Pharmacokinetics and Pharmacodynamics of LCZ696, a Novel Dual-Acting Angiotensin Receptor–Neprilysin Inhibitor (ARNi)

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Angiotensin receptor blockade and neprilysin (NEP) inhibition together offer potential benefits for the treatment of hypertension and heart failure. LCZ696 is a novel single molecule comprising molecular moieties of valsartan and NEP inhibitor prodrug AHU377 (1:1 ratio). Oral administration of LCZ696 caused dose-dependent increases in atrial natriuretic peptide immunoreactivity (due to NEP inhibition) in Sprague-Dawley rats and provided sustained, dose-dependent blood pressure reductions in hypertensive double-transgenic rats. In healthy participants, a randomized, double-blind, placebo-controlled study (n = 80) of single-dose (200-1200 mg) and multiple-dose (50-900 mg once daily for 14 days) oral administration of LCZ696 showed that peak plasma concentrations were reached rapidly for valsartan (1.6-4.9 hours), AHU377 (0.5-1.1 hours), and its active moiety, LBQ657 (1.8-3.5 hours). LCZ696 treatment was associated with increases in plasma cGMP, renin concentration and activity, and angiotensin II, providing evidence for NEP inhibition and angiotensin receptor blockade. In a randomized, open-label crossover study in healthy participants (n = 56), oral LCZ696 400 mg and valsartan 320 mg were shown to provide similar exposure to valsartan (geometric mean ratio [90% confidence interval]: $AUC_{0-\infty}$ 0.90 [0.82-0.99]). LCZ696 was safe and well tolerated. These data support further clinical development of LCZ696, a novel, orally bioavailable, dual-acting angiotensin receptor–NEP inhibitor (ARNi) for hypertension and heart failure.

Keywords: *angiotensin receptor blocker; hypertension; heart failure; neprilysin inhibitor; valsartan*

Journal of Clinical Pharmacology, 2010;50:401-414

The potent cardiovascular and renal effects of the natriuretic peptides present a potentially important therapeutic option for the treatment of hypertension and heart failure.[1,2] The natriuretic peptides constitute a family of similar but genetically distinct peptides, which include the atrial, brain-type, and C-type natriuretic peptides (ANP, BNP, and CNP, respectively).[3] ANP and BNP exert their physiological actions through the natriuretic peptide receptors type A (NPR-A) and type B (NPR-B), which are coupled to guanylyl cyclase. NPR activation thus increases intracellular cyclic GMP (cGMP), which in turn mediates biologic effects that include vasodilatation, natriuresis and diuresis, inhibition of the renin–angiotensin-aldosterone system (RAAS), endothelin and vasopressin, and lipid mobilization.[2,4]

ANP has a short half-life in the circulation, and the major enzyme responsible for degrading it is neprilysin (neutral endopeptidase 24.11, NEP).[5] Inhibitors of NEP have been shown to increase levels of ANP[6,7] and may therefore have beneficial effects for the treatment of a number of cardiovascular diseases, including hypertension and heart failure. However, clinical trials with NEP inhibitors have failed to demonstrate consistent lowering of blood pressure (BP) in patients with hypertension, possibly because NEP is also

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DOI:10.1177/0091270009343932

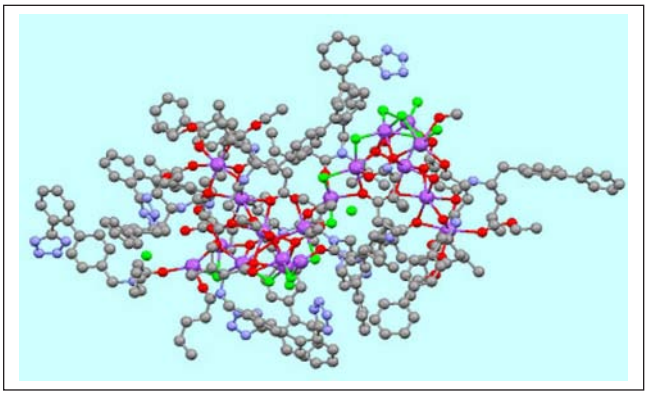

Figure 1. Ball-and-stick representation of structure of LCZ696 ($C_{48}H_{55}N_6O_6Na_3 \cdot 2.5H_2O$). Atoms shown are carbon (grey), sodium (purple), carboxylate and carbonyl oxygen (red), and water oxygen (green). Hydrogen atoms are not shown for clarity.

responsible for the degradation of the potent vasoconstrictor, angiotensin II (Ang II).[8] Dual-acting compounds that simultaneously inhibit NEP and block the generation or action of Ang II have therefore been developed. Omapatrilat, a dual inhibitor of NEP and angiotensin-converting enzyme (ACE), demonstrated superior antihypertensive efficacy to the ACE inhibitor enalapril but was also associated with an increased incidence of angioedema.[9] Omapatrilat also inhibits aminopeptidase P (APP), and it is possible that the observed increased incidence of angioedema was related to inhibition of the breakdown of bradykinin and substance P by APP and ACE, with NEP inhibition having only a minor effect.[10] A dual-acting ARB–NEP inhibitor (ARNi), which would not directly affect ACE or APP activity, may therefore be a safer approach to inhibit the RAAS and increase natriuretic peptide levels.

LCZ696 (trisodium [3-((1*S*,3*R*)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-butylcarbamoyl) propionate-(*S*)-3′-methyl-2′-(pentanoyl{2″-(tetrazol-5-ylate)biphenyl-4′-ylmethyl}amino)butyrate] hemipentahydrate) comprises molecular moieties of valsartan, a well-established ARB,[11] and of the NEP inhibitor prodrug AHU377 ((2R, 4S)-5-biphenyl-4-yl-5-(3-carboxy-propionylamino)-2-methyl-pentanoic acid ethyl ester; Figure 1), which is metabolized to the active NEP inhibitor LBQ657 by enzymatic cleavage of its ethyl ester.[12] LCZ696 is a novel single molecule in which the molecular moieties of valsartan and the molecular moieties of AHU377 are present in a 1:1 molar ratio. The details of the molecular structure of LCZ696 will be presented elsewhere (manuscript submitted).

Here we report the pharmacokinetic and pharmacodynamic effects of LCZ696 in animal studies and in 2 studies conducted in healthy human participants. Studies in dogs compared the pharmacokinetics and exposures of valsartan and AHU377 following administration of the 2 individual compounds or following LCZ696 administration. Studies in rats examined the effects of LCZ696 on BP and biomarkers of NEP inhibition. The clinical studies examined the single- and multiple-dose pharmacokinetics and pharmacodynamics of LCZ696 following oral administration.

METHODS

Animal Studies

The pharmacokinetics and pharmacodynamics of LCZ696 were studied in beagle dogs and rats, respectively. All animal procedures were conducted in accordance with approved Institutional Animal Care and Use Committee protocols and the *Guide for the Care and Use of Laboratory Animals* by Novartis (East Hanover, New Jersey).

Pharmacokinetics

The pharmacokinetics and relative bioavailability of LCZ696 were determined in male beagle dogs (n = 3; 8.8-13.2 kg in weight). Dogs received single doses of LCZ696 tablets (300 mg, 354 μmol/kg) or of valsartan (160 mg, 367 μmol/kg) and AHU377 (100 mg, 232 μmol/kg) tablets in a crossover design. Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 30, and 48 hours after dosing. Samples were assayed for valsartan, LBQ657, and AHU377 using a validated high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analytical method where the components were separated using an ACE 5 C_{18} column (2.1 × 50 mm) and detected using an API3000 mass spectrometer (Applied Biosystems, Foster City, California) with electrospray ionization in selected reaction monitoring mode. The analytical assay was linear within a concentration range of 1 to 10000 ng/mL for all 3 analytes. The lower limit of quantification was 1 ng/mL for valsartan, AHU377, and LBQ657.

Pharmacodynamics

The pharmacodynamic effects of LCZ696 were assessed in conscious, chronically instrumented rats. BP-lowering effects were evaluated in double-transgenic rats (dTGR) that overexpress both human renin

and angiotensinogen and, consequently, exhibit an Ang II–dependent hypertension.[13] The dTGR (n = 6 per treatment group) were surgically implanted with an aortic catheter and radio transmitter (Data Sciences, Inc, Laurel, Maryland) for measurement of mean arterial pressure. The NEP-inhibitory effect of LCZ696 was determined in chronically cannulated Sprague-Dawley rats (n = 4 per treatment group) infused (450 ng/kg/min) with exogenous ANP (1-28) (American Peptide Company, Sunnyvale, California).[14] Potentiation of plasma ANP immunoreactivity (ANPir) levels was used as an index of NEP inhibition in vivo. Plasma ANPir concentrations were assayed with a commercially available Atrial Natriuretic Factor (1-28) (human) EIA kit (Peninsula Laboratories, Inc, San Carlos, California). The antibody used exhibits 100% cross-reactivity with rat ANP (1-28) but 0% cross-reactivity with rat BNP-45, rat CNP, or rat endothelin-1. In both studies, LCZ696 (2, 6, 20, or 60 mg/kg) was administered orally as powder in gelatin mini-capsules (Torpac, Fairfield, New Jersey); vehicle control rats received empty capsules. Each rat received only 1 treatment.

Statistical Analyses

Assessments of the effects of LCZ696 on BP included the time courses of mean arterial pressure (MAP) and of change in MAP from baseline (ΔMAP), as well as 24-hour time-weighted average (TWA) ΔMAP. The 24-hour TWA was calculated by dividing the area between the ΔMAP curve (determined using the trapezoidal rule) and ΔMAP baseline (by definition, 0) by 24 hours. Differences in 24-hour TWA ΔMAP between LCZ696 groups and vehicle were compared using a 1-way analysis of variance (ANOVA) followed by Tukey HSD (honestly significant difference) as a post hoc test. Plasma ANPir assessments included the time courses of ANPir concentrations and of ANPir expressed as a percentage of baseline ANPir and 8-hour TWA ANPir. A paired Student *t* test was used to compare group responses to the baseline values (100%). One-way ANOVA was used to determine the significance of the dose–response relationships. Statistical analyses were conducted using Microsoft Excel or S-PLUS 7 (Insightful Corporation, Seattle, Washington). All differences were considered statistically significant at a level of $P \leq .05$.

Clinical Studies in Healthy Participants

The pharmacokinetics, safety, and tolerability of LCZ696 were investigated in 2 studies involving healthy human volunteers under an IND. A dose escalation study examined the single- and multiple-dose pharmacokinetics and pharmacodynamics of ascending oral doses of LCZ696, and a bioequivalence study evaluated the relative exposure of valsartan following administration of LCZ696 or valsartan. Both studies were conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki, and received approval from the local institutional review board (MDS Pharma Services Institutional Review Board, Lincoln, Nebraska). All participants provided written informed consent before participation in the study.

Study Design

Dose escalation study. The dose escalation study was a randomized, double-blind, placebo-controlled, parallel-group study. Participants were divided into 8 cohorts of 10 individuals. Four cohorts received a single dose of LCZ696, and 4 received multiple oral doses of LCZ696 (once daily for 14 days), with each cohort receiving a different dosage: 200, 600, 900, and 1200 mg for single-dose administration and 50, 200, 600, and 900 mg for multiple-dose administration. Within each cohort, participants were randomized to LCZ696 (n = 8) or placebo (n = 2). Participants who terminated the study prematurely were replaced by individuals who underwent the same treatment as the replaced participant. Escalation to the next dose was allowed only if all safety and tolerability data from the preceding dose had been satisfactory. Multiple-dose administration was started only after the equivalent single-dose assessment had been completed.

For single-dose administration, participants were domiciled at the study center from the day before dosing until 72 hours postdose. LCZ696 was administered with 240 mL of water between 7:00 and 9:00 AM following an overnight fast of at least 10 hours, and fasting continued for 4 hours after dosing. For multiple-dose administration, participants were domiciled for 4 days before dosing until 72 hours after the final dose. During this period, they followed a weight maintenance diet with no more than 3 g Na^+/d (130 mEq/d) and 5 g K^+/d (128 mEq/d). On the first and last days of LCZ696 administration (days 1 and 14), dosing occurred as for single-dose administration. On the other study days, LCZ696 was administered 30 minutes before breakfast, except on day 13 (1 hour before breakfast).

Bioavailability study. The bioavailability study was a single-center, randomized, open-label, crossover

study. After screening, eligible participants were randomized to a single dose of either LCZ696 400 mg or valsartan 320 mg (Diovan), administered with 240 mL of water between 7:00 and 9:00 AM following an overnight fast of at least 10 hours; fasting continued for 4 hours after dosing. Following a washout period of at least 5 days, participants crossed over to receive the other treatment. During each study period, participants were domiciled at the study center from the evening before dosing until at least 48 hours postdose, then returned to the study center after an overnight fast for study assessments at 72 hours after dosing.

Study Participants

Both studies enrolled healthy male and female volunteers aged 18 to 55 years, of at least 50 kg in weight, and with a body mass index (BMI) of 18 to 30 kg/m^2. Female volunteers had to be postmenopausal (no hormone replacement therapy in the past 6 months) or had to have undergone ovarectomy (with or without hysterectomy) for inclusion.

Major exclusion criteria for both studies included history of angioedema; use of any over-the-counter medication (eg, vitamins, dietary supplements) or prescription drugs within 2 or 4 weeks, respectively, before dosing; and any surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of drugs. In addition, individuals who had smoked during the previous 3 months were excluded from the bioavailability study. Light smokers (up to 5 cigarettes per day) were eligible for the dose escalation study, but individuals had to refrain from smoking for at least 2 days before dosing and for the duration of the study.

Pharmacokinetic Measurements

In the dose escalation study, blood samples for the evaluation of pharmacokinetic parameters were collected predose and at 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 48, and 72 hours postdose in the single-dose cohorts. In the multiple-dose cohorts, samples were collected at the same intervals after the first dose (not 48 and 72 hours) and the last (day 14) dose. In addition, samples were taken predose on days 9, 11, and 13 to assess trough levels. In the bioavailability study, samples were collected predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours postdose. In both studies, blood samples (2.5 mL) were taken either by direct venipuncture or by an indwelling cannula inserted in a forearm vein. Samples were collected into EDTA-containing tubes and centrifuged at 1500 *g* for 10 minutes at 3°C to 5°C, and the plasma was removed and frozen at −15°C or below until plasma drug concentrations were determined.

Plasma concentrations for valsartan, AHU377, and LBQ657 were determined using a validated HPLC/MS/MS analytical method. Following liquid–liquid extraction, reconstituted samples underwent HPLC using an ACE 5 C_{18} column (2.1 × 50 mm) with gradient elution (0.1% formic acid in water/0.1% formic acid in acetonitrile) over 3.2 minutes (dose escalation study) or 5 minutes (bioavailability study). Detection was performed by MS/MS with electrospray ionization (ESI) using an API 3000 mass spectrometer. Internal standards for the assays were D_9-valsartan, $^{13}C_4$-AHU377, and $^{13}C_4$-LBQ657. The general settings used in the dose escalation study were as follows: ESI N_2 gas settings (nebulizer 10, curtain 12, collision 6, source temperature 350°C, voltage −3500 V); selected reaction monitoring; negative ion mode; collision gas N_2; collision energy −26 eV (valsartan), −42 eV (AHU377), and −20 eV (LBQ657); and unit mass resolution. In the bioavailability study, the general settings were as follows: ESI N_2 gas settings (nebulizer 12, curtain 12, collision 7, source temperature 350°C, voltage 5500 V); multiple reaction monitoring; positive ion mode; collision gas N_2; collision energy 27 eV (valsartan), 25 eV (AHU377), and 23 eV (LBQ657); and unit mass resolution. In both studies, dwell time was 150 ms for valsartan, AHU377, and LBQ657 and 100 ms for each internal standard. In the dose escalation study, the respective masses for the precursor and product ions (m/z) were 434.3 and 350.0 for valsartan, 410.2 and 167.0 for AHU377, and 382.2 and 265.0 for LBQ657. In the bioavailability study, the respective masses for the precursor and product ions (m/z) were 436.3 and 235.0 for valsartan, 412.2 and 266.1 for AHU377, and 384.1 and 266.1 for LBQ657. The lower limit of quantification (LLOQ) was 1 ng/mL for all 3 analytes in both studies, except for LBQ657 in the bioavailability study (LLOQ 10 ng/mL). Within-study assay validation across both studies showed an assay precision (% coefficient of variation [CV]) of 2.2% to 14.0% for valsartan, 1.5% to 7.9% for AHU377, and 2.2% to 11.5% for LBQ657, as well as a bias of −4.0% to 3.0% for valsartan, −3.6% to 3.0% for AHU377, and −6.0% to 3.1% for LBQ657.

Pharmacodynamic Assessments

In the dose escalation study, blood samples were also collected from the multiple-dose cohorts to assess the effects of LCZ696 administration on biomarkers related to NEP inhibition (cGMP) and

the RAAS (renin concentration, plasma renin activity [PRA], Ang II). Blood samples were collected predose and at 4, 12, and 24 hours postdose on days 6 and 12. Samples were collected at corresponding time points at baseline (day −1), that is, at −24, −20, and −12 hours predose. For cGMP analysis, blood samples (1.2 mL) were collected into prechilled EDTA-containing tubes, then immediately centrifuged at 1000 *g* for 10 minutes at 4°C and put on ice, before the plasma was removed into chilled tubes and stored at −80°C until analysis. Blood samples (6 mL) for biomarker determinations were collected into EDTA-containing tubes and centrifuged at 3000 rpm for 20 minutes at room temperature, and the plasma was removed and stored at −20°C until analysis. cGMP was measured by radioimmunoassay (RIA) with ^{125}I-cGMP, using a kit for the determination of cGMP in human plasma (Amersham International, Amersham, UK). This method was validated at SGS CEPHAC Europe (Paris, France); the quantification range was 0.10 to 7.5 nmol/L. The assays for renin concentration, PRA, and Ang II were performed by Dr Erik Frandsen (Department of Clinical Physiology and Nuclear Medicine, Glostrup University Hospital, Glostrup, Denmark). Renin concentration was measured using a DSL-25100 renin immunoradiometric assay (Diagnostic Systems Laboratories, Inc, Webster, Texas; quantification range [WHO renin], 0-1000 mIU/L). PRA was measured using a combined enzyme kinetic assay and radioimmunoassay using ^{125}I-Ang I and Ang I antiserum raised in rabbits (quantification range, 0.1-1000 mIU/L). Plasma (25 μL) was incubated for 3 hours at 37°C with 50 pmol sheep angiotensinogen in a total reaction volume of 100 μL; the buffer was Tris 0.5M containing 10 mM EDTA and 20 mg/L chlorhexidine. The reaction was stopped by transferring the tube to an ice bath and 1 mL 0°C ^{125}I-Ang I tracer and 100 μL Ang I antiserum added, so that the Ang I radioimmunoassay was performed in the same tube as the enzymatic reaction. Ang II was measured by radioimmunoassay using rabbit Ang II antiserum and ^{125}I-Ang II following ethanol extraction from plasma samples (quantification range, 0.4-830 pg/mL). Preliminary experiments confirmed that the presence of study drug did not interfere with any of the biomarker assays.

Safety and Tolerability Assessments

Adverse events (AEs) were monitored and recorded throughout both studies and were evaluated for severity and likely relationship to study drug by the investigators. Standard laboratory tests for hematology, blood chemistry, and urine were monitored regularly, and physical examinations, electrocardiogram (ECG) recordings, and monitoring of vital signs were performed at regular intervals during the study. In the dose escalation study, to assess gastrointestinal tolerance of LCZ696, we tested participants in the multiple-dose cohorts for occult blood in feces prior to dosing and on 3 separate occasions during the 14-day treatment period.

Statistical Analyses

Pharmacokinetic parameters (area under the concentration–time curve between first and last observation [$AUC_{0\text{-last}}$], area under the concentration–time curve extrapolated to infinity [$AUC_{0\text{-}\infty}$], maximum plasma concentration [C_{max}], time to achieve maximum plasma concentration [t_{max}], terminal half-life [$t_{1/2}$], plasma clearance corrected for bioavailability [CL/F], and apparent volume of distribution corrected for bioavailability [$V_{\lambda z}$/F]) were determined for valsartan in the bioavailability study and valsartan, AHU377, and LBQ657 in the dose escalation study by noncompartmental methods using WinNonlin Pro.

In the dose escalation study, an exploratory analysis of dose proportionality was performed by assuming a linear mixed effects model with a fixed continuous dose-effect $E(\ln(PK)) = \alpha + \beta \ln(Dose)$ and calculating 90% confidence limits for β and for the estimated range of dose linearity. Pairwise comparisons between doses were also evaluated, and plots were made to assess the dose-proportionality range visually. The biomarker data (log-transformed change from baseline) were analyzed by an analysis of covariance (ANCOVA) model, with treatment (different dose levels) as a factor and baseline biomarker (also log transformed) as a covariate. Point estimates and the associated 95% confidence intervals (CIs) for the treatment–placebo ratios were back-transferred from the respective values for the differences of mean logarithms obtained from the ANCOVA.

In the bioavailability study, the pharmacokinetic variables $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, and C_{max} were log transformed and analyzed separately using a linear mixed effects model, with fixed effects from sequence, treatment, and period. Random effects were generated by subject nested in sequence. The resulting 90% CI of the treatment mean ratios was used to evaluate relative bioavailability of the LCZ696 tablet and the valsartan capsules. Sample size for the bioavailability study was determined based on data from a previous pharmacokinetic study in human volunteers indicating that the relative bioavailability

Table I Summary of the Pharmacokinetic Parameters of Valsartan, AHU377, and LBQ657 Following Administration of Single Oral Doses of LCZ696 300 mg or Valsartan 160 mg plus AHU377 100 mg in Beagle Dogs (n = 3)

Treatment	t_{max}, h	C_{max}, ng/mL	$AUC_{0\text{-}last}$, ng·h/mL	$AUC_{0\text{-}\infty}$, ng·h/mL	C_{max}/Dose	$AUC_{0\text{-}last}$/Dose	$AUC_{0\text{-}\infty}$/Dose
Valsartan							
LCZ696 300 mg	1.3 ± 0.6	4720 ± 1920	21000 ± 10300	21200 ± 10300	148 ± 51	642 ± 187	648 ± 186
Valsartan 160 mg + AHU377 100 mg	4.0 ± 3.5	1110 ± 828	7200 ± 3050	7990[a]	32 ± 18	214 ± 39	220[a]
AHU377							
LCZ696 300 mg	0.8 ± 0.3	2860 ± 2340	3070 ± 2380	3130 ± 2360	86 ± 67	91 ± 59	93 ± 58
Valsartan 160 mg + AHU377 100 mg	0.7 ± 0.3	818 ± 643	1160 ± 764	1280 ± 713	36 ± 20	53 ± 22	59 ± 18
LBQ657							
LCZ696 300 mg	1.3 ± 0.6	1820 ± 1560	3680 ± 2670	3820 ± 2740	53 ± 34	109 ± 54	113 ± 56
Valsartan 160 mg + AHU377 100 mg	0.8 ± 0.3	767 ± 351	2110 ± 927	2190 ± 938	36 ± 8	99 ± 21	103 ± 21

Dose: LCZ696, 354 μmol/kg (300 mg tablet/dog); valsartan, 367 μmol/kg (160 mg tablet/dog); AHU377, 232 μmol/kg (100 mg tablet/dog). Values are mean ± SD. $AUC_{0\text{-}last}$, area under the concentration–time curve between first and last observation; $AUC_{0\text{-}\infty}$, area under the plasma concentration–time curve extrapolated to infinity; $AUC_{0\text{-}last}$/Dose, dose (μmol/kg) normalized $AUC_{0\text{-}last}$; $AUC_{0\text{-}\infty}$/Dose, dose (μmol/kg) normalized $AUC_{0\text{-}\infty}$; C_{max}, maximum plasma concentration; C_{max}/Dose, dose (μmol/kg) normalized C_{max}; t_{max}, time to reach maximum plasma concentration.
[a]n = 2 (the value for 1 animal could not be estimated due to fluctuations in terminal phase).

of valsartan following administration of LCZ696 compared with valsartan was 1.67 (data on file). A sample size of 22 participants in each sequence group (44 in total) was sufficient to provide overall 80% power that the 90% CIs for the ratio of the 2 treatments for $AUC_{0\text{-}last}$, $AUC_{0\text{-}\infty}$, and C_{max} for 200 mg LCZ696 and 320 mg valsartan were contained within the interval (0.80-1.25). A total of 56 participants (28 in each sequence group) were enrolled into the study to ensure that 44 participants completed the protocol.

RESULTS

Pharmacokinetics of LCZ696 in Beagle Dogs

Oral administration of LCZ696 to beagle dogs (n = 3) led to a rapid increase in plasma concentrations of valsartan. The t_{max} for valsartan following LCZ696 dosing was 1.3 hours, whereas combined administration of valsartan (free acid) and AHU377 (calcium salt) tablets yielded a t_{max} for valsartan of 4.0 hours (Table I). Systemic exposure (AUC and C_{max}) to valsartan following LCZ696 administration was about 3-fold higher than that observed following administration of approximately equimolar doses of valsartan and AHU377. LCZ696 or valsartan plus AHU377 delivered similar exposure to LBQ657 (Table I). All pharmacokinetic parameters were derived from 3 beagle dogs per treatment group, with the exception of valsartan $AUC_{0\text{-}\infty}$ in the valsartan + AHU377 group, which was determined in 2 dogs.

Pharmacodynamics of LCZ696 in Rats

The antihypertensive effects of LCZ696 were studied in conscious, chronically instrumented dTGR (n = 6 per treatment group) expressing the genes for human renin and angiotensinogen. Oral administration of LCZ696 (2-60 mg/kg) induced a dose-dependent ($P < .05$ for 24-hour TWA ΔMAP at the 3 highest doses) and long-lasting reduction in MAP (Figure 2A,B). Oral administration of LCZ696 (2-60 mg/kg) to Sprague-Dawley rats (n = 4 per treatment group) stimulated a rapid (within 15 minutes; $P < .05$ for all 4 doses) and dose-dependent augmentation of plasma ANP immunoreactivity (Figure 2C,D).

Clinical Studies in Healthy Participants

Baseline Characteristics

Dose escalation study. In all, 83 participants were randomized and received treatment: 41 participants in the single-dose cohorts and 42 in the multiple-dose cohorts. Of these, 65 participants received LCZ696 (single dose, n = 32; multiple dose, n = 33). Five participants discontinued from the study, 2 for withdrawal of consent (placebo) and 2 for administrative

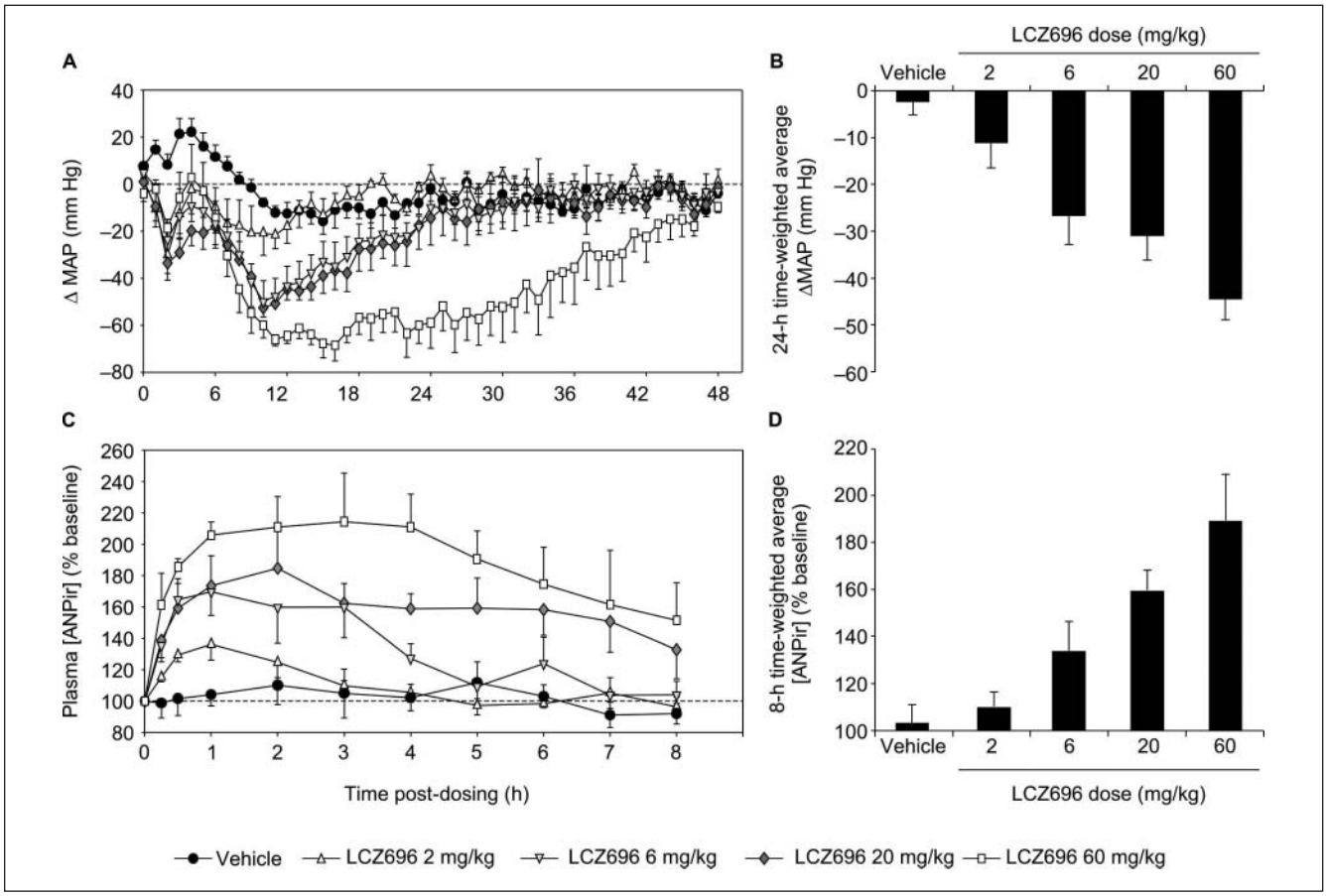

Figure 2. Effects of oral administration of LCZ696 in conscious rats on (A, B) change in mean arterial pressure (ΔMAP) in double-transgenic rats (dTGR) overexpressing human renin and angiotensinogen and (C, D) plasma atrial natriuretic peptide immunoreactivity (ANPir) as a percentage of pre-LCZ696 plasma ANPir in ANP-infused (450 ng/kg/min) Sprague-Dawley rats. Dose-response results are expressed as the (B) 24-hour or (D) 8-hour time-weighted average (TWA; area under the curve divided by the integration time) ΔMAP or plasma ANPir, respectively. Data are mean ± SEM.

reasons (one each receiving multiple-dose 600-mg and 900-mg LCZ696). A second participant receiving multiple-dose LCZ696 900 mg was discontinued on day 10 due to elevated heart rate outside study inclusion criteria on day –4.

Baseline and demographic characteristics were well balanced between treatment groups in the multiple-dose (Table II) and single-dose cohorts. Mean age ranged from 26 to 38 years for single-dose treatments and 31 to 42 years for the multiple-dose groups; mean body weight ranged from 72 to 86 kg. All except one of the participants were male, and most participants were either black (n = 57; 69%) or white (n = 20; 24%).

Bioavailability study. In the bioavailability study, 56 participants were randomized and received treatment, and 51 participants completed the study. Four participants discontinued because of abnormal laboratory values, and 1 participant withdrew consent after day 3 of the first treatment period. Most participants were male (n = 52; 93%) and white (n = 50; 89%). Mean age was 29 years, and mean body weight was 78 kg.

Pharmacokinetic Analyses

Dose escalation study. Mean plasma concentration–time profiles for valsartan, AHU377, and LBQ657 following oral administration of LCZ696 in the single-dose cohorts are shown in Figure 3. Valsartan and AHU377 were absorbed rapidly following administration, with maximum plasma concentrations reached within 1.7 to 2.2 and 0.5 to 1.1 hours,

Table II. Baseline and Demographic Characteristics of Healthy Participants in the Multiple-Dose Cohort

	LCZ696				
	50 mg (n = 8)	**100 mg (n = 8)**	**600 mg (n = 9)**	**900 mg (n = 8)**	**Placebo (n = 9)**
Age, y	37 ± 14	34 ± 7	36 ± 10	31 ± 8	42 ± 7
Gender, male/female, n/n	7/1	8/0	9/0	8/0	9/0
Race, n (%)					
White	4 (50)	2 (25)	2 (22)	0	1 (11)
Black	3 (38)	6 (75)	6 (67)	8 (100)	8 (89)
Asian	0	0	1 (11)	0	0
Other	1 (13)	0	0	0	0
Height, cm	173 ± 9	175 ± 9	174 ± 6	173 ± 4	177 ± 6
Weight, kg	72 ± 8	82 ± 12	77 ± 6	74 ± 8	85 ± 8
Body mass index, kg/m^2	24.3 ± 2.7	26.5 ± 2.2	25.3 ± 1.8	24.9 ± 3.3	27.0 ± 2.2
Mean supine systolic blood pressure, mm Hg	112 ± 8	114 ± 10	114 ± 8	119 ± 20	114 ± 12
Mean supine diastolic blood pressure, mm Hg	66 ± 8	64 ± 8	67 ± 6	70 ± 11	68 ± 5
Serum potassium,[a] mmol/L	4.4 ± 0.3	4.3 ± 0.2	4.2 ± 0.3	4.5 ± 0.2	4.3 ± 0.4
Serum creatinine,[a] mg/dL	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.2

Values are mean ± SD unless otherwise stated.
[a]Day –4 values are presented.

respectively, after dosing. Conversion of AHU377 to LBQ657 was also rapid, with peak LBQ657 concentrations reached 1.9 to 3.5 hours after LCZ696 administration. Mean $t_{1/2}$ values for AHU377 ranged from 1.1 to 3.6 hours, whereas mean $t_{1/2}$ values for valsartan and LBQ657 were 8.9 to 16.6 and 9.9 to 11.1 hours, respectively.

Within an LCZ696 single-dose range of 200 to 1200 mg, the increases in AUC and C_{max} values for LBQ657 were approximately proportional to the LCZ696 dose. For AHU377, the increases in AUC values were approximately dose proportional across the range of LCZ696 doses evaluated, whereas the increases for C_{max} values were approximately proportional only across the 200- to 900-mg dose range. There was a linear, albeit less than proportional, relationship between LCZ696 dose and valsartan AUC and C_{max}.

During the multiple-dosing period, peak plasma concentrations were achieved rapidly after LCZ696 dosing for valsartan (1.6-4.9 hours), AHU377 (0.6-0.9 hours), and LBQ657 (1.8-2.7 hours) (Table III). Comparison of C_{max} and $AUC_{0\text{-}24}$ values on days 1 and 14 showed no significant accumulation of valsartan or AHU377 and minimal accumulation of LBQ657 (R = 1.2).

Bioavailability study. Mean plasma concentration-time profiles for valsartan were similar following the administration of a single oral dose of LCZ696 400 mg or valsartan 320 mg (Figure 4). Peak plasma concentrations of valsartan were achieved rapidly following dosing with LCZ696 (median 2.0 hours) or valsartan (median 4.0 hours), and mean terminal half-life (approximately 18 hours) was similar between the 2 treatments (Table IV).

Systemic exposure to valsartan following dosing with LCZ696 400 mg met bioequivalence criteria compared with valsartan 320 mg for C_{max} (geometric mean ratio [90% CI]: 0.98 [0.87-1.10]) and AUC (geometric mean ratio [90% CI]: $AUC_{0\text{-last}}$ 0.90 [0.81-1.00]; $AUC_{0\text{-}\infty}$ 0.90 [0.82-0.99]). Analysis of dose-normalized values for systemic exposure showed that a 40% higher valsartan exposure was achieved following LCZ696 administration than after administration of valsartan alone (C_{max} 1.52 [1.35-1.71]; $AUC_{0\text{-}\infty}$ 1.40 [1.27-1.55]).

Pharmacodynamic Assessments

Dose escalation study. Following multiple-dose administration of LCZ696 to healthy volunteers, biomarkers of NEP inhibition (cGMP) and angiotensin receptor (AT_1) blockade (renin concentration, PRA, and Ang II) were significantly increased. All doses of LCZ696 treatment increased 24-hour mean cGMP levels, with a maximum 40% increase in cGMP observed with the 200-mg dose on day 12 (geometric

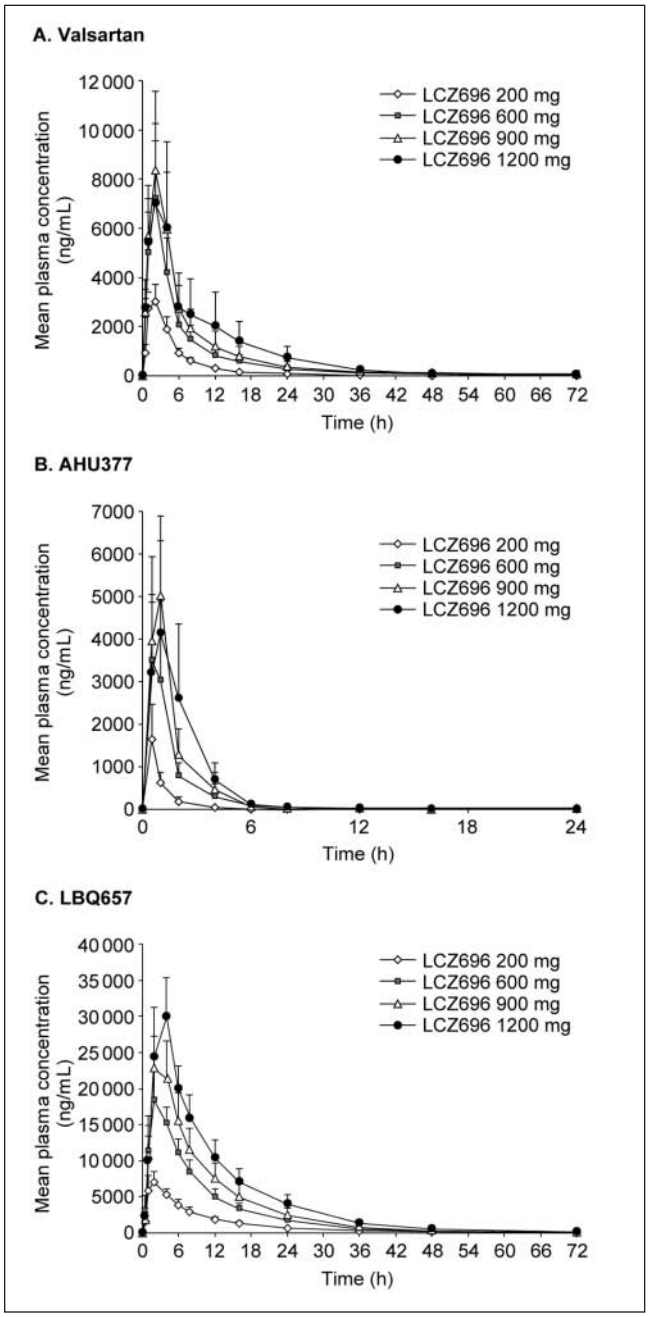

Figure 3. Plasma concentration–time profiles for (A) valsartan, (B) AHU377, and (C) LBQ657 following administration of a single oral dose of LCZ696 200, 600, 900, or 1200 mg in healthy participants. Data are mean ± SD.

mean ratio relative to placebo 1.40 [95% CI: 1.14, 1.71]). cGMP levels were significantly increased at 4 hours (all doses) and 12 hours postdose (LCZ696 600 and 900 mg) after administration of LCZ696 and returned to baseline levels at 24 hours after administration of all doses of LCZ696 (Figure 5).

LCZ696 treatment induced significant, dose-dependent increases in renin concentration (93%-634% increase vs placebo), PRA (280%-1768% increase), and Ang II (241%-1188% increase) on day 12 (Figure 5). All biomarkers showed a maximum increase 4 hours after administration of LCZ696, and significant increases in levels of all RAAS biomarkers relative to placebo were observed 24 hours after dosing.

Safety and Tolerability

LCZ696 was generally well tolerated in both studies. AEs were mostly mild in intensity, and there were no serious AEs or discontinuations due to AEs reported in either study. No clinically relevant changes in vital signs (with the exception of hypotension), ECG assessments, or clinical laboratory values were reported over the course of either the dose escalation or bioavailability study. In addition, in the dose escalation study, no clinically significant results were noted with occult blood testing.

Dose escalation study. AEs were reported by 4 participants in the single-dose cohorts: dizziness (n = 2), back pain (n = 1), and vasovagal syncope during blood draw (n = 1). All AEs were mild in intensity, and only 1 (dizziness) was considered potentially related to study medication. None of the participants receiving the highest LCZ696 dose (1200 mg) reported AEs.

Nineteen participants reported a total of 48 AEs during multiple-dose LCZ696 treatment (Table V). Orthostatic hypotension, either with or without accompanying symptoms such as tachycardia (postural orthostatic hypotension), was the most common AE, but all hypotension events were transient and resolved without intervention. Dizziness was experienced by 4 participants during the study and was associated with orthostatic hypotension in one of these participants. All AEs of dizziness and orthostatic hypotension were considered related to study medication.

During multiple LCZ696 dosing, 3 participants experienced elevated creatine kinase levels, and 1 participant experienced elevated γ-glutamyl transpeptidase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values, which rapidly returned to normal. Thirteen participants experienced elevated serum potassium levels during multiple LCZ696 dosing, but these were isolated occurrences (normal values recorded on repeat

Table III Summary of the Pharmacokinetic Parameters of Valsartan, AHU377, and LBQ657 Following Multiple-Dose Administration of LCZ696 (50, 200, 600, and 900 mg Once Daily) in Healthy Participants in the Dose Escalation Study

LCZ696 Dose	t_{max}, h	C_{max}, ng/mL	$AUC_{0\text{-last}}$, ng·h/mL	$AUC_{0\text{-}\infty}$, ng·h/mL	$t_{1/2}$, h
Valsartan					
50 mg (n = 8)	1.6 ± 0.5	1233 ± 325	7681 ± 1794	6935 ± 1940	15.2 ± 9.4
200 mg (n = 8)	1.8 ± 0.5	3990 ± 685	24097 ± 4680	21079 ± 4087	22.1 ± 16.5
600 mg (n = 9)	2.2 ± 0.8	8563 ± 2652	81639 ± 68 333	58876 ± 34189	22.6 ± 21.8
900 mg (n = 8)	4.9 ± 8.5	8960 ± 3012	72743 ± 28 929	54920 ± 15954	15 ± 5.6
LBQ657					
50 mg (n = 8)	2.1 ± 0.8	2094 ± 448	22773 ± 6594	18771 ± 4871	14.7 ± 2.9
200 mg (n = 8)	1.8 ± 0.5	8529 ± 1659	82903 ± 16136	70450 ± 112623	13.0 ± 3.0
600 mg (n = 9)	2.7 ± 1.1	23513 ± 3176	258155 ± 39112	215366 ± 22554	12.9 ± 3.8
900 mg (n = 8)	2.3 ± 0.8	38257 ± 9620	384594 ± 102026	326628 ± 71017	12.3 ± 1.8
AHU377					
50 mg (n = 8)	0.7 ± 0.3	508 ± 179	525 ± 103	527 ± 104	0.9 ± 0.2
200 mg (n = 8)	0.6 ± 0.2	1974 ± 678	2083 ± 803	2087 ± 803	1.7 ± 0.9
600 mg (n = 9)	0.6 ± 0.3	4309 ± 1686	6858 ± 1853	6863 ± 1853	2.2 ± 0.6
900 mg (n = 8)	0.9 ± 0.6	6524 ± 2054	9996 ± 2208	9999 ± 2208	3.3 ± 1.3

Values are mean ± SD. $AUC_{0\text{-}\infty}$, area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration–time curve between first and last observation; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; t_{max}, time to achieve maximum plasma concentration.

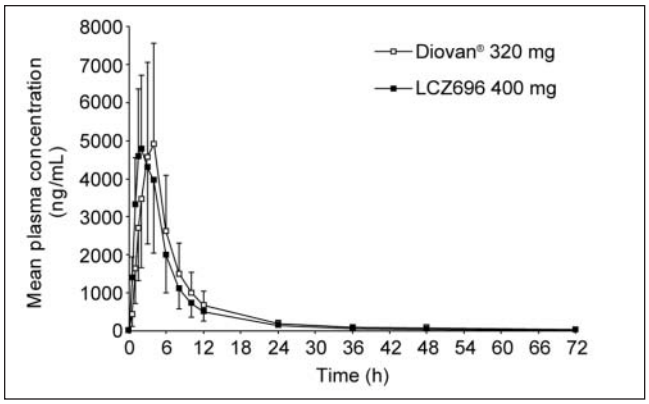

Figure 4. Plasma concentration–time profiles for valsartan following administration of a single oral dose of LCZ696 400 mg or valsartan 320 mg in healthy participants. Data are mean ± SD.

assessment) and were not considered related to treatment. One participant in the 200-mg multiple-dose group showed a slight decrease in serum potassium (3.3-3.6 mEq/L) that had returned to normal at the end of the study.

Bioavailability study. Overall, 10 participants reported AEs following LCZ696 administration and 8 following valsartan administration. Headache was the most frequently reported AE, experienced by 6 and 2 participants after LCZ696 and valsartan dosing, respectively. Dizziness (2 participants) and nausea (2 participants) were the only other events reported by more than 1 participant during the study.

Four participants (2 from the valsartan group and 2 from the LCZ696 group) discontinued the bioavailability study before the second treatment period because of abnormal laboratory values. Of the 2 participants who had received valsartan, 1 exhibited elevated AST and ALT; the other exhibited elevated ALT, bilirubin, and triglycerides. Of the 2 participants who had received LCZ696, 1 exhibited elevated creatine kinase and AST; the other exhibited low hemoglobin and hematocrit. Of these events, only elevated creatine kinase was considered clinically significant by the investigator. However, the participant was asymptomatic when evaluated by the investigator, with no reported AEs and normal ECG assessments, and had likely violated the exercise restriction for the study.

DISCUSSION

We report the results of preclinical and clinical studies conducted to evaluate the pharmacokinetics and pharmacodynamic effects of LCZ696, a novel single molecule comprising molecular moieties of the ARB valsartan and the NEP inhibitor prodrug AHU377. Pharmacokinetic studies in beagle dogs and a dose escalation study in human participants showed a rapid rise in plasma concentrations of valsartan and

Table IV Summary of the Pharmacokinetic Parameters of Valsartan Following Administration of Single Oral Doses of LCZ696 400 mg or Valsartan 320 mg in Healthy Volunteers in the Bioavailability Study

Treatment	t_{max}, h	C_{max}, ng/mL	$AUC_{0\text{-last}}$, ng·h/mL	$AUC_{0\text{-}\infty}$, ng·h/mL	CL/F, L/h	$V_{\lambda z}$/F, L	$t_{1/2}$, h
Valsartan 320 mg	3.4 ± 0.8	5204 ± 2570	36449 ± 18022	37354 ± 18415	10.2 ± 4.4	262 ± 241	17.5 ± 11.0
LCZ696 400 mg	2.2 ± 0.9	5138 ± 2039	32973 ± 13491	33707 ± 13421	7.2 ± 3.4	191 ± 238	17.7 ± 20.7

Values are mean ± SD. $AUC_{0\text{-}\infty}$, area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration–time curve between first and last observation; CL/F, plasma clearance corrected for bioavailability; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; t_{max}, time to achieve maximum plasma concentration; $V_{\lambda z}$/F, apparent volume of distribution corrected for bioavailability.

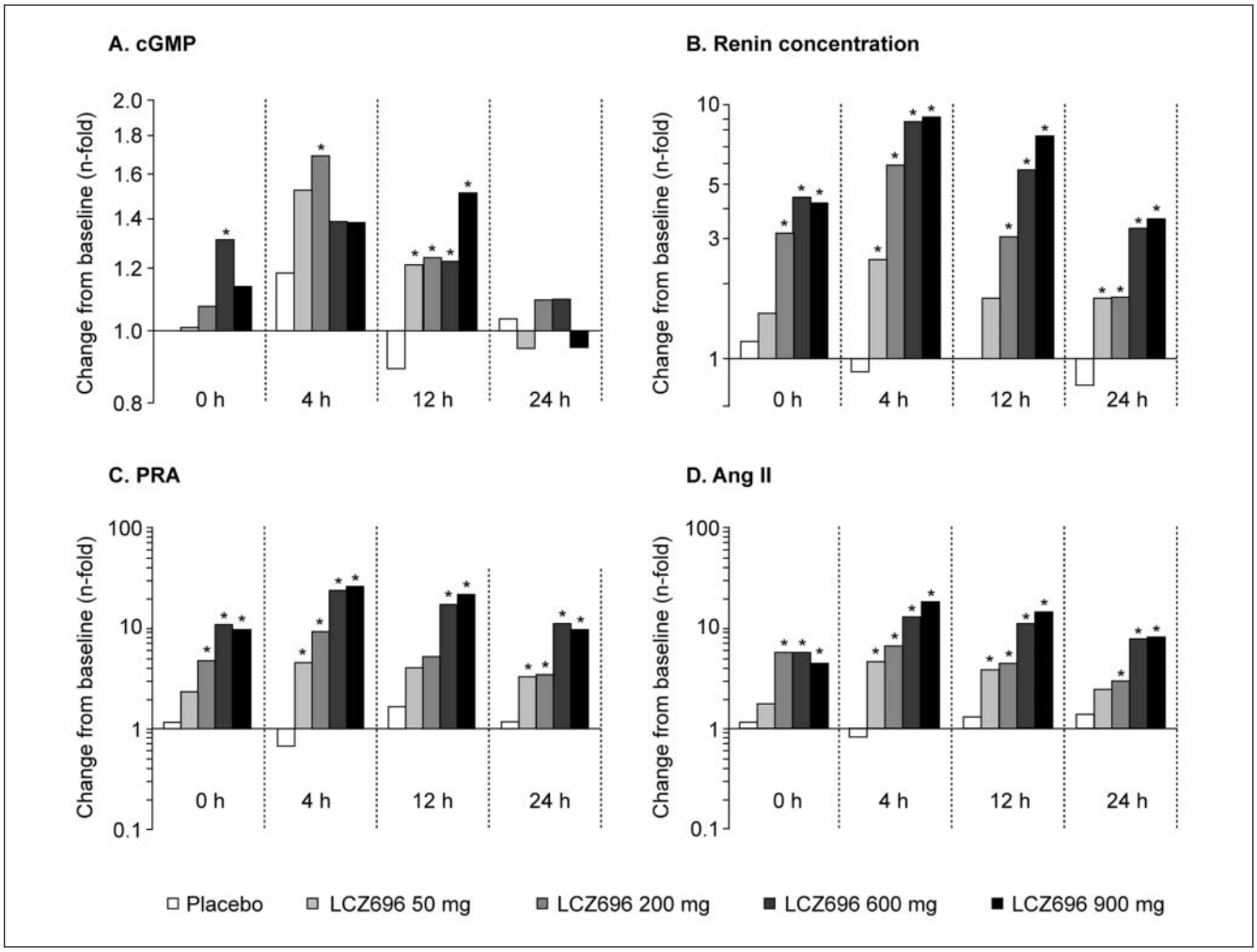

*Figure 5. Change from baseline in (A) plasma cGMP, (B) renin concentration, (C) plasma renin activity (PRA), and (D) angiotensin (Ang) II levels after once-daily oral administration of LCZ696 50, 200, 600, or 900 mg in healthy participants. Data are n-fold change from baseline (logarithmic scale) at the postdose time points indicated. *P < .05 vs placebo.*

AHU377 following oral administration of LCZ696. The short $t_{1/2}$ of AHU377 and t_{max} of LBQ657 demonstrated rapid metabolism of the prodrug AHU377 to the active NEP inhibitor moiety. Although there was evidence of dose proportionality for AHU377 and LBQ657 across the assessed oral dose range for LCZ696, no statistically significant dose-exposure relationship was observed. The increases in AUC

Table V Adverse Events (AEs) During Multiple-Dose Oral Administration of LCZ696 50, 200, 600, or 900 mg or Placebo in the Dose Escalation Study

	LCZ696				
	50 mg (n = 8)	200 mg (n = 8)	600 mg (n = 9)	900 mg (n = 8)	Placebo (n = 9)
Participants reporting an AE	3	2	4	7	3
Discontinuations due to an AE	0	0	0	0	0
Most frequently reported AEs (≥2 participants receiving any treatment)					
Postural orthostatic hypotension[a]	1	0	1	5	1
Orthostatic hypotension[b]	1	1	2	1	0
Headache	0	1	1	2	1
Dizziness	0	1	3	0	0

Values are the number of participants reporting an individual AE.
[a]Symptomatic (all participants reported tachycardia).
[b]Asymptomatic.

values for AHU377, as well as AUC and C_{max} values for LBQ657, were approximately proportional to the LCZ696 dose, whereas C_{max} values for LBQ657 showed approximate dose proportionality over the 200- to 900-mg dose range. There was a linear but less than proportional relationship between LCZ696 and the AUC and C_{max} for valsartan. Once-daily administration of LCZ696 for 14 days led to minimal accumulation of valsartan, AHU377, or LBQ657 irrespective of the dose of LCZ696.

The bioavailability study demonstrated that systemic exposure to valsartan following a single 400-mg oral dose of LCZ696 was equivalent to that following administration of 320 mg of valsartan, a dose that has proven antihypertensive efficacy in clinical trials.[11] Analysis of dose-normalized pharmacokinetic data from either preclinical or clinical studies showed that the exposure of valsartan following administration of LCZ696 was approximately 40% higher than the exposure following administration of valsartan alone.

Inhibition of NEP activity increases levels of ANP, which in turn stimulates the synthesis of cGMP via guanylyl cyclase–linked receptors. Preclinical pharmacodynamic studies demonstrated a rapid and dose-dependent increase in plasma ANP immunoreactivity following oral administration of LCZ696, whereas multiple-dose administration of LCZ696 in the dose escalation study in healthy participants significantly increased plasma cGMP levels. These findings are consistent with inhibition of NEP activity. The LCZ696-stimulated increase in cGMP peaked at 4 and 12 hours after dosing, shortly after the maximum plasma concentrations of LBQ657 were reached, and were similar to those reported with omapatrilat in healthy volunteers.[15] Marked reductions in BP would not be expected with administration of LCZ696 in normotensive healthy participants; indeed, previous studies in normotensive volunteers have shown little effect on BP of valsartan[16] or the NEP inhibitor candoxatril,[7] likely due to normal compensatory physiologic mechanisms to prevent hypotension. Studies in a dTGR model of Ang II–dependent hypertension[13] demonstrated sustained, dose-dependent decreases in BP following administration of LCZ696, supporting further studies of the antihypertensive efficacy of this agent in patients with hypertension.

The clinical dose escalation study also assessed markers of RAAS blockade. ARBs such as valsartan block the activity of Ang II at the angiotensin II type 1 (AT_1) receptor, reducing the normal feedback inhibition of renin release from the kidneys by Ang II and thereby increasing renin concentration, PRA, and Ang II levels.[17] LCZ696 stimulated significant, dose-dependent increases in renin concentration, PRA, and Ang II concentration compared with placebo, indicative of blockade of the AT_1 receptor by LCZ696. Notably, LCZ696 200 mg increased renin concentration by 3.1-fold, PRA by 4.9-fold, and Ang II by 3.7-fold relative to placebo. These increases are of a similar order of magnitude to those observed previously with administration of valsartan 320 mg in healthy participants not receiving a low-sodium diet.[18,19] Significant increases in all RAAS biomarkers were sustained 24 hours after LCZ696 administration, consistent with the observed long plasma $t_{1/2}$ of valsartan (15-22 hours).

Pharmacokinetic data from the clinical dose escalation study showed that the peak concentrations of valsartan and LBQ657 were reached at about the same time following both single- and multiple-dose LCZ696 administration (1.5-4.5 hours). This is reflected in the similar timeframe observed for pharmacodynamic effects, with the maximum concentrations for both cGMP and RAAS biomarkers reached by 4 hours after dosing with multiple-dose administration. In contrast, studies with omapatrilat suggested that the pharmacodynamic effects of NEP inhibition were delayed compared with ACE inhibition. In healthy volunteers, t_{max} for omapatrilat was 0.5 to 2.0 hours; near complete ACE inhibition was observed by 2 hours postdose,[15] whereas peak cGMP levels (indicative of NEP inhibition) were not reached until 4 to 8 hours postdose with single- or multiple-dose administration.[15,20] The concurrent effects of LCZ696 on NEP inhibition and AT_1 receptor blockade may have benefits for clinical efficacy. Furthermore, the sustained pharmacodynamic effects following LCZ696 administration, together with the long observed plasma $t_{1/2}$ for both valsartan and LBQ657, indicate the potential suitability of LCZ696 for once-daily dosing.

LCZ696 was well tolerated when administered as single doses of up to 1200 mg or as multiple doses of up to 900 mg once daily. Most AEs were mild and transient in nature, and there were no serious AEs or discontinuations due to AEs reported with LCZ696 administration in either study. Orthostatic hypotension, with or without symptoms, and dizziness were the most common AEs observed with LCZ696. These AEs might be expected following the administration of a potent BP-lowering agent to normotensive healthy participants. No clinically relevant trends in clinical laboratory values or ECG changes were observed with LCZ696 administration. These findings are consistent with the good safety and tolerability profile for valsartan observed in clinical trials.[11] Longer term clinical studies are required to evaluate whether the Ang II receptor blockade and NEP inhibition provided by LCZ696 will avoid the increased incidence of angioedema associated with the dual-acting ACE and NEP inhibitor omapatrilat.[9]

In summary, the pharmacokinetic and pharmacodynamic properties of LCZ696 indicate that it is a potent dual-acting angiotensin receptor–NEP inhibitor (ARNi). Following oral administration in healthy participants, LCZ696 rapidly delivers NEP inhibition and angiotensin receptor blockade. The time course and duration of AT_1 receptor blockade and NEP inhibition support once-daily dosing in hypertension. Antihypertensive efficacy of LCZ696 was demonstrated in a renin-angiotensin-driven genetic rat model of fulminant hypertension. These results support further development of LCZ696, a novel dual-acting angiotensin receptor–NEP inhibitor, for the treatment of hypertension and heart failure.

The authors appreciate the efforts of Michael Beil, Fumin Fu, Creton Kalfoglou, Silvia Pomposiello, Yinong Zhou, and Wenyu Hu in conducting the nonclinical studies. All authors participated in the writing and approval of study protocols and participated in the collection, analysis, and interpretation of data for one or more of the studies described in this article. All authors participated in the writing of the manuscript and approved the final manuscript for publication. The authors take full responsibility for the content of the paper but thank Dr Richard White (Oxford PharmaGenesis Ltd) for assistance in collating the comments of the authors and editing the final manuscript.

Financial disclosure: These studies were supported by Novartis Institutes for BioMedical Research, Inc, Cambridge, Massachusetts. All authors are employees of Novartis and are eligible for Novartis stock and stock options.

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