Angiotensin Receptor Neprilysin Inhibitor LCZ696 Attenuates Cardiac Remodeling and Dysfunction After Myocardial Infarction by Reducing Cardiac Fibrosis and Hypertrophy

Thomas G. von Lueder, MD, PhD*; Bing H. Wang, PhD*; Andrew R. Kompa, PhD; Li Huang, BSc; Randy Webb, PhD; Pierre Jordaan, PhD; Dan Atar, MD, PhD; Henry Krum, MBBS, PhD

Background—Angiotensin receptor neprilysin inhibitors (ARNi), beyond blocking angiotensin II signaling, augment natriuretic peptides by inhibiting their breakdown by neprilysin. The myocardial effects of ARNi have been little studied until recently. We hypothesized that LCZ696 attenuates left ventricular (LV) remodeling after experimental myocardial infarction (MI), and that this may be contributed to by inhibition of hypertrophy and fibrosis in cardiac cells.

Methods and Results—One week after MI, adult male Sprague–Dawley rats were randomized to treatment for 4 weeks with LCZ696 (68 mg/kg body weight perorally; MI-ARNi, n=11) or vehicle (MI-vehicle, n=6). Five weeks after MI, MI-ARNi versus MI-vehicle demonstrated lower LV end-diastolic diameter (by echocardiography; 9.7±0.2 versus 10.5±0.3 mm), higher LV ejection fraction (60±2 versus 47±5%), diastolic wall strain (0.23±0.02 versus 0.13±0.02), and circular strain (−9.8±0.5 versus −7.3±0.5%; all P<0.05). LV pressure–volume loops confirmed improved LV function. Despite similar infarct size, MI-ARNi versus MI-vehicle had lower cardiac weights (P<0.01) and markedly reduced fibrosis in peri-infarct and remote myocardium. Angiotensin II–stimulated incorporation of 3[H]leucine in cardiac myocytes and 3[H]proline in cardiac fibroblast was used to evaluate hypertrophy and fibrosis, respectively. The neprilysin inhibitor component of LCZ696, LBQ657, inhibited hypertrophy but not fibrosis. The angiotensin receptor blocker component of LCZ696, valsartan inhibited both hypertrophy and fibrosis. Dual valsartan+LBQ augmented the inhibitory effects of valsartan and the highest doses completely abrogated angiotensin II–mediated effects.

Conclusions—LCZ696 attenuated cardiac remodeling and dysfunction after MI. This may be contributed to by superior inhibition of LCZ696 on cardiac fibrosis and cardiac hypertrophy than either stand-alone neprilysin inhibitor or angiotensin receptor blocker. (Circ Heart Fail. 2015;8:71-78. DOI: 10.1161/CIRCHEARTFAILURE.114.001785.)

Key Words: angiotensins ■ brain natriuretic peptide ■ cardiac hypertrophy ■ left ventricular remodeling ■ myocardial infarction

Hypertension and heart failure (HF) are major causes of death and morbidity in the Western world, and their prevalence is projected to increase. Increasing recognition that sustained overdrive of neurohormonal systems such as the renin–angiotensin–aldosterone system (RAAS) is involved in HF pathophysiology has led to the introduction of drugs inhibiting key components of the RAAS into clinical practice. The success of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARB) underscores the importance of RAAS as a target in these (and other) cardiovascular disorders.

In addition to the RAAS, counter-regulatory hormonal pathways are also activated in HF. The natriuretic peptide (NP) system counteracts the RAAS by promoting vasodilation, natriuresis, and inhibition of fibrosis and hypertrophy. Biologically active NPs are degraded by the enzyme neutral endopeptidase or neprilysin; consequently, neprilysin inhibition represents an important pharmacological approach to augment the salutary actions of NPs. Presumably because of its affinity toward multiple substrate peptides beyond the NPs such as endothelin-1 and angiotensin II, stand-alone neprilysin inhibitor (NEPi) has not been proven to be clinically efficacious. Simultaneous blockade of RAAS and neprilysin

Received August 20, 2014; accepted October 29, 2014.

From the Department of Epidemiology and Preventive Medicine, Monash Center of Cardiovascular Research and Education in Therapeutics, Monash University, Alfred Hospital, Melbourne, Australia (T.G.v.L., B.H.W., A.R.K., L.H., H.K.); Department of Cardiology B, Oslo University Hospital Ullevål; Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway (T.G.v.L., D.A.); Department of Medicine, University of Melbourne, St Vincent’s Hospital, Fitzroy, Australia (A.R.K.); Development Franchise-Critical Care, Novartis Pharma, East Hanover, NJ (R.W., P.J.); and Oncology and Drug Safety & Epidemiology, Novartis Pharma, Basel, Switzerland (P.J.).

*Drs von Lueder and Wang contributed equally to this work.

Correspondence to Henry Krum, MBBS, PhD, Monash Centre of Cardiovascular Research and Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University, Alfred Hospital, Melbourne, VIC 3004, Australia. E-mail henry.krum@med.monash.edu.au

© 2014 American Heart Association, Inc.

Circ Heart Fail is available at http://circheartfailure.ahajournals.org

DOI: 10.1161/CIRCHEARTFAILURE.114.001785

71
through dual-acting ACE and NEPi (vasopeptidase inhibitors) has been evaluated a decade ago. Its prototypic drug, omapatrilat, showed favorable effects in clinical trials of hypertension and HF, but further development was halted because of increased rates of angioedema, presumably caused by accumulation of bradykinin in at-risk patients.\textsuperscript{11,12} Recently, a new drug class, angiotensin receptor neprilysin inhibitors (ARNi), have been developed to simultaneously block the RAAS and augment NPs through neprilysin inhibition at a presumed lower risk of bradykinin-induced angioedema.\textsuperscript{13,14} In a preclinical model, ARNi (using valsartan–candoxatril) provided similar antihypertensive efficacy as omapatrilat without inducing tracheal plasma extravasation (a surrogate of angioedema).\textsuperscript{15}

LCZ696 has been the first ARNi to be evaluated in patients with hypertension and HF.\textsuperscript{16-18} LCZ696 combines a moiety of the ARB valsartan and the NEPi precursor, AHU377. After intake, LCZ696 is metabolized into active valsartan and inactive AHU377, whereas AHU377 is further cleaved into the active NEPi LBQ657. LCZ696 thus far has shown promising efficacy without significant occurrence of off-target effects. However, the effects of ARNi (LCZ696) on cardiac fibrosis, hypertrophy, and remodeling after myocardial infarction (MI) remain to be established.

We therefore hypothesized that LCZ696 would have beneficial effects on cardiac remodeling after experimental MI, and that this may be contributed to by inhibition of hypertrophy and fibrosis in cardiac cells.

Methods

Induction of MI, Treatment, and Study Protocol

Adult 6- to 8-week-old male Sprague–Dawley rats (220–250 g body weight) were subjected to induction of MI by left anterior descending ligation as routinely performed in our laboratory.\textsuperscript{19} Rats were randomized in a 2:1 fashion to 4 weeks of treatment with LCZ696 (68 mg/kg body weight perorally; MI-ARNi, n=11) or vehicle (MI-Vhc, n=6). LCZ696 was a kind gift of Novartis, Basel, Switzerland. Treatment commenced 1 week after surgery (baseline) to avoid interference with reparative scar formation. Glomerular filtration rate (GFR), proteinuria, serum, and urinary creatinine were determined and echocardiography performed before treatment was commenced. Five weeks after MI (end point), systolic blood pressure was measured in conscious rats using the tail-cuff method before being placed in a metabolic cage to allow rest. On the following day, cardiac function was assessed by echocardiography and invasive left ventricular (LV) catheterization, respectively, before rats were euthanized and organs harvested.\textsuperscript{19}

Echocardiography and Hemodynamic Measurements

Standard 2-dimensional and Doppler echocardiography was performed before and after 4 weeks of treatment in lightly anesthetized animals (40 mg/kg ketamine and 5 mg/kg IP xylazine) using a commercially available system (GE Vingmed Vivid 7, Horten, Norway) with a 10-MHz phased array probe, as routinely used in our laboratory.\textsuperscript{19} All animals were anesthetized with pentobarbitone (60 mg/kg IP) and intubated for cardiac catheterization procedures, as previously described.\textsuperscript{19} Briefly, animals were ventilated and a 2-F miniaturized combined catheter/micromanometer (model SPR838; Millar Instruments, Houston, TX) was inserted into the right common carotid artery to obtain aortic blood pressure and then advanced into the left ventricle to obtain LV pressure–volume loops. Pressure–volume loops were recorded at steady state and during transient preload reduction, achieved by occlusion of the inferior vena cava and portal vein with the ventilator turned off and animal aneoxic. The following validated parameters were assessed using Millar conductance data acquisition and analysis software PVAN 3.5: LV end-systolic pressure, LV end-diastolic pressure, maximal and minimal conductance data acquisition and analysis software PVAN 3.5: LV end-systolic pressure, LV end-diastolic pressure, maximal and minimal change in pressure over time ($dP/dt_{max}$ and $dP/dt_{min}$), $\tau$ (t Logistic), and the slope of the preload recruitable stroke work relationship. At the conclusion of the measurements, tissues were harvested and fixed in neutral buffered formalin and processed for histopathology.

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (PHS Approved Animal Welfare Assurance No. A5587-01). All animal usage was also approved by St. Vincent’s Hospital’s Animal Ethics Committee in accordance with National Health and Medical Research Council’s Guide for the Care and Use of Laboratory Animals (Animal Ethics Committee No. 028/11).

Histological Analysis of Cardiac Tissue

Paraffin-embedded LV tissue sections (4 µm) were stained with picrosirius red to determine interstitial fibrosis using Aperio ScanScope Console v.8.0.0.1058 (AperioTechnologies, Inc). Picrosirius red staining in the noninfarct zone and peri-infarct zone of the LV was selected for its intensity of staining, and the percentage area was calculated using an established algorithm.\textsuperscript{19} Similarly, perivascular fibrosis was defined as the area of picrosirius red staining immediately surrounding the adventitia of the intramural coronary arteries. The intensity and algorithm were preset and maintained constant for analysis of all sections. Infarct size was expressed as an averaged percentage of the endocardial and epicardial scarred circumferences of the LV.

Cellular Cardiac Hypertrophy and Fibrosis In Vitro

Rat neonatal cardiac myocytes and fibroblasts were obtained from 1- to 2-day-old Sprague–Dawley rat pups by enzymatic collagenase digestion and prepared for in vitro assays as routinely used in our laboratory.\textsuperscript{20} Cardiac myocyte hypertrophy was assessed by AngII-stimulated (100 nM) neonatal cardiac myocytes with 3[H]leucine incorporation for 60 hours. AngII-stimulated (100 nM) collagen synthesis was determined by 3[H]proline incorporation in neonatal cardiac fibroblasts for 48 hours. Cells were preincubated with valsartan, AHU377, LBQ657, or valsartan+LBQ657 (ARNi) for 1 hour before stimulation. Dose ranges used and NEPi to ARB ratios aimed to replicate as far as possible doses of LCZ696 used clinically. The drugs were a kind gift of Novartis, Basel, Switzerland. In addition, exogenous B-type natriuretic peptide (BNP) was added at different concentrations into the cell culture media just before AngII stimulation to assess the effect of direct augmentation of NP signaling. Experiments were repeated 2 to 4× in triplicate each time.

Statistical Analysis

Values are expressed as mean±SEM. For in vivo experiments, unpaired Student $t$ test was used. For cell studies, 1-way ANOVA with Bonferroni post hoc analysis was used for comparison among all groups and paired $t$ test was used for comparisons between treated groups using GraphPad Prism software version 5 as described.\textsuperscript{21} A 2-tailed $P$ value of $<0.05$ was considered significant.

Results

Effects of ARNi on Cardiac Hypertrophy and Fibrosis After Experimental MI

After 4 weeks of treatment, MI-ARNi exhibited significantly smaller weights compared with MI-Vhc in all cardiac chambers,
consistent with reduced cardiac hypertrophy (Table 1). Lung and kidney weights were similar between groups. Although heart rate was comparable, blood pressure trended to be lower with LCZ696 versus MI-Vhc. The degree of fibrosis both in noninfarcted remote myocardium (Figure 1A) and in the peri-infarct zone (Figure 1B) was significantly reduced in MI-ARNi versus MI-Vhc. In contrast, no differences in perivascular fibrosis in noninfarcted (Figure 1C) or peri-infarct (Figure 1D) myocardium were discerned.

Effects of ARNi on LV Remodeling and Function
Infarct size was substantial and consistent with large MI, and similar in both groups (Figure 1E). Echocardiography 1 week after MI before treatment demonstrated similar LV dimensions and function in both groups (Table 2).

At end point, MI-ARNi exhibited smaller LV cavity dimensions with unaltered wall thickness versus MI-Vhc (Table 3). Computed LV mass with MI-ARNi was lower than MI-Vhc, consistent with organ weights. Ejection fraction and fractional area change were increased, as was diastolic wall strain.

Doppler echocardiography analysis revealed similar early and reduced late diastolic filling and a trend toward higher E/A ratios in MI-ARNi versus MI-Vhc. This was reflected by a similar pattern of early and late myocardial velocities by TDI. E/e ratios (an estimate of LV filling pressure) and systolic myocardial velocities were similar between groups. At similar degree of basal rotation, circular strain was increased in MI-ARNi versus MI-Vhc.

Hemodynamics in ARNi- Versus Vehicle-Treated Rats After MI
Analysis of steady-state LV pressure–volume loops in vivo revealed no statistically significant differences in heart rate, LV volumes, LV pressures, cardiac output, or arterial pressures (Table 1). However, LV unloading by caval vein occlusion revealed a significantly higher end-systolic pressure–volume relationship ($P<0.001$), lower $\tau$ ($P<0.05$), and a trend for lower end-diastolic pressure–volume relationship ($P=0.10$) in MI-ARNi versus MI-Vhc animals.

Renal Function After MI
Renal function in MI-Vhc and MI-ARNi at baseline was similar, and neither urinary creatinine nor proteinuria changed significantly in either group. Despite small increases in plasma creatinine in both groups during the study period, the difference between values at end point versus baseline was significant only in MI-Vhc (not shown). GFR significantly decreased in both groups and to a greater extent in MI-Vhc; accordingly, both absolute GFR values at end point (MI-Vhc $7.1\pm0.5$ versus MI-ARNi $8.3\pm0.3 \text{ mL/min per kilogram}$; $P=0.07$) and the difference in changes in GFR over time (MI-Vhc $-4.7\pm0.7$ versus MI-ARNi $-3.6\pm0.2 \text{ mL/min per kilogram}$; $P=0.09$) nearly reached significance.

Effects of Stand-Alone NEPi on Cellular Cardiac Fibrosis and Hypertrophy
AngII induced profound collagen accumulation in fibroblasts (Figure 2A) and cardiac myocyte hypertrophy (Figure 2B), respectively, as has been shown previously. The inactive NEPi precursor, AHU377, did not inhibit collagen accumulation in fibroblasts nor cardiac myocyte hypertrophy (data not shown). In cardiac fibroblasts, the active NEPi LBQ657 had no discernible effects (Figure 2A). In contrast, LBQ657 modestly inhibited cardiac myocyte hypertrophy (Figure 2B).

Effects of Stand-Alone ARB and ARNi on Cardiac Fibrosis and Hypertrophy
Valsartan dose dependently inhibited collagen accumulation in fibroblasts (Figure 3A) and cardiac myocyte hypertrophy (Figure 3B), respectively. In cardiac fibroblasts, addition of LBQ657 ($10 \mu\text{mol/L}$) significantly augmented the inhibitory effects of valsartan except for the highest dose where both valsartan and valsartan+LBQ657 values were reduced to unstimulated negative control values (Figure 4A). In cardiac myocytes, addition of LBQ657 ($10 \mu\text{mol/L}$) significantly augmented the inhibitory effects of the lowest dose of valsartan; however, only the highest combined dose of ARNi (valsartan+LBQ657) afforded complete inhibition of AngII-induced hypertrophy to values that were similar to unstimulated negative controls (Figure 4B).

BNP Reduced AngII-Stimulated Cellular Cardiac Hypertrophy and Cardiac Fibrosis
Increasing doses of BNP into culture media dose dependently reduced AngII-stimulated collagen synthesis in cardiac...
fibroblasts (Figure 5A) as well as cardiac myocyte hypertrophy (Figure 5B). The highest BNP dose (ie, $10^{-8}$ mol/L) resulted in inhibition at levels that were not different from unstimulated negative controls.

**Discussion**

We herein present novel data on the effects of LCZ696, a first-in-class ARNi, on post-MI cardiac remodeling and on AngII-induced hypertrophy and fibrosis in cardiac cell lines.

First, our data show that treatment with LCZ696 versus vehicle resulted in attenuation of cardiac dysfunction, fibrosis and remodeling, and somewhat attenuated decline in kidney function in rats after experimental MI. Second, although the inactive NEPi precursor, AHU377, did not affect AngII-stimulated cellular cardiac hypertrophy and fibrosis, its active metabolite LBQ657 in vitro modestly inhibited cardiac hypertrophy, but not cardiac fibrosis. Third, valsartan potently inhibited AngII-stimulated cellular cardiac hypertrophy and cardiac fibrosis. Fourth, concomitant LBQ657 and valsartan augmented the effects of the stand-alone ARB valsartan in cell culture. Finally, BNP dose dependently inhibited AngII-mediated effects in both cell types, providing further rationale for augmentation of salutary NP effects in cardiac hypertrophy and fibrosis.

Cardiac dysfunction and remodeling were attenuated by 4 weeks treatment with LCZ696, commencing 1 week after experimental MI. The delay in commencement of treatment aimed to avoid interference with reparative scar tissue formation and hemodynamic stabilization. Despite large and similar infarct size in both groups, organ weights and cardiac dimensions (by echocardiography) consistently showed a 15% to 20% reduction in LV size by LCZ696. Moreover, echocardiography and in vivo hemodynamic measurements revealed better preservation of cardiac function. Although early diastolic LV inflow and myocardial velocities were not different, lower late diastolic LV inflow, reduced diastolic wall strain (ie, an index of LV diastolic stiffness), lower $\tau$, higher end-diastolic pressure–volume relationship, and reduced atrial size all support better-preserved diastolic function. This is consistent with effects of LCZ696 to reduce atrial adverse remodeling in patients with heart failure with preserved ejection fraction even in the absence of discernible effects on LV diastolic filling. Here, MI rats did not exhibit signs of overt HF, for example, elevated lung weights but it may be reasonable to assume that HF would have developed during longer observation time, although the study was not designed to assess evolution to HF or mortality. The observed improvements in cardiac function afforded by LCZ696 may be related both to reduced cardiac hypertrophy (as manifest by decreased LV weight and mass) and a reduction in interstitial fibrosis in the peri-infarct and remote myocardium, but not perivascular fibrosis. Further mechanistic studies are required to elucidate the precise effect on profibrotic signaling cascades.

A previous report investigating dual pathway inhibition with omapatrilat versus the ARB candesartan demonstrated that both agents attenuated LV remodeling and dysfunction as well as several profibrotic peptides in rats after ischemia–reperfusion injury. In addition, Bäcklund et al showed superior efficacy of omapatrilat versus captopril on
cardiomyocyte apoptosis and cardiac remodeling after MI. LCZ696 also seems to show similar increased efficacy beyond stand-alone RAAS blockade as omapatrilat in this study. The clinical introduction of omapatrilat had been halted because of increased rates of angioedema in clinical trials leading to the development of ARNi.

Our in vitro studies were designed to test whether effects of LCZ696 on LV remodeling were contributed to by direct actions on relevant cardiac cell types. Furthermore, we were specifically interested in whether NEPi resulted in incremental effects on cardiac fibrosis as well as hypertrophy, in addition to that achieved by ARB. Our data did not demonstrate an in vitro effect of the inactive NEPi prodrug AHU377 in either cell type. This is consistent with the established mode of action of AHU377 requiring enzymatic cleavage to LBQ657, the active inhibitor of neprilysin. LBQ657, however, showed a distinct pattern of action in the different cell types. In cardiac fibroblasts, no discernible effect of LBQ657 at any tested dose was found despite clear add-on effects (when added to valsartan) and despite similar efficacy of exogenous BNP to inhibit AngII-mediated signaling in that cell type. Although not specifically tested, increase in other, potentially unfavorable neprilysin substrates such as endothelin or even AngII itself may have counteracted NEPi-induced augmentation of BNP, resulting in neutral effects on collagen accumulation. This is consistent with neutral net effects of NEPi in humans.9,10,25 In contrast, stand-alone LBQ657 was found to attenuate hypertrophy in cardiac myocytes.

Predictably, valsartan potently attenuated AngII-induced signaling in both cell types. Addition of LBQ657 to valsartan (replicating an ARNi) further augmented inhibitory effects. This was also observed in cardiac fibroblasts in which stand-alone LBQ657 did not show discernible effects despite cardiac fibroblasts having been shown to produce BNP in response to various noxious stimuli.26 In contrast, in cardiac myocytes, the net effect of stand-alone neprilysin inhibition (by LBQ657) was that of attenuation of hypertrophy. Both in cardiac fibroblasts and in myocytes only the highest ARNi dose afforded complete inhibition of AngII-mediated signaling. In line with important effects of NP augmentation by LBQ657, BNP potently inhibited AngII-induced signaling in both cardiac cell lines.

In patients with HF, elevated NP levels are frequently found in concert with the degree of cardiac dysfunction and Table 2. Baseline Echocardiography in Rats 1 Week After MI Before Treatment With Vhc or ARNi

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MI-Vhc (n=6)</th>
<th>MI-ARNi (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>8.1±0.1</td>
<td>7.7±0.4</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>5.6±0.4</td>
<td>6.2±0.2</td>
</tr>
<tr>
<td>Anterior wall thickness, mm</td>
<td>1.29±0.08</td>
<td>1.40±0.05</td>
</tr>
<tr>
<td>Diastolic wall strain</td>
<td>0.15±0.02</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>57±3</td>
<td>51±1</td>
</tr>
<tr>
<td>E/A</td>
<td>1.84±0.24</td>
<td>1.98±0.21</td>
</tr>
<tr>
<td>E/Ea</td>
<td>20.8±2.1</td>
<td>23.8±2.0</td>
</tr>
</tbody>
</table>

No significant differences, MI-ARNi vs MI-Vhc; unpaired t test. ARNi indicates angiotensin receptor neprilysin inhibitor; LV, left ventricular; MI, myocardial infarction; and Vhc, vehicle.

Table 3. Left Ventricular Function by Echocardiography in MI Rats After 4 Weeks of Treatment With Vhc or ARNi

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MI-Vhc (n=5)</th>
<th>MI-ARNi (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>10.5±0.3</td>
<td>9.7±0.2*</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>8.4±0.7</td>
<td>7.6±0.2</td>
</tr>
<tr>
<td>Anterior wall thickness, mm</td>
<td>1.12±0.06</td>
<td>1.09±0.02</td>
</tr>
<tr>
<td>Diastolic wall strain</td>
<td>0.13±0.03</td>
<td>0.23±0.02†</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>0.55±0.03</td>
<td>0.50±0.03</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>0.30±0.03</td>
<td>0.20±0.03*</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>47±5</td>
<td>60±2*</td>
</tr>
<tr>
<td>Computed LV mass, mg</td>
<td>959±68</td>
<td>793±32*</td>
</tr>
<tr>
<td>E, m/s</td>
<td>0.79±0.04</td>
<td>0.69±0.03</td>
</tr>
<tr>
<td>A, m/s</td>
<td>0.55±0.05</td>
<td>0.41±0.02*</td>
</tr>
<tr>
<td>E/A</td>
<td>1.46±0.10</td>
<td>1.72±0.11</td>
</tr>
<tr>
<td>E/Ea</td>
<td>21.1±1.1</td>
<td>20.3±1.8</td>
</tr>
<tr>
<td>E/Em</td>
<td>1.14±0.17</td>
<td>1.46±0.16</td>
</tr>
<tr>
<td>Circular strain, %</td>
<td>−7.3±0.5</td>
<td>−9.8±0.5†</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.01; MI-ARNi vs MI-Vhc; unpaired t test. ARNi indicates angiotensin receptor neprilysin inhibitor; LV, left ventricular; MI, myocardial infarction; and Vhc, vehicle.

Figure 2. Effects of LBQ657 in cardiac fibroblasts and myocytes. A, Effects of LBQ657 in cardiac fibroblasts. B, Effects of LBQ657 in cardiac myocytes. AngII indicates angiotensin II; AngII vs control; #P<0.05; ##P<0.01; ####P<0.0001; AngII vs AngII.
symptom status. Despite their natriuretic and diuretic effects, administration of synthetic NPs has failed to improve clinical outcomes, at least in the acute setting. Conceptually ARNi offers the advantage of augmenting endogenous NPs, combining their benefits with those of ARBs, well-established drugs in hypertension and HF. Contemporary clinical data underscore the efficacy of ARNi in patients with hypertension and heart failure with preserved ejection fraction and important surrogate end points, with an acceptable safety profile.16,17,27 Recently, the prospective comparison of ARNi with angiotensin-converting enzyme inhibitors to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF), a large (n=8422) outcome study assessing efficacy and safety of LCZ696 versus the angiotensin-converting enzyme inhibitors, enalapril, in patients with HF and reduced ejection fraction was stopped prematurely after interim analysis suggested superiority over standard therapy with angiotensin-converting enzyme inhibitors, the current gold standard.18,28 PARADIGM-HF will likely provide definite answers on the efficacy and safety of LCZ696 in HF. Given that many patients in PARADIGM-HF have an ischemic basis to their LV dysfunction, the findings of the present study will likely provide mechanistic insight into the benefits of LCZ696 in PARADIGM.29

Limitations
This study used only male rats, thus sex differences in efficacy, if any, cannot be determined from this work. The PARADIGM-HF trial did not show heterogeneity in efficacy response across sexes.

The use of sedation and anesthesia for echocardiography and invasive hemodynamic assessment, respectively, may have influenced the results. However, we used a consistent level of anesthesia across all animals reflected by similar

---

**Figure 3.** Effects of single angiotensin receptor blocker, valsartan on cellular cardiac fibrosis and hypertrophy. As expected, angiotensin (Ang) II–stimulated collagen synthesis in cardiac fibroblasts (A) and cardiac myocyte hypertrophy (B), respectively, were robustly and dose dependently inhibited by angiotensin receptor blockade with valsartan. Yet, the degree of inhibition did not increase much further from the second highest to highest valsartan concentration consistent with leveling off of valsartan efficacy at either cell type. Even at the highest valsartan concentration a small but significant residual AngII-stimulated effect still could be observed. ****P<0.0001, AngII vs control; ###P<0.001, ####P<0.0001, valsartan vs AngII.

**Figure 4.** Effects of angiotensin receptor nephrilysin inhibitor (ARNi) compared with stand-alone angiotensin receptor blocker (ARB) on cellular cardiac fibrosis and hypertrophy. Although inefficient as stand-alone, addition of fixed-dose LBQ657 (10 μmol/L) significantly augmented the inhibitory effects of valsartan (VAL) in cardiac fibroblasts. This was true for all VAL doses except for the highest dose where the difference between ARB (VAL) and ARNi (VAL+LBQ657) values were smaller than their differences with unstimulated negative control values (A). Accordingly, ARNi with both the second highest and the highest VAL concentration abrogated angiotensin (Ang) II signaling completely. In cardiac myocytes, addition of fixed-dose LBQ657 (10 μmol/L) significantly augmented the inhibitory effects of the lowest, but not the 2 middle-range VAL doses. Only the highest combined dose of ARNi (VAL+LBQ) afforded complete inhibition of AngII-induced hypertrophy to values not different from unstimulated negative controls (B). ****P<0.0001, AngII vs control; ###P<0.001, ####P<0.0001, LBQ657 vs AngII; $P<0.05, VAL vs LBQ+VAL.
von Lueder et al

ARNi Attenuates Cardiac Fibrosis and Hypertrophy

77

References


2. Radford MJ, Arnold JM, Bennett SJ, Cinquegrani MP, Cleland JG, Havranek EP, Heidenreich PA, Rutherford JD, Spertus JA, Stevenson LW, Golf DC, Grover FL, Malekna DJ, Peterson ED, Redberg RF; American College of Cardiology; American Heart Association Task Force on Clinical Data Standards; American College of Chest Physicians; International Society for Heart and Lung Transplantation; Heart Failure Society of America. ACC/AHA key data elements and definitions for measuring the clinical management and outcomes of patients with chronic heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (Writing Committee to Develop Heart Failure Clinical Data Standards): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Failure Society of America. Circulation. 2005;112:1888–1916.


Sources of Funding

This work was supported by National Health Medical Research Council of Australia Program Grant ID 546272. Dr von Lueder was supported by research grant ID 2011062 from the South-Eastern Norway Regional Health Authority.

Disclosures

Dr Krum has research contracts and has served as a consultant to Novartis. Dr von Lueder has received lecture honoraria from Novartis. The other authors report no conflicts.

Figure 5. Effects of exogenous B-type natriuretic peptide (BNP) on angiotensin (Ang) II-stimulated cellular cardiac hypertrophy and fibrosis in vitro. Coincubation with increasing doses of BNP in cell culture media dose dependently reduced AngII-stimulated collagen accumulation in cardiac fibroblasts (A) and hypertrophy of cardiac myocytes (B). No further inhibition was observed between 10^{-9} and 10^{-8} mol/L consistent with plateau of response. The highest BNP dose (ie, 10^{-6} mol/L) resulted in inhibition at levels that were not different from unstimulated negative controls. *P<0.05, **P<0.01, and ***P<0.001, BNP (logM) vs AngII.

heart rates so any effects should be like-for-like across both study groups.

In summary, we have shown that the ARNi LCZ696 attenuated cardiac remodeling and dysfunction after experimental MI and inhibited cardiac fibrosis and cardiac hypertrophy in vivo after MI, as well as in vitro beyond that achieved by stand-alone ARB. Our data may offer novel mechanistic insight into the benefits observed with LCZ696 in clinical studies. The present findings suggest that LCZ696, by combining RAAS blockade with augmentation of beneficial NP effects, has potential as a novel therapeutic agent after MI as well as in a broader spectrum of cardiovascular disorders.


**CLINICAL PERSPECTIVE**

Natriuretic peptides, among the most potent endogenous vasodilators in man, are activated on increased cardiac stress occurring in heart failure and other cardiovascular diseases. Natriuretic peptides exert their actions on the kidneys and protect the heart and vasculature through volume and pressure unloading. This mechanism seems to become deficient in heart failure at least in part by increased activity of nephrilysin, the endopeptidase that breaks down natriuretic peptides (and angiotensin II). Recently, the first-in-class angiotensin receptor nephrilysin inhibitor (ARNi) LCZ696 showed efficacy in a phase II study in patients with heart failure (PARAMOUNT [Prospective comparison of ARNI with ARB on Management Of heart failure with preserved ejection fraction]) and preserved ejection fraction as well as in those with reduced ejection fraction (PARADIGM-HF [Prospective comparison of ARNI with angiotensin-converting enzyme inhibitors to Determine Impact on Global Mortality and morbidity in Heart Failure trial]). The present study evaluated effects of ARNI on cardiac remodeling after myocardial infarction, as well as potential direct effects on cardiac hypertrophy and fibrosis. Our data demonstrate a significant reduction in post–myocardial infarction cardiac remodeling and dysfunction, accompanied by a marked reduction in pathological fibrosis. In addition, ARNI exhibited potent antifibrotic and antihypertrophic effects in cardiac cells beyond either single nephrilysin inhibition or stand-alone angiotensin receptor blocker. This is suggestive of synergistic effects of dual blockade of these 2 key therapeutic targets. B-type natriuretic peptide replicated the actions of ARNI on angiotensin II–stimulated cardiac cells, suggesting, additional to hemodynamic unloading, that direct cardioprotective effects of natriuretic peptides are exerted in conditions of neurohormonal activation. Our data therefore provide novel mechanistic insight into efficacy of ARNI in recent clinical trials and suggest evaluation in a broader setting of cardiovascular disease associated with fibrosis and hypertrophy such as post–myocardial infarction and in cardiorenal syndrome.
Angiotensin Receptor Neprilysin Inhibitor LCZ696 Attenuates Cardiac Remodeling and Dysfunction After Myocardial Infarction by Reducing Cardiac Fibrosis and Hypertrophy

Thomas G. von Lueuder, Bing H. Wang, Andrew R. Kompa, Li Huang, Randy Webb, Pierre Jordaan, Dan Atar and Henry Krum

Circ Heart Fail. 2015;8:71-78; originally published online October 31, 2014; doi: 10.1161/CIRCHEARTFAILURE.114.001785

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circheartfailure.ahajournals.org/content/8/1/71

Data Supplement (unedited) at:
http://circheartfailure.ahajournals.org/content/suppl/2014/10/31/CIRCHEARTFAILURE.114.001785.DC1
http://circheartfailure.ahajournals.org/content/suppl/2016/12/26/CIRCHEARTFAILURE.114.001785.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/
Supplemental figure: Changes in key echocardiography parameters shown as differences (deltas) in values at final minus at baseline assessment. Changes in LV end-diastolic volume were not statistically different between groups (A). Compared to MI-Vhc, MI-ARNi showed significant differences of changes in LV end-systolic volumes (B), ejection fraction (C), and computed LV mass (D) consistent with attenuation of adverse LV remodelling and dysfunction. While the difference in changes in diastolic wall strain (E) did not reach statistical significance, changes in LV fractional area change did (F). **p<0.01, MI-ARNi vs MI-Vhc, unpaired t-test; ns, no significant differences.
LCZ696은 심근경색 후 심근 섬유화 및 비후(심실 재형성)를 개선한다

김응주 교수 · 고려대학교 구로병원 순환기내과

초록

배경
안지오텐신수용체 네프릴리신 억제제(angiotensin receptor nepriyisin inhibitors, ARNi)는 안지오텐신 II의 역제뿐만 아니라 나트륨이뇨펩티드(natriuretic peptide)를 분해시 키는 네프릴리신을 억제해 나트륨이뇨펩티드를 증가시킨다. ARNi의 심근 효과는 아직까지 연구된 바가 거의 없다. 따라서, LCZ696이 심장세포의 비후와 섬유화를 억제해 심근경색 후 좌심실의 재형성을 완화시킬 수 있는지 알아보고자 하였다.

방법 및 결과
심근경색 1주 후, Sprague-Dawley 쥐를 치료군(LCZ696 68mg/kg 경구 투여, 4주간, 11마리)과 대조군(6마리)으로 무작위 배정하였다. 심근경색 5주 후, 치료군은 대조군에 비해 좌심실 확장기일 직경(심초음파상 9.7±0.2 vs. 10.5±0.3mm)은 작았고, 좌심실 구혈률(60±2 vs. 47±5%), 확장기벽 스트레인(0.23±0.02 vs. 0.13±0.02), 원형 스트레인(-9.8±0.5 vs. -7.3±0.5%; 모두 편=0.05)은 컸다. 좌심실 압력-용적 곡선(pressure-volume loops)로 좌심실기능의 개선을 확인한 결과, 심근경색의 크기 비슷한 것만 치료군은 심장무게가 더 작았고(P<0.01), 섬근경색 주위뿐만 아니라 원위부에서도 심근의 섬유화가 현저히 적었다. 안지오텐신 II 자극으로 심근세포의 3[H]leucine과 심장섬유아세포(cardiac fibroblast)의 3[H]proline에서 비후와 섬유화 정도를 각각 평가하였다. LCZ696 중 네프릴리신 억제 부분인 LBQ657은 비후만 저해한 반면, 안지오텐신수용체 억제 부분인 발살탄은 비후와 섬유화 모두를 억제하였다. 발살탄과 LBQ를 함께 사용했을 때는 발살탄에 의한 억제 효과가 증폭되었으며, 최고 농도에서는 안지오텐신 II에 의한 효과가 완전히 무력화되었다.

결론
LCZ696은 심근경색 후 심장의 재형성과 기능장애를 완화시켰다. 이러한 결과는 네프릴리신 억제제와 안지오텐신수용체 억제제의 동시 사용(LCZ696)이 각각의 단독 사용에 비해 심장 섬유화 및 비후 억제에 더 우월하게 작용했기 때문으로 생각된다.