (LCZ696) is a first-in-class angiotensin receptor neprilysin inhibitor approved for the treatment of heart failure and reduced ejection fraction.18 After oral administration, sacubitril/valsartan delivers systemic exposure to sacubitril (AHU377), a neprilysin inhibitor prodrug, and valsartan, an angiotensin receptor blocker. Sacubitril is rapidly metabolized into the active neprilysin inhibitor sacubitrilat (LBQ657).19 Sacubitril/valsartan provides simultaneous blockade, thereby facilitating beneficial effects of natriuretic peptides and other neprilysin substrates while inhibiting detrimental effects of the RAAS.20

Previous studies have shown significant reductions in office and ambulatory BP with sacubitril/valsartan compared with valsartan alone or placebo in patients with mild-to-moderate hypertension.17,21 Based on its mechanisms of action, sacubitril/valsartan is expected to counteract sodium and volume retention and improve vasodilatory responses, which would be beneficial in patients with SSH. The present study was conducted to evaluate the effects of sacubitril/valsartan compared with valsartan alone on natriuresis, diuresis, serum sodium and potassium levels, office and ambulatory BP, and biomarkers in Asian patients with SSH.

Methods

Methods and statistical analysis are briefly summarized below. Further details are provided in the online-only Data Supplement.

This was a multicenter, randomized, double-blind, crossover study (Clinical Trial registry NCT01681576) in patients with SSH and ≥18 years of age. The study consisted of 3 periods: a screening/washout period, a diagnostic qualification period, and a double-blind crossover treatment period (Figure 1A). The study population included patients aged ≥18 years with hypertension, either untreated (newly diagnosed patients or those who had not been taking any antihypertensive drug for ≥4 weeks before screening) or treated with antihypertensive drugs for ≥4 weeks before screening, and mean sitting systolic BP (SBP) either ≥140 to <180 mm Hg at screening (untreated patients only) or ≥120 to ≤180 mm Hg at screening and ≤180 mm Hg at the end of the washout period (treated patients only). SSH was confirmed in the diagnostic qualification period by an increase in the mean arterial pressure (MAP) by ≥10% when switching from a 7-day, low-sodium (<50 mmol/d) to high-sodium (320 mmol/d) diet.22 Dietary compliance was measured by 24-hour urine sodium excretion, targeting a sodium excretion of ≤100 mmol/d for a low-sodium diet and ≥200 mmol/d for a high-sodium diet.

Patients then switched to a normal sodium diet (150–200 mmol/d) from the beginning of a 1- to 2-week run-in period before the first crossover treatment period and maintained the sodium intake throughout the remainder of the study period. Compliance was supported with regular dietary counseling and confirmation of a stable 24-hour urinary sodium excretion before the start of each treatment period (140.5 mmol/24 hours at baseline of period 1; 152.7 mmol/24 hours at baseline of period 2; and P=0.10 for the adjusted baseline difference between periods).

During the double-blind treatment period, patients were randomized (1:1) to receive either sacubitril/valsartan 400 mg or valsartan 320 mg once daily in the morning for 4 weeks, followed by a washout period of 1 to 2 weeks. Patients were then crossed over to receive the other treatment.

Changes in the effects of sacubitril/valsartan versus valsartan on cumulative sodium excretion (natriuresis) were measured at 6 and 24 hours after the first dose administration (primary outcome measures). In addition, effects on natriuresis at 6 and 24 hours after dosing on day 28 and cumulative urine excretion (diuresis) at 6 and 24 hours after dosing on days 1 and 28 were evaluated.

Triplicate seated office BP (each at a 2-minute interval) and pulse rates were recorded at baseline, day 14, and day 28 of each treatment period using an automated calibrated BP device (Omron Healthcare; centrally provided to all study centers by CoreLab Partners Inc) by the study nurse. All measurements were made in the presence of the study nurse or designated study personnel and at approximately the same time of the day before dosing with the study drug, after 15 minutes of rest in a seated position with the back supported and both feet on the floor. The resting period was implemented to minimize confounding factors to the greatest extent.

Furthermore, for 24-hour ambulatory BP monitoring, a calibrated ambulatory BP monitoring device (Omron Healthcare; centrally provided to all study centers and centrally read by CoreLab Partners Inc.) was attached to the nondominant arm of the patient. Daytime and nighttime were defined as the periods from 9 AM to 9 PM and 1 AM to 6 AM, respectively. Dipping status was defined as dipper when the reduction in nighttime ambulatory SBP and diastolic BP (DBP) was >10% compared with that in daytime values and as nondipper when the reduction in nighttime ambulatory SBP or DBP was ≤10% compared with daytime values.23

Statistical Analysis

Enrolling 140 patients into the diagnostic qualification period was hypothesized to lead to 56 patients with SSH completing the study, assuming a prevalence of salt sensitivity of 50% in the study population and a 20% drop-out rate. This sample size was targeted to provide an 80% statistical power to detect a treatment difference of ≥30 mmol/24 hours in urine sodium excretion at a 2-sided significance level of 2.5%, accounting for multiplicity (one prespecified interim analysis for the primary outcome measure with an option to prematurely end the study).

In addition, the aforementioned sample size was adequate to detect a 26 mm Hg and ≥5 mm Hg treatment differences in change from baseline in mean sitting SBP and mDBP, respectively (for secondary outcome measures). This calculation was based on an assumed intrapatient standard deviation (SD) of 50 mmol/24 hours for urine sodium excretion and an SD of 10 and 8 mm Hg for SBP and DBP, respectively. The change from baseline in seated office BP was analyzed for each day by using a mixed-effects model with sequence, period, and treatment as fixed factors and patient as the random factor. The 24-hour ambulatory BP monitoring data were analyzed using a repeated-measures analysis of covariance model with sequence, period, treatment, hours post dose, and treatment by hours post dose interaction as fixed factors; patient as the random factor; and baseline as the covariate. The point estimate and 97.5% confidence interval (CI) for the difference along with the P value for equality of the 2 treatments were reported for the purpose of 2-sided hypothesis of equality. The significance level of 2.5% was selected to account for multiplicity because of the interim analysis.

For plasma and urinary biomarker data, log-transformed analysis was conducted using a mixed-effects model, with sequence, period, and treatment as fixed factors and patient as the random effects for each day and time interval. BP covariate analysis for N-terminal pro B-type natriuretic peptide (NT-proBNP) was conducted using change from baseline NT-proBNP as the random effect. The adjusted difference in the mean values between treatments was presented along with respective 95% CIs. The number and proportion of patients with adverse events (AEs) were listed by preferred term.

Results

Patient Disposition and Demographics

Of the 209 patients who entered the diagnostic qualification period, 74 (35.4%) fulfilled the SSH criteria; of these, 72 entered the double-blind crossover treatment period, and...
65 (90.3%) completed the study. Reasons for discontinuation were AEs (hypersensitivity), noncompliance with study treatment, protocol deviation in one patient each, and withdrawal of consent in 4 patients (Figure 1B). The mean office BP at baseline was 147/90 mm Hg, and the mean MAP increased by ≈16% from 98 to 114 mm Hg when patients switched from a low- to a high-sodium diet (Table). Other baseline characteristics are summarized in Table.

Pharmacodynamic Assessments

Natriuresis

On day 1, treatment with sacubitril/valsartan but not valsartan resulted in a significant increase in 6-hour natriuresis (from 38.4 mmol at baseline to 61.3 mmol on day 1) and in 24-hour natriuresis (from 148.8 mmol at baseline to 188.9 mmol on day 1). The adjusted difference between sacubitril/valsartan and valsartan treatments was 24.5 mmol for 6-hour natriuresis.
and 50.3 mmol for 24-hour natriuresis (P<0.001 for both time points; Figure 2). Thus, the primary end point of the study was met. The relative contribution of 6-hour natriuresis to 24-hour natriuresis after sacubitril/valsartan administration was greater on day 1 compared with that on baseline.

On day 28, both 6- and 24-hour natriuresis after sacubitril/valsartan treatment were comparable with those at baseline. Although 6-hour natriuresis on day 28 after valsartan treatment was higher compared with that on baseline (P=0.002), the adjusted treatment differences between sacubitril/valsartan and valsartan in 6- and 24-hour natriuresis on day 28 were not statistically significant. No significant changes in serum sodium and potassium levels were observed with either treatment on day 28 (Table S1 in the online-only Data Supplement).

**Diuresis**

On day 1, treatment with sacubitril/valsartan but not valsartan resulted in a significant increase from baseline in 6-hour diuresis (from 855.1 mL at baseline to 1215.9 mL) and in 24-hour diuresis (from 2752.8 mL at baseline to 3172.3 mL). The adjusted difference between sacubitril/valsartan and valsartan treatments was 291.2 mL for 6-hour diuresis (P<0.001) and 356.4 mL for 24-hour diuresis (P=0.002; Figure 2).

On day 28, both 6- and 24-hour diuresis after sacubitril/valsartan treatment were comparable with those at baseline. Similar to natriuresis, 6-hour diuresis with valsartan was significantly greater compared with that at baseline (P<0.001). However, the adjusted treatment differences between sacubitril/valsartan and valsartan in 6- and 24-hour diuresis on day 28 were not statistically significant.

**Blood Pressures**

Treatment with sacubitril/valsartan and valsartan for 4 weeks resulted in greater reductions from baseline in office BP with sacubitril/valsartan than with valsartan (Figure 3; Figure S1). The reduction from baseline in office SBP was significantly greater with sacubitril/valsartan (13.3 mm Hg) than with valsartan (5.8 mm Hg), with an adjusted mean between-treatment difference of −7.6 mm Hg (P=0.002). The reduction from baseline in office DBP was −6.2 mm Hg with sacubitril/valsartan (P<0.0001) and −4.2 mm Hg with valsartan (P=0.0002). However, the adjusted mean between-treatment difference of −2.0 mm Hg was not statistically significant.

Baseline ambulatory BP values were comparable between sacubitril/valsartan and valsartan (SBP/DBP: 139.2/86.2 mm Hg versus 135.6/84.7 mm Hg, 128.8/79.1 mm Hg versus 127.7/78.7 mm Hg, and 136.3/84.5 mm Hg versus 133.7/83.5 mm Hg for daytime, nighttime, and 24 hours, respectively). On day 28, sacubitril/valsartan resulted in significantly greater decreases in ambulatory BP values compared with valsartan (Figure 4; Figure S1). The adjusted mean treatment differences between sacubitril/valsartan and valsartan were statistically significant for reductions in daytime, nighttime, and 24-hour ambulatory SBP, DBP, MAP, and pulse pressure. The adjusted treatment differences for ambulatory BP monitoring were −4.9 (P<0.001), −4.7 (P<0.001), and −4.0 (P<0.001) mm Hg for daytime, nighttime, and 24-hour SBP, respectively. The corresponding differences were −2.4 (P=0.012), −2.6 (P=0.008), and −1.7 (P<0.001) for daytime, nighttime, and 24-hour DBP, respectively; −3.5 (P=0.001), −3.8 (P<0.001), and −2.8 (P<0.001) for daytime, nighttime, and 24-hour MAP, respectively; and −2.8 (P<0.001), −2.2 (P=0.006), and −2.2 (P<0.001) for daytime, nighttime, and 24-hour pulse pressure, respectively.

Both sacubitril/valsartan and valsartan achieved greater ambulatory BP reductions from baseline at nighttime than at daytime, despite dosing in the morning (Figure 4; Figure S1). Although the diurnal BP reduction patterns were comparable, the absolute reductions in nighttime ambulatory BP values were greater with sacubitril/valsartan than with valsartan.

**Biomarkers**

Plasma NT-proBNP, urinary cyclic guanosine monophosphate (cGMP), and plasma aldosterone levels were comparable between treatments at baseline (Figure 5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sacubitril/Valsartan 400 mg QD/Valsartan 320 mg QD (N=36)</th>
<th>Valsartan 320 mg QD</th>
<th>Sacubitril/Valsartan 400 mg QD (N=36)</th>
<th>All Patients (N=72)</th>
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<tr>
<td>Age, y</td>
<td>55.7 (12.5)</td>
<td>58.9 (7.5)</td>
<td>57.3 (10.3)</td>
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<td>Male, n (%)</td>
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<td>23 (64)</td>
<td>46 (64)</td>
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<td>Asian, n (%)</td>
<td>36 (100)</td>
<td>36 (100)</td>
<td>72 (100)</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<td>25.7 (2.9)</td>
<td>26.1 (3.4)</td>
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<tr>
<td>BP parameters, mm Hg</td>
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<td></td>
</tr>
<tr>
<td>Mean seated SBP*</td>
<td>147.0 (9.7)</td>
<td>147.5 (12.1)</td>
<td>147.3 (10.09)</td>
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</tr>
<tr>
<td>Mean seated DBP*</td>
<td>90.2 (6.9)</td>
<td>90.4 (7.2)</td>
<td>90.3 (7)</td>
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<tr>
<td>MAP with low-sodium diet</td>
<td>97.6 (7.1)</td>
<td>98.8 (8.2)</td>
<td>98.2 (7.6)</td>
<td></td>
</tr>
<tr>
<td>MAP with high-sodium diet</td>
<td>112.6 (8.4)</td>
<td>114.7 (8.6)</td>
<td>113.6 (8.5)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation) or number (percentage) as indicated. BP indicates blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; QD, once daily; and SBP, systolic blood pressure.

*SBP and DBP are taken from vital sign evaluations at screening for untreated patients and at washout for pretreated patients.
A significant reduction from baseline in NT-proBNP was observed on day 28 with sacubitril/valsartan (from 34.1 to 20.0 pg/mL; \( P < 0.001 \)) and valsartan (from 35.4 to 25.4 pg/mL; \( P < 0.001 \)). A between-treatment analysis on day 28 showed significantly lower concentrations of NT-proBNP with sacubitril/valsartan (geometric mean ratio between treatments and 95% CI; 0.80 [0.71–0.89]; \( P = 0.001 \); Figure 5A). The between-treatment analysis of NT-proBNP concentrations was repeated by exploring the effects of change from baseline in office SBP and DBP as covariates. A significant covariate effect was observed (\( P \) value for the covariate effect: SBP, \( P = 0.020 \); DBP, \( P = 0.005 \)), implying a correlation between changes in SBP/DBP and log-transformed NT-proBNP values. However, inclusion of change from baseline in office SBP and DBP as covariates in the analysis of NT-proBNP concentrations was repeated by exploring the effects of change from baseline in office SBP and DBP as covariates. A significant covariate effect was observed (\( P \) value for the covariate effect: SBP, \( P = 0.020 \); DBP, \( P = 0.005 \)), implying a correlation between changes in SBP/DBP and log-transformed NT-proBNP values. However, inclusion of change from baseline in office SBP and DBP as covariates in the analysis of NT-proBNP suggested that the greater reduction in NT-proBNP with sacubitril/valsartan than with valsartan is independent from changes in office BP (covariate-adjusted analysis of NT-proBNP after treatment with sacubitril/valsartan presented as geometric mean ratio [95% CI]; change from baseline SBP as a covariate: 0.85 [0.74–0.98], \( P = 0.022 \); change from baseline DBP as covariate: 0.82 [0.72–0.94], \( P = 0.005 \)).

On day 28, treatment with sacubitril/valsartan resulted in a statistically significant increase in urinary cGMP excretion from baseline (from an adjusted geometric mean of 465.5
nmol/24 hours to 690.9 nmol/24 hours; \( P < 0.001 \)), whereas a statistically significant decrease from an adjusted geometric mean of 483.6 nmol/24 hours to 426.0 nmol/24 hours (\( P = 0.013 \)) was observed with valsartan treatment (Figure 5B and 5C). A between-treatment comparison showed 62% (95% CI, 1.50–1.74) higher urinary cGMP excretion with sacubitril/valsartan, which was statistically significant (\( P < 0.001 \)).

Treatment with both sacubitril/valsartan and valsartan resulted in significantly decreased concentrations of plasma aldosterone by \( \approx 25\% \) (Figure 5D) with no statistically significant between-treatment differences.

Safety Results

Both sacubitril/valsartan and valsartan were generally well tolerated by patients with SSH in this study. No deaths and one serious AE of pneumonia reported in this study were not considered to be related to the study drug by the investigator. One AE of facial allergy resulted in study discontinuation, which was resolved and not considered to be related to the study drug by the investigator. Of the 72 patients, 39 (54.2%) experienced at least one treatment-emergent AE. The overall incidence of AEs was similar between sacubitril/valsartan (32.4%) and valsartan (32.8%) treatments. The most common AEs experienced by the patients were dizziness (n=9, 12.5%), hematuria (n=5, 6.9%), headache (n=5, 6.9%), nasopharyngitis (n=5, 6.9%), cough (n=4, 5.6%), and hypokalemia (n=1, 1.5% with valsartan; Table S2). No case of hyponatremia or hypernatremia was reported. The adjusted mean differences in plasma potassium and sodium levels did not vary significantly between the 2 treatments (Table S1).

Discussion

This is the first study to assess the pharmacodynamic effects of sacubitril/valsartan and valsartan in Asian patients with SSH identified through rigorous clinical testing. The study met its primary end point by showing that sacubitril/valsartan treatment resulted in a significant increase in 6- and 24-hour natriuresis after first dose administration versus valsartan. This initial natriuretic effect was accompanied with increased diuresis; both were not sustained after 4 weeks of sacubitril/
valsartan treatment and did not result in changes in serum sodium or potassium levels. Irrespective of its short-term natriuretic and diuretic effects, sacubitril/valsartan provided significant improvements in office and ambulatory BPs at 4 weeks of treatment and a significantly greater reduction in NT-proBNP levels in this patient population.

Because this is a mechanistic study to evaluate the contribution of neprilysin inhibition to the effect of sacubitril/valsartan, valsartan was selected as a comparator. This selection was further supported by the fact that sacubitril/valsartan 400 mg once daily provides bioequivalent exposure to valsartan compared with valsartan 320 mg once daily, which is also the highest approved dose of valsartan for the treatment of hypertension.

The observed short-term natriuretic and diuretic effects are consistent with those observed in previous studies that investigated the renal effects of ANP,22 the key substrate of neprilysin that has been shown to increase with sacubitril/valsartan.23 Prolonged infusion of ANP in rats was associated with increased natriuresis and diuresis during the first hour, which returned to baseline after 2 hours of infusion.25 Urinary excretion of cGMP, the second messenger of ANP, remained elevated during ANP infusion, suggesting that the diminishing natriuretic and diuretic effects in response to ANP are independent of cGMP signaling. In addition, a transient increase in natriuresis and diuresis after prolonged ANP infusion was confirmed in healthy men.26 Similar to earlier findings, the present study also showed a sustained increase in urinary cGMP excretion, which did not parallel the return to baseline of natriuresis and diuresis. Therefore, desensitization of the natriuretic peptide receptor A; decreased activity of the ANP-converting enzyme corin, which is involved in ANP release; or increased activity of phosphodiesterases involved in cGMP degradation are unlikely to explain the observed return to baseline of natriuresis and diuresis after ANP infusion or continued sacubitril/valsartan treatment because all these mechanisms would have been associated with a return to baseline levels of cGMP. A potential hypothesis is a cGMP-dependent compensatory sodium and water reabsorption involving epithelial sodium channels and aquaporin 2 in the renal medullary collecting duct after prolonged ANP exposure.27–31 These short-term natriuretic and diuretic effects are likely to prevent hypernatremia or hyponatremia and hyperkalemia or hypokalemia during long-term treatment with sacubitril/valsartan.
This favorably differentiates sacubitril/valsartan from diuretics such as thiazide diuretics that are often used to treat patients with SSH.\textsuperscript{9–11}

Another cGMP-dependent mechanism that may explain the apparent dissociation of short-term effects of sacubitril/valsartan on natriuresis and diuresis but sustained significant BP reduction is related to the vasodilatory effects of ANP.\textsuperscript{13} ANP exhibits a potent vasodilatory effect on large arteries through the natriuretic peptide A receptor/cGMP pathway in both animal models and in humans.\textsuperscript{32–34} The vasodilatory effects, along with initial natriuresis, could reset sodium and water homeostasis and prevent sustained natriuresis and diuresis. According to a previous study, heterozygous ANP deficiency resulted in SSH in mice, which further strengthens the link between the natriuretic peptide system and SSH.\textsuperscript{14}

Adequate BP control in patients with SSH is challenging because salt sensitivity is a heterogeneous phenotype characterized by impaired renal sodium excretion and suboptimal vasodilatory response as a consequence of multiple mechanisms, such as paradoxical mineralocorticoid receptor activation, increased renal sympathetic activity and RAAS, decreased activity of the natriuretic peptide system, and genetic polymorphisms.\textsuperscript{1,3} Therefore, it is conceivable that sacubitril/valsartan, by simultaneously inhibiting degradation of natriuretic peptides and blocking the AT\textsubscript{1} receptor, may provide effective BP control in patients with SSH. In the present study, treatment with sacubitril/valsartan for 4 weeks in Asian patients with SSH resulted in significantly greater reductions in office SBP, DBP, and ambulatory BP measurements versus valsartan. The effect size was comparable to the reduction in office BP with sacubitril/valsartan reported in Caucasian patients with hypertension\textsuperscript{31} and slightly smaller than that reported in Asian patients with hypertension.\textsuperscript{17} However, this comparison across studies needs to be interpreted with caution because the baseline BP was \(\approx8/10\) mm Hg lower in the present study, the treatment duration was 4 weeks shorter, and the sample size was significantly smaller than that of the other 2 studies. Considering these limitations, we may underestimate the true ambulatory BP reductions that may be achieved with sacubitril/valsartan treatment in patients with SSH.

The consistently greater reductions in nighttime ambulatory SBP, DBP, and MAP with sacubitril/valsartan versus valsartan are noteworthy. Given the predominant nondipper phenotype in patients with SSH (\(\approx70\%\) in the present study), this finding is of clinical significance because the abnormal dipping status (nondipper) often coexists with SSH, and nighttime BP is more closely associated with increased cardiovascular morbidity in patients with hypertension.\textsuperscript{35,36} Previous studies have shown the value of administering antihypertensive agents in the evening that resulted in better target organ protection and cardiovascular outcomes.\textsuperscript{37–39} The present study showed for the first time that targeting central underlying mechanisms of the nondipping status, such as impaired renal sodium excretion, by once-daily administration of sacubitril/valsartan in the morning can reduce both daytime and nighttime BP. An additional benefit of once-daily dosing is related to better treatment compliance, which is of paramount importance in chronic disease management.

The increase in urine cGMP excretion after treatment with sacubitril/valsartan for 4 weeks in the present study is an indication of sustained nephrilysin inhibition. As expected, valsartan had no effect on urine cGMP. Moreover, the significant reduction in plasma NT-proBNP with sacubitril/valsartan versus valsartan in the current study suggests a greater reduction in left ventricular wall stress with sacubitril/valsartan. This finding is corroborated by a prior observation that treatment with valsartan and a nephrilysin inhibitor compared with valsartan alone was associated with significantly less cardiac fibrosis, smaller media/lumen ratio of intramyocardial coronary arteries, and decreased macrophage infiltration in the vessel wall of aorta, mesenteric arteries, and intramyocardial coronary arteries in stroke-prone spontaneous hypertensive rats.\textsuperscript{40} Although it cannot be excluded that certain effects may be attributed to a greater BP reduction with sacubitril/valsartan, results of the covariate analysis suggest that the effects may be largely independent of BP reduction. Although the baseline NT-proBNP levels in the present study were low and within normal limits, the extent of reduction in NT-proBNP levels was numerically greater with sacubitril/valsartan than with amlodipine or atenolol, despite a greater BP reduction, in the ASCOT study (Anglo-Scandinavian Cardiac Outcomes Trial).\textsuperscript{41} The decrease in plasma aldosterone concentrations after treatment with both sacubitril/valsartan and valsartan is considered to be related to AT\textsubscript{1} receptor blockade and indicates that both treatments are conferring cardiovascular benefits owing to RAAS inhibition.

This study has several limitations. Although the study was adequately powered to detect relevant differences in natriuresis and diuresis between treatments, the sample size remained small. Study participants were exclusively Asian patients, which precludes the generalizability of the pharmacodynamic results to other ethnic groups. Natriuresis and diuresis were only measured on days 1 and 28. Therefore, the exact time of normalization of increased natriuresis and diuresis could not be determined. The treatment duration of 4 weeks was adequate to address the primary and secondary end points of this trial but was shorter than the treatment duration in other sacubitril/valsartan hypertension studies, in which patients with hypertension regardless of salt sensitivity were enrolled. Therefore, the comparison of BP results across studies is of limited value. Moreover, urine ANP levels could not be reliably analyzed because the assay performance was not optimal, leading to assay outputs falling outside the quality-control acceptance criteria.

**Perspectives**

This study demonstrated that sacubitril/valsartan, a first-in-class angiotensin receptor nephrilysin inhibitor that simultaneously enhances natriuretic peptides and blocks the AT\textsubscript{1} receptor, was associated with short-term increases in natriuresis and diuresis, which did not disturb serum electrolyte levels, and better office and ambulatory BP control, including a more pronounced BP reduction at nighttime, compared with valsartan. The biomarker changes were consistent with the mechanisms of action of sacubitril/valsartan and valsartan and suggest a greater hemodynamic unloading of the heart with sacubitril/valsartan compared with valsartan. Further
confirmatory studies are required to investigate if concomitant nesiritide inhibition and AT1 receptor blockade could present a potentially superior pharmacological alternative to standard-of-care therapy available to patients with SSH.

Acknowledgments
All authors participated in the development and writing of the article and approved the final article for publication. The authors take full responsibility for accuracy and content of this article and acknowledge Rohan Mitra (Novartis Healthcare Pvt Ltd, Hyderabad, India) for providing medical writing and editorial support.

Sources of Funding
This study was funded by Novartis Pharma AG, Basel, Switzerland.

Disclosures
M.Y. Rhee received lecture honoraria from Pfizer Inc, LG Life Sciences Ltd, Bayer Korea Ltd, Hanmi Pharm Co Ltd, Yuhan Co Ltd, and Boryung Pharmaceutical Co Ltd and research grant from Boryung Pharmaceutical Co Ltd and Dong-A Pharmaceutical Co, Ltd. B. Tomlinson received research funding from Amgen, AstraZeneca, Merck Serono, Merck Sharp and Dohme, Novartis, Pfizer, and Boehringer-Ingelheim. R. Mitra (Novartis Healthcare Pvt Ltd, Hyderabad, India) was full-time employee of Novartis at the time of study conduct and eligible to receive Novartis stocks. The authors report no conflicts.

References

**Novelty and Significance**

**What Is New?**
- This is the first study assessing the pharmacodynamic effects of sacubitril/valsartan compared with valsartan in Asian patients with salt-sensitive hypertension as diagnosed by ≥10% increase in mean arterial pressure when switching from a low-sodium (50 mmol/d) to a high-sodium (320 mmol/d) diet.

**What Is Relevant?**
- High dietary sodium intake and genetic predisposition to salt sensitivity of blood pressure are of particular clinical relevance in Asian populations.
- Compared with valsartan, sacubitril/valsartan treatment was associated with significant short-term increases in natriuresis and diuresis on day 1, without affecting serum electrolyte levels, and greater reductions in office blood pressure, ambulatory blood pressure (particularly at nighttime despite of morning dosing), and N-terminal pro B-type natriuretic peptide levels (independent of office blood pressure changes between treatments) on day 28.

**Summary**

Based on the results from this study, which reflect its unique mechanisms of action, sacubitril/valsartan may constitute a novel therapeutic approach for Asian patients with salt-sensitive hypertension. Further confirmatory studies are necessary to test this hypothesis.
Effects of Sacubitril/Valsartan (LCZ696) on Natriuresis, Diuresis, Blood Pressures, and NT-proBNP in Salt-Sensitive Hypertension

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Hypertension. 2017;69:32-41; originally published online November 14, 2016; doi: 10.1161/HYPERTENSIONAHA.116.08484

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

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Supplementary Methods

Effects of Sacubitril/Valsartan (LCZ696) on Natriuresis, Diuresis, Blood Pressures, and NT-proBNP in Salt-Sensitive Hypertension

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Study Patients
The study population included patients aged ≥18 years with hypertension, either untreated (newly diagnosed patients or those who had not been taking any antihypertensive drug for ≥4 weeks before screening) or treated with antihypertensive drugs for ≥4 weeks before screening, and mean sitting systolic blood pressure (msSBP) either ≥140 to <180 mmHg at screening (untreated patients only) or ≥120 to ≤160 mmHg at screening and <180 mmHg at the end of the washout period (treated patients only). Patients were excluded if they had severe hypertension (msSBP ≥180 mmHg and/or mean sitting diastolic blood pressure [msDBP] ≥100 mmHg), or required concomitant use of antihypertensive drugs, diuretics, or drugs that would affect diuresis, natriuresis, or BP during the study.

Study Design
Patients followed a normal sodium diet (150–200 mmol/day) during the screening/washout period. Patients maintained a low sodium diet (50 mmol/day) for 7 days, switched to a high sodium diet (320 mmol/day) for the next 7 days, returned to their normal sodium diet in the diagnostic qualification period, and maintained this diet for the rest of the study period. Dietary compliance was measured by 24-hour urine sodium excretion targeting a sodium excretion of ≤100 mmol/day for a low sodium diet and ≥200 mmol/day for a high sodium diet. SSH was diagnosed if the mean arterial pressure (MAP) based on office BP measurements increased by ≥10% when patients switched from a low to high sodium diet. Patients then switched to a standardized sodium intake of 150–200 mmol/day from the beginning of a 1–2 week run-in period prior to the first crossover treatment period and maintained the sodium intake throughout the remainder of the study period. All participants provided informed consent prior to any study assessment. The study protocol was approved by the independent ethics committee or institutional review board of each center. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Biomarkers
At baseline and on Day 28, plasma N-Terminal pro B-type natriuretic peptide (NT-proBNP) was measured using the Elecsys proBNP assay (Roche Diagnostics, Mannheim, Germany), urine cyclic guanosine monophosphate (cGMP) was measured using the Habersham cGMP enzyme-immunoassay (GE Healthcare, Buckinghamshire, UK), and plasma aldosterone was measured using the Coat-a-Count aldosterone radioimmunoassay (Siemens, Tarrytown, NY, USA).

Safety Assessments
Safety assessments included evaluation of adverse events (AEs) and serious adverse events, including their intensity and association with the study drug, and regular monitoring of hematology, blood chemistry, and urine analyses as well as vital signs and body weight.
Supplementary Table S1—Change in Plasma/Serum Electrolyte and Body Weight at Week 4 from Baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>n</th>
<th>Day −1</th>
<th>Day 28</th>
<th>Change</th>
<th>Day −1</th>
<th>Day 28</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma/serum potassium (mmol/L)</td>
<td>Sacubitril/valsartan</td>
<td>71</td>
<td>4.17</td>
<td>4.13</td>
<td>−0.03</td>
<td>0.38</td>
<td>0.37</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Valsartan</td>
<td>65</td>
<td>4.26</td>
<td>4.09</td>
<td>−0.16</td>
<td>0.46</td>
<td>0.28</td>
<td>0.41</td>
</tr>
<tr>
<td>Plasma/serum sodium (mmol/L)</td>
<td>Sacubitril/valsartan</td>
<td>71</td>
<td>139.92</td>
<td>140.00</td>
<td>0.08</td>
<td>2.53</td>
<td>2.49</td>
<td>2.71</td>
</tr>
<tr>
<td></td>
<td>Valsartan</td>
<td>66</td>
<td>140.65</td>
<td>139.48</td>
<td>−1.17</td>
<td>2.67</td>
<td>2.58</td>
<td>2.84</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Sacubitril/valsartan</td>
<td>71</td>
<td>71.69</td>
<td>71.33</td>
<td>−0.36</td>
<td>13.57</td>
<td>13.65</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Valsartan</td>
<td>66</td>
<td>71.50</td>
<td>71.17</td>
<td>−0.33</td>
<td>17.88</td>
<td>13.78</td>
<td>6.77</td>
</tr>
</tbody>
</table>

SD, standard deviation

Supplementary Table S2—Incidence of Adverse Events (≥2% of Patients) – Safety Analysis Set

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LCZ696 400 mg QD N=71</th>
<th>Valsartan 320 mg QD N=67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Patients with AEs</td>
<td>23 (32.4)</td>
<td>22 (32.8)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5 (7.0)</td>
<td>5 (7.5)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>3 (4.2)</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (1.4)</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>2 (2.8)</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>Cough</td>
<td>4 (5.6)</td>
<td>0</td>
</tr>
<tr>
<td>Back Pain</td>
<td>0</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Dry Mouth</td>
<td>2 (2.8)</td>
<td>0</td>
</tr>
<tr>
<td>Flank Pain</td>
<td>0</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Pyuria</td>
<td>2 (2.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

AE, adverse event
Supplementary Figure S1 – Comparison of changes from baseline in 24-hour (A) maSBP, (B) maDBP, (C) maMAP, and (D) maPP between both treatments. h, hour; maDBP, mean ambulatory diastolic blood pressure; maMAP, mean ambulatory arterial pressure; maPP, mean ambulatory pulse pressure; maSBP, mean ambulatory systolic blood pressure; QD, once daily.