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

von Lueder et al: ARNi Attenuates Cardiac Fibrosis and Hypertrophy

Thomas G. von Lueder, MD, PhD1,2; Bing H. Wang, PhD1; Andrew R. Kompa, PhD1,3; Li Huang, BSc1; Randy Webb, PhD4; Pierre Jordaan, PhD5,6; Dan Atar, MD, PhD2; Henry Krum, MBBS, PhD1

1Monash Centre of Cardiovascular Research and Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University, Alfred Hospital, Melbourne, Australia; 2Department of Cardiology B, Oslo University Hospital Ullevål, and Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; 3Department of Medicine, University of Melbourne, St Vincent’s Hospital, Fitzroy, Australia; 4Novartis Institutes for BioMedical Research, East Hanover, NJ; 5Novartis Institutes for BioMedical Research, Basel, Switzerland

*The first two authors contributed equally.

Correspondence to
Prof. 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
Fax +61 3 990 30556
Phone +61 3 990 30042
E-mail: henry.krum@med.monash.edu.au

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Abstract

**Background**—Angiotensin-receptor neprilysin inhibitors (ARNi), beyond blocking angiotensin II (AngII)-signalling, augment natriuretic peptides by inhibiting their breakdown by neprilysin (NEP). 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 four weeks with LCZ696 (68 mg/kg body weight PO; MI-ARNi, n=11) or vehicle (MI-Vhc, n=6). Five weeks after MI, MI-ARNi versus MI-Vhc demonstrated lower LV end-diastolic diameter (LVEDD, by echocardiography; 9.7±0.2 vs 10.5±0.3 mm), higher LV ejection fraction (LVEF, 60±2 vs 47±5%), diastolic wall strain (0.23±0.02 vs 0.13±0.02), and circular strain (CS, -9.8±0.5 vs -7.3±0.5%, all P<0.05). LV pressure-volume loops confirmed improved LV function. Despite similar infarct size, MI-ARNi versus MI-Vhc had lower cardiac weights (P<0.01), and markedly reduced fibrosis in peri-infarct and remote myocardium. AngII-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 NEP inhibitor component of LCZ696, LBQ657, inhibited hypertrophy but not fibrosis. The ARB component of LCZ696, valsartan (VAL) inhibited both hypertrophy and fibrosis. Dual VAL+LBQ augmented the inhibitory effects of VAL and the highest doses completely abrogated AngII-mediated effects.

**Conclusions**—LCZ696 attenuated cardiac remodeling and dysfunction post-MI. This may be contributed to by superior inhibition of LCZ696 on cardiac fibrosis and cardiac hypertrophy than either stand-alone NEPi or ARB.

**Key Words:** angiotensin, natriuretic peptide, cardiac hypertrophy, myocardial infarction, remodeling
Hypertension and heart failure (HF) are major causes of death and morbidity in the Western world, and their prevalence is projected to increase\(^1\)-\(^3\). 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\(^4\)-\(^6\). The success of angiotensin-converting enzyme inhibitors (ACEi) 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\(^7\), \(^8\). Biologically active NPs are degraded by the enzyme neutral endopeptidase or neprilysin (NEP); consequently, NEP inhibition represents an important pharmacological approach to augment the salutary actions of NPs. Presumably due to its affinity towards multiple substrate peptides beyond the NPs such as endothelin-1 and angiotensin-II, stand-alone NEPi has not been proven to be clinical efficacious\(^9\), \(^10\). Simultaneous blockade of RAAS and NEP through dual-acting ACE and NEP inhibitors (vasopeptidase inhibitors) has been evaluated a decade ago. Its prototypic drug, omapatrilat, showed favourable effects in clinical trials of hypertension and HF, but further development was halted due to increased rates of angioedema, presumably due to accumulation of bradykinin in at-risk patients\(^11\), \(^12\). Recently, a new drug class, angiotensin receptor neprilysin inhibitors (ARNi), have been developed to simultaneously block the RAAS and augment NPs through NEP inhibition at a presumed lower risk of bradykinin-induced angioedema\(^13\), \(^14\). In a preclinical model, ARNi (using valsartan-candoxatril) provided similar anti-hypertensive efficacy as omapatrilat without inducing tracheal plasma extravasation (a surrogate of angioedema)\(^15\).
LCZ696 has been the first ARNi to be evaluated in patients with hypertension and heart failure. LCZ696 combines a moiety of the ARB valsartan (Val) and the NEPi precursor, AHU377. After intake, LCZ696 is metabolized into active Val and inactive AHU377, whilst 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 post-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 myocardial infarction (MI), treatment and study protocol**

Adult 6–8 week old male Sprague-Dawley rats [220–250 g body weight (BW)] were subjected to induction of MI by left anterior descending ligation as routinely performed in our laboratory. Rats were randomized in a 2:1 fashion to four weeks of treatment with LCZ696 (68 mg/kg body weight PO; MI-ARNi, n=11) or vehicle (MI-Vhc, n=6). LCZ696 was a kind gift of Novartis, Basel, Switzerland. Treatment commenced one week after surgery (baseline) in order 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 (endpoint), systolic blood pressure (BP) was measured in conscious rats using the tail-cuff method before being placed in a metabolic cage for a 24-h period with free access to food and water. GFR and renal functional parameters were then measured, and animals were allowed to rest.
following day, cardiac function was assessed by echocardiography and invasive LV catheterization, respectively, before rats were euthanized and organs harvested19.

**Echocardiography and hemodynamic measurements**

Standard two-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 laboratory19. All recordings were analysed off-line by an experienced specialist using commercially available customized software within a personal computer workstation (EchoPac vers108.1.0, GE Vingmed, Horten, Norway). LV basal rotation and systolic circular LV strain (CS) were determined by speckle tracking analysis of LV basal short axis views20. Diastolic wall strain (DWS), an index of diastolic stiffness based on the linear elastic theory, was defined as the difference between systolic and diastolic posterior wall thickness divided by systolic posterior wall thickness21. Animals were anesthetized with pentobarbitone (60 mg/kg ip) and intubated for cardiac catheterization procedures, as previously described19. 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 BP and then advanced into the left ventricle to obtain left ventricular 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 apnoeic. The following validated parameters were assessed using Millar conductance data acquisition and analysis software PVAN 3.5: left ventricular end-systolic pressure, left ventricular 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 harvest 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 (AEC) in accordance with National Health and Medical Research Council’s Guide for the Care and Use of Laboratory Animals (AEC No. 028/11).

**Histological analysis of cardiac tissue**

Paraffin-embedded LV tissue sections (4µm) 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 non-infarct 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. 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 was pre-set 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 (NCM) and fibroblasts (NCF) 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. Cardiac myocyte hypertrophy was assessed by AngII-stimulated (100nM) NCM with 3[H]leucine-incorporation over 60 h. AngII-stimulated (100nM) collagen synthesis was determined by 3[H]proline-incorporation in NCF over 48 h.
Cells were pre-incubated with valsartan (VAL), AHU377, LBQ657, or VAL+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 BNP was added at different concentrations into the cell culture media just before AngII stimulation in order to assess the effect of direct augmentation of NP signalling. Experiments were repeated 2-4 times in triplicate each time.

**Statistical analysis**

Values are expressed as mean ± SEM. For *in vivo* experiments, unpaired Student’s t-test was used. For cell studies, one-way ANOVA with Bonferroni’s *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. A two-tailed p-value of <0.05 was considered significant.

**Results**

**Effects of ARNi on cardiac hypertrophy and fibrosis after experimental MI**

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

Infarct size was substantial and consistent with large MI, and similar in both groups (Fig. 1E). Echocardiography one week after MI prior to 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 vs MI-Vhc (Table 3). Computed LV mass with MI-ARNi was lower than MI-Vhc, consistent with organ weights. EF and FAC were increased, as was DWS.

Doppler echocardiography analysis revealed similar early and reduced late diastolic filling, and a trend towards 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/A 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 post-MI

Analysis of steady-state LV pressure-volume (PV) 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 PV relationship (p<0.001), lower Tau (p<0.05), and a trend for lower end-diastolic PV relationship (p=0.10) in MI-ARNi versus MI-Vhc animals.

Renal function following 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 over the study period, the difference between values at endpoint vs baseline were 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 endpoint (MI-Vhc 7.1±0.5 vs MI-ARNi 8.3±0.3 ml/min/kg, p=0.07) and the difference in changes in GFR over time (MI-Vhc -4.7±0.7 vs MI-ARNi -3.6±0.2 ml/min/kg, p=0.09) nearly reached significance.

Effects of stand-alone NEPi on cellular cardiac fibrosis and hypertrophy

AngII induced profound collagen accumulation in fibroblasts (Fig. 2A) and cardiac myocyte hypertrophy (Fig. 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 (Fig. 2A). In contrast, LBQ657 modestly inhibited cardiac myocyte hypertrophy (Fig. 2B).

Effects of stand-alone ARB and ARNi on cardiac fibrosis and hypertrophy

Valsartan dose-dependently inhibited collagen accumulation in fibroblasts (Fig. 3A) and cardiac myocyte hypertrophy (Fig. 3B), respectively. In cardiac fibroblasts, addition of LBQ657 (10µM) significantly augmented the inhibitory effects of Val except for the highest dose where both valsartan and valsartan + LBQ657 values were reduced to unstimulated negative control values (Fig. 4A). In cardiac myocytes, addition of LBQ657 (10µM) significantly augmented the inhibitory effects of the lowest dose of valsartan, however, only the highest combined dose of ARNi (VAL+LBQ657) afforded complete inhibition of AngII-induced hypertrophy to values that were similar to unstimulated negative controls (Fig. 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 (Fig. 5 A) as well as cardiac myocyte hypertrophy (Fig. 5B). The highest BNP dose (i.e. 10\(^{-8}\) M) 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 shows that treatment with LCZ696 versus vehicle resulted in attenuation of cardiac dysfunction, fibrosis and remodeling, and somewhat attenuated decline in kidney function in rats following experimental MI. Second, while 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 one week after experimental MI. The delay in commencement of treatment aimed to avoid interference with reparative scar tissue formation and hemodynamic stabilisation. Despite large and similar infarct size in both groups, organ weights and cardiac dimensions (by echocardiography) consistently showed a 15-20% 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 DWS (i.e. an index of LV diastolic stiffness), lower Tau, higher EDPVR 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 HFpEF even in the absence of discernible effects on LV diastolic filling\(^{17}\). Here, MI rats did not exhibit signs of overt HF e.g. elevated lung weights but it may be reasonable to assume that HF would have developed over 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) as well as 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 signalling 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 a number of profibrotic peptides in rats following ischemia-reperfusion injury\(^{23}\). In addition, Backlund et al showed superior efficacy of omapatrilat versus captopril on cardiomyocyte apoptosis and cardiac remodeling post-MI\(^{24}\). LCZ696 also appears to show similar increased efficacy beyond stand-alone RAAS blockade as omapatrilat in this study. The clinical introduction of omapatrilat had been halted due to increased rates of angioedema in clinical trials leading to the development of ARNi.

Our \textit{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 NEP. LBQ657, on the other hand 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 signalling in that cell type. Although not specifically tested, increase in other, potentially unfavourable NEP 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 signalling 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 produce 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 NEP inhibition (by LBQ657) was of attenuation of hypertrophy. Both in cardiac fibroblasts and myocytes only the highest ARNi dose afforded complete inhibition of AngII-mediated signalling. In line with important effects of NP augmentation by LBQ657, BNP potently inhibited AngII-induced signalling in both cardiac cell lines.

In patients with HF, elevated NP levels are frequently found in concert with the degree of cardiac dysfunction and 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
heart failure. Contemporary clinical data underscores the efficacy of ARNi in patients with hypertension and HFrEF to alter blood pressure and important surrogate endpoints, with an acceptable safety profile\textsuperscript{16, 17, 27}. Very recently, the Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF), a large (n=8,422) outcome study assessing efficacy and safety of LCZ696 versus the ACEi, enalapril, in patients with HF and reduced EF (HFrEF) was stopped prematurely after interim analysis suggested superiority over standard therapy with ACEi, the current “gold standard”\textsuperscript{18, 28}. PARADIGM-HF will likely provide definite answers regarding 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\textsuperscript{29}.

**Limitations**

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

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 anaesthesia across all animals reflected by similar 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 following experimental MI, and inhibited cardiac fibrosis and cardiac hypertrophy \textit{in vivo} post-MI, as well as \textit{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 post-MI as well as in a broader spectrum of cardiovascular disorders.

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**Disclosures**

HK has research contracts and has served as a consultant to Novartis. TvL has received lecture honoraria from Novartis. The other authors report no relevant conflicts of interest.

**References**


29. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz M, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR. Baseline characteristics and treatment of patients in Prospective comparison of ARNI with ACEI to Determine Impact on
Table 1. Hemodynamics and organ weights in MI-rats after four weeks of treatment with vehicle (Vhc) or LCZ696 (ARNi)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MI-Vhc (n=6)</th>
<th>MI-ARNi (n=11)</th>
</tr>
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<tbody>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117 ± 8</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>266 ± 11</td>
<td>254 ± 10</td>
</tr>
<tr>
<td>Cardiac output (mL/min)</td>
<td>59.5 ± 4.5</td>
<td>64.4 ± 7.1</td>
</tr>
<tr>
<td>PRSW</td>
<td>65.3 ± 11.2</td>
<td>80.3 ± 8.2</td>
</tr>
<tr>
<td>LVEDP</td>
<td>9.9 ± 3.5</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>dP/dt_max</td>
<td>5036 ± 368</td>
<td>5204 ± 482</td>
</tr>
<tr>
<td>dP/dt_min</td>
<td>-4446 ± 439</td>
<td>-4289 ± 350</td>
</tr>
<tr>
<td>Tau</td>
<td>12.0 ± 1.7</td>
<td>8.9 ± 0.38*</td>
</tr>
<tr>
<td>Ea</td>
<td>0.48 ± 0.46</td>
<td>0.16 ± 0.11</td>
</tr>
<tr>
<td>EDPVR</td>
<td>0.032 ± 0.007</td>
<td>0.019 ± 0.004</td>
</tr>
<tr>
<td>ESPVR</td>
<td>0.27 ± 0.04</td>
<td>0.46 ± 0.02**</td>
</tr>
<tr>
<td><strong>Body and organ weights</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>385 ± 5</td>
<td>380 ± 7</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>887 ± 20</td>
<td>803 ± 25*</td>
</tr>
<tr>
<td>RV weight (mg)</td>
<td>272 ± 11</td>
<td>241 ± 8*</td>
</tr>
<tr>
<td>Atrial weight (mg)</td>
<td>160 ± 12</td>
<td>125 ± 8*</td>
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<tr>
<td>Total heart weight (mg)</td>
<td>1319 ± 21</td>
<td>1168 ± 35**</td>
</tr>
<tr>
<td>Lung weight (mg)</td>
<td>1552 ± 44</td>
<td>1544 ± 56</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>2615 ± 76</td>
<td>2687 ± 119</td>
</tr>
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PRSW, preload-recruitable stroke work; LVEDP, left ventricular enddiastolic pressure; dP/dt_max, maximum pressure decay; dP/dt_min, minimum pressure decay; Ea, aterial elastance; DEPVR and ESPVR, endsystolic and enddiastolic pressure-volume relationship, respectively; LV, left ventricular; RV, ventricular. *p<0.05, **p<0.01; MI-ARNi versus MI-Vhc; unpaired t-test.
Table 2. Baseline echocardiography in rats one week after MI prior to treatment with vehicle (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>LVEDD, LV end-diastolic diameter (mm)</td>
<td>8.1 ± 0.1</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>LVSD, LV end-systolic diameter (mm)</td>
<td>5.6 ± 0.4</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>AWT, 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>LVEF, 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/Em</td>
<td>20.8 ± 2.1</td>
<td>23.8 ± 2.0</td>
</tr>
</tbody>
</table>

No significant differences, MI-ARNi versus MI-Vhc; unpaired t-test.
Table 3. Left ventricular function by echocardiography in MI-rats after four weeks of treatment with vehicle (Vhc) or ARNi

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MI-Vhc</th>
<th>MI-ARNi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter N=5</td>
<td>N=11</td>
<td></td>
</tr>
<tr>
<td>LVEDD, LV end-diastolic diameter (mm)</td>
<td>10.5 ± 0.3</td>
<td>9.7 ± 0.2*</td>
</tr>
<tr>
<td>LVSD, LV end-systolic diameter (mm)</td>
<td>8.4 ± 0.7</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>AWT, 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>LVEDV, LV end-diastolic volume (ml)</td>
<td>0.55 ± 0.03</td>
<td>0.50 ± 0.03</td>
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<tr>
<td>LVSV, LV end-systolic volume (ml)</td>
<td>0.30 ± 0.03</td>
<td>0.20 ± 0.03*</td>
</tr>
<tr>
<td>LVEF, LV ejection fraction (%)</td>
<td>47 ± 5</td>
<td>60 ± 2*</td>
</tr>
<tr>
<td>LVM, 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/E_m</td>
<td>21.1 ± 1.1</td>
<td>20.0 ± 1.8</td>
</tr>
<tr>
<td>E_m/A_m</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 versus MI-Vhc; unpaired t-test.
Figure Legends

Figure 1. Effects of chronic administration of LCZ696 on cardiac fibrosis following MI
Paraffin-embedded LV tissue sections stained with picrosirius red revealed markedly and significantly reduced interstitial fibrosis with LCZ696 (MI-ARNi) both in the non-infarct zone (Fig. 1A) and peri-infarct zone (Fig. 1B) compared to MI-VHC. In contrast, perivascular fibrosis was not different between groups in non-infarct zone (Fig 1C) and peri-infarct zone (Fig. 1D). Infarct size as expressed as an averaged percentage of the endocardial and epicardial scarred circumferences of the LV was substantial and similar in both groups (Fig. 1E). *p<0.05, **p<0.01; MI-ARNi vs MI-VHC.

Figure 2. Effects of stand-alone NEPi LBQ657 on cardiac fibrosis and hypertrophy in vitro.
Similar to the inactive NEPi prodrug AHU377, the active NEPi LBQ657 did not show discernible effects on AngII-induced collagen accumulation in cardiac fibroblasts (Fig. 2A). In contrast, LBQ657 modestly attenuated AngII-induced cardiac myocyte hypertrophy although without a clear dose-response (Fig. 2B). ****p<0.0001, AngII vs control; #p<0.05, ##p<0.01, ####p<0.0001, LBQ657 vs AngII.

Figure 3. Effects of single ARB, valsartan on cellular cardiac fibrosis and hypertrophy
As expected, AngII-stimulated collagen synthesis in cardiac fibroblasts (Fig. 3A) and cardiac myocyte hypertrophy (Fig. 3B), 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 levelling-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 ARNi compared to stand-alone ARB on cellular cardiac fibrosis and hypertrophy

Although inefficient as stand-alone, addition of fixed-dose LBQ657 (10µM) significantly augmented the inhibitory effects of valsartan in cardiac fibroblasts. This was true for all valsartan doses except for the highest dose where the difference between ARB (valsartan) and ARNi (valsartan + LBQ657) values were smaller than their differences with unstimulated negative control values (Fig. 4A). Accordingly, ARNi with both the second-highest and highest valsartan concentration abrogated AngII signalling completely. In cardiac myocytes, addition of fixed-dose LBQ657 (10µM) significantly augmented the inhibitory effects of the lowest, but not the two middle-range valsartan 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 (Fig. 4B). ****p<0.0001, AngII vs control; ###p<0.001, ####p<0.0001, LBQ657 vs AngII; $p<0.05, VAL vs LBQ+VAL.

Figure 5. Effects of exogenous BNP on AngII-stimulated cellular cardiac hypertrophy and fibrosis in vitro

Co-incubation with increasing doses of BNP in cell culture media dose-dependently reduced AngII-stimulated collagen accumulation in cardiac fibroblasts (Fig. 5 A) and hypertrophy of cardiac myocytes (B). No further inhibition was observed between 10⁻⁹ and 10⁻⁸M consistent with plateau of response. The highest BNP dose (i.e. 10⁻⁸ M) resulted in inhibition at levels that were not different from unstimulated negative controls. *p<0.05, **p<0.01, ***p<0.001, BNP (logM) vs AngII.
Figure 1

A
Cardiac Fibrosis - Non-Infarct Zone

B
Cardiac Fibrosis - Peri-Infarct Zone

C
Perivascular Fibrosis Non-Infarct Zone

D
Perivascular Fibrosis Peri-Infarct Zone

E
Infarct Size
Figure 2

A. Effects of LBQ657 in cardiac fibroblasts

B. Effects of LBQ657 in cardiac myocytes
Figure 3

A. Effects of valsartan in cardiac fibroblasts

B. Effects of valsartan in cardiac myocytes
Figure 4

A. Effects of valsartan vs ARNi in cardiac fibroblasts

B. Effects of valsartan vs ARNi in cardiac myocytes
The Angiotensin-Receptor Neprilysin Inhibitor LCZ696 Attenuates Cardiac Remodeling and Dysfunction After Myocardial Infarction by Reducing Cardiac Fibrosis and Hypertrophy
Thomas G. von Lueder, Bing H. Wang, Andrew R. Kompa, Li Huang, Randy Webb, Pierre Jordaan, Dan Atar and Henry Krum

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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 nepriylisin inhibitors, ARNI)는 안지오텐신 II의 억제뿐만 아니라 나트륨이뇨펩티드(natriuretic peptide)를 분해하는 네프릴리신을 억제해 나트륨이뇨펩티드를 증가시킨다. ARNi의 심장 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 확장기말 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 P<0.05)은 높았다. 좌심실 압력-용적 고라(pressure-volume loops)로 좌심실기능의 개선을 확인한 결과, 심근경색의 크기는 비슷하였지만 치료군은 심장무게가 더 작았고(P<0.01), 심근경색 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 영역에 더 우월하게 작용했기 때문으로 생각된다.