

Direct Renin Inhibitor Attenuates Left Ventricular Remodeling in Post-Myocardial Infarction Heart Failure Mice

Ning-I Yang, Chia-Chi Liao, Ming-Jui Hung and Wen-Jin Cherng

Background: The role of direct renin inhibitors in myocardial ischemia-induced heart failure is controversial. We hypothesized that direct renin inhibitors play a positive role, affecting in vivo myocardial function as well as in vitro extracellular matrix change.

Methods: Ten-week-old C57BL/6J male mice with 2-kidney 1-clip (2K1C) model were enrolled in this study. The mice were divided into 3 groups each with 18 mice; group 1 sham-operated, group 2 coronary artery ligation-induced heart failure, and group 3 coronary artery ligation-induced heart failure receiving aliskiren minipump infusion. These mice were assessed for systemic hemodynamics and left ventricular function by 2-dimensional echocardiography (iE33, Philips). Myocardial tissue was stained and crude protein was isolated from the non-ischemic viable left ventricle. Myocardial tissue contents of anti-angiotensin II type 1 (AT1) receptor, matrix metalloproteinase (MMP)-2 and MMP-9 were examined.

Results: There were 54 mice that received 2K1C and were followed up for three weeks. Baseline characteristics showed no difference. At follow-up, the heart failure-only group had greater left ventricular mass and worse systolic function as compared to the sham group. Whereas the heart failure-aliskiren group had lower left ventricle mass and better systolic function as compared to the heart failure-only group. AT1 receptor, MMP-2 and MMP-9 levels were increased in the heart failure-only model while direct renin inhibitor attenuated this significantly.

Conclusions: Direct renin inhibitors improved myocardial function in a myocardial ischemia-induced heart failure mouse model. The improvement seen is present in myocardial mass, left ventricular systolic function and also in myocardial interstitial tissue.

Key Words: Direct renin inhibitor • Echocardiography • Heart failure

INTRODUCTION

Renin-angiotensin-aldosterone system (RAAS) plays a major role in maintaining cardiovascular homeostasis. It is activated in organic heart disease including hyper-

tension, ischemic heart disease or heart failure (HF). Several pathway blocking agents, particularly blocking in mid-pathway or at the terminal receptors, have proven efficient in controlling blood pressure, preventing ventricular remodeling and diminishing HF progression. However, these pathway blocking agents, such as angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), might reactivate angiotensin II and aldosterone escape or breakthrough which diminishes their efficacy.¹⁻³ In recent years, a blocking agent acting at the start of the RAAS pathway appears to represent a new development. This novel direct renin inhibitor, aliskiren, blocks the RAAS without inducing a

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Division of Cardiology, Chang Gung University College of Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan.

Address correspondence and reprint requests to: Dr. Wen-Jin Cherng, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, No. 222, Mai-Chin Road, Keelung 20401, Taiwan. Tel: 886-2-2431-3131 ext. 3168; Fax: 886-2-2433-5342; E-mail: cwenjin@cgmh.org.tw

compensatory reaction in angiotensin I, angiotensin II or plasma renin activity. The effect of aliskiren on the heart has been controversial. In HF, the Aliskiren Observation of Heart Failure (ALOFT) study showed that although aliskiren reduced plasma b-type natriuretic peptide (BNP) levels, no clinical benefit was seen.⁴ In the Aliskiren Study in Post-Myocardial Infarction Patients to Reduce Remodeling (ASPIRE) study, the addition of aliskiren to standard treatment following acute myocardial infarction did not attenuate left ventricular (LV) remodeling.⁵ Other studies have shown that in rats with cardiomyopathy, aliskiren attenuates myocardial apoptosis and oxidative stress,⁶ while in mice, cardiac function and remodeling is improved after myocardial infarction, independent of blood pressure.⁷ As HF progresses, several compensatory mechanisms can be elicited. Adverse cardiac remodeling induces LV dilation, eccentric hypertrophy, increased extracellular collagen matrix and interstitial fibrosis.⁸ The increased production of extracellular collagen matrix, matrix metalloproteinases (MMPs) and impaired fibroblast/cardiomyocyte interaction might lead to remodeling and dysfunction.⁸ Our hypothesis is that direct renin inhibitors may play a positive role in HF mice not only favorably affecting hemodynamics and LV function, but also generating effects at the extra myocyte interstitial level.

METHODS

Animal preparation

We chose C57BL/6J male mice which are prototypes of strains with the same single renin gene found in humans, together with the 2-kidney 1-clip (2K1C) model in which one renal artery is constricted to chronically reduce renal perfusion, and the other kidney remains untouched.⁹

Inducing myocardial infarction

Ten-week-old C57BL/6J male mice were put under general anesthesia with Forane inhalation (Isoflurane 1-2% in oxygen, Abbott Lab, Queenborough, England), the left kidney was exposed through a flank incision. After separating the left renal artery and vein, a silver clip with an internal diameter of 0.12 mm was placed around the renal artery, and the right renal artery was

kept patent.⁹ Three days after the operation, a second general anesthesia was administered with ketamine 50 mg kg⁻¹ and xylazine 60 mg kg⁻¹ (Nan-Kuang Chem. Co. Taiwan) intraperitoneal injection, and anesthesia was maintained with isoflurane after endotracheal intubation. A left parasternal thoracotomy incision was performed, and the left coronary artery was ligated 2 mm from its origin with 7-0 silk nylon suture.¹⁰ Prior to commencing the study, we induced myocardial infarction with this method on 20 2K1C mice and established that by our method, we were able to achieve at least 20% of infarction area, thus giving us sufficient confidence to use this model. In the sham-operated mice, the left coronary artery suture was not tied. The mice were maintained on standard rodent food with water ad libitum. Flunixin 1.1 to 2.5 mg kg⁻¹, subcutaneous twice daily was given for pain relief. Mice with a resultant infarct size of < 20% were excluded from the analysis. Blood sampling was performed and tail blood pressure and heart rate were recorded before the operation. Blood pressure was measured using a non-invasive blood pressure analyzer, BP-2000 Visitech system (Visitech System, Inc., Apex, NC, USA).¹¹

Treatment

Animals were divided into 3 groups, including group 1, the sham-operated group, and groups 2 and 3, the HF groups. Both group 2 and 3 were randomized to receive either vehicle or aliskiren infusion via Alzet osmotic minipump (model 1004, Alza Corp., Vacaville, CA, USA) for 3 weeks. The drug-treated mice received 25 mg/kg/day of aliskiren beginning the second day after surgery.

Hemodynamics study

At 3 weeks after the surgical procedure, the aliskiren osmotic minipumps were removed. To assess the effects of aliskiren and myocardial infarction, the mice were reanesthetized and ventilated the second day. Tail blood pressure, systolic LV function and heart rate were recorded, and LV function was measured by echocardiography. After completion of hemodynamic measurements and blood sampling, the heart was immediately excised and placed in an aerated Krebs-Henseleit solution.

Echocardiographic study

At baseline and at 3 weeks follow-up, the mice had

their LV function assessed by 2-dimensional echocardiography (Philips Medical Systems, Andover, MA, USA) with a 15-7 L-io probe (7-15 MHz). LV dimension, wall thickness, mass and ejection fraction were all measured (Figure 1).

Infarct size determination

Within the Krebs-Henseleit solution, the heart was dissected free of adjacent tissues. The ventricles were separated from the atria, and the right ventricle free wall was dissected free of the septum. The LV was opened with an incision along the interventricular septum from base to apex. Both ventricles were blotted dry and then immediately weighed. The circumferences of the LV and the region of infarction were outlined on a clear plastic sheet for both the endocardial and epicardial surfaces. Infarct size was analyzed by summing the internal circumferential length of the scar in each segment and dividing by the sum of the total internal circumference of the LV in each segment.¹² The same measurement was done for the external circumferential length of the scar. The two percentages, internal and external, were then averaged for the final infarct size expressed as a percentage of the LV. All of the lung, aorta and kidney were removed and weighed.

Staining of heart tissue

One quarter of peri-infarcted LV tissue were separated and fixed in 10% formalin solution, whereafter the tissue were then embedded in paraffin wax. Subsequently, 4 μ m-thick heart sections were stained with Masson's trichrome stains to distinguish the areas of connective tissue. The other part of the LV tissue was frozen rapidly in liquid nitrogen, and stored at -80 °C until use.

Western blotting

Crude protein was extracted from the non-ischemic viable LV. BCA protein assay kit (Thermo) was used for protein quantification. After both homogenate of heart samples were centrifuged and supernatant collected, primary antibodies (anti-MMP-2, anti-MMP-9, anti-angiotensin II type 1 (AT1) receptor, anti-alpha tubulin, dilutes: 1/1000, 1/1000, 1/800, 1/500, 1/5000, 1/50000) and secondary antibody (horseradish peroxidase (HRP) – conjugated goat anti-rabbit IgG antibody, diluted: 1/10000) were added. Reacting with immobilized western chemiluminescent HRP substrate (Millipore), we used a Bio-Rad Versa Dec 4000 Imaging Densitometer for quantification analysis.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean \pm SEM. One-way ANOVA was used for multiple comparisons and paired-T test was used for comparing pre- and post-samples. Differences were considered statistically significant at a value of $p < 0.05$.

RESULTS

All mice except the sham-operated group received 2K1C and coronary artery ligation. A total of 65 mice received coronary artery ligation-induced heart failure, with 36 surviving the procedure; thus surgical mortality was 45%. The 36 mice were then separated into 2 groups: HF-only and HF-aliskiren. At the 3 week follow-up examination, 54 mice were assessed including 18 sham-operated mice (group 1), 18 HF-only mice (group 2) and 18 HF-aliskiren treatment mice (group 3). There

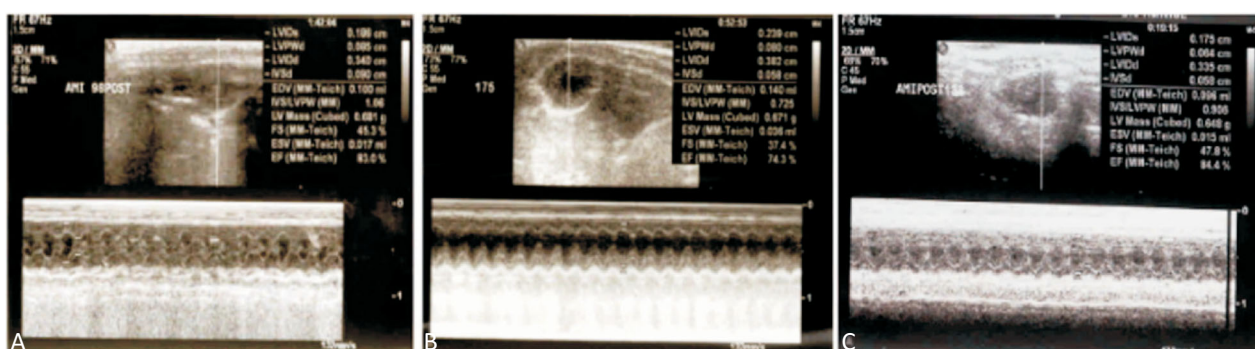


Figure 1. Echocardiography of sham-operated (A), heart failure-only (B) and heart failure with aliskiren treatment group (C).

were no differences among these 3 groups in terms of baseline body weight, blood pressure, heart rate, LV size, LV mass, LV fractional shortening and LV ejection fraction (Table 1).

Hemodynamic data

After the 3 week follow-up, weight of heart and LV indexed by body weight were increased in the HF group as compared to the sham group (0.54 ± 0.01 vs. 0.48 ± 0.01 , $p < 0.05$ and 0.38 ± 0.01 vs. 0.34 ± 0.01 , $p < 0.05$), whereas the HF-aliskiren group had lower values compared to the HF-only group (0.5 ± 0.01 vs. 0.54 ± 0.01 , $p < 0.05$ and 0.36 ± 0.01 vs. 0.38 ± 0.01 , $p < 0.05$). Hemodynamic data, including mean blood pressure and heart rate, showed no significant difference among these 3 groups (Table 2).

Echocardiography data

Both LV internal diameters were increased in the HF-only group compared to the sham group in diastole (3.49 ± 0.09 vs. 2.63 ± 0.13 , $p < 0.05$) and systole ($2.16 \pm$

0.07 vs. 1.39 ± 0.07 , $p < 0.05$). In the HF-aliskiren group, only the LV systolic dimension was larger than the sham group (1.67 ± 0.08 vs. 1.39 ± 0.07 , $p < 0.05$), whereas both LV systolic and diastolic dimensions were smaller when compared with the HF-only group (1.67 ± 0.08 vs. 2.16 ± 0.07 , $p < 0.05$ and 3.02 ± 0.12 vs. 3.49 ± 0.09 , $p < 0.05$). With regard to LV volumes, the HF-only group had larger diastolic and systolic volumes compared to the sham group at follow-up, whereas the HF-aliskiren group did not show any change. When compared with the HF-only group, LV volumes were significantly lower in the HF-aliskiren group. A similar picture was also observed with LV mass, with the HF-only group having an increased mass compared with the sham group (674 ± 4 vs. 649 ± 4 , $p < 0.05$) and the HF-aliskiren group (674 ± 4 vs. 650 ± 3 , $p < 0.05$), and no significant difference seen between the sham and HF-aliskiren groups. LV systolic

Table 1. Baseline characteristics of hemodynamics and cardiac function

Parameters	Sham (n = 18)	HF-only (n = 18)	HF-aliskiren (n = 18)
Body weight (g)	22.6 ± 0.4	22.8 ± 0.3	23.2 ± 0.4
Systolic BP (mmHg)	113 ± 2	112 ± 1	112 ± 2
Diastolic BP (mmHg)	90 ± 2	84 ± 2	81 ± 2
Mean BP (mmHg)	101 ± 2	98 ± 2	96 ± 2
Heart rate (bpm)	524 ± 17	509 ± 16	548 ± 12
LVIDd (mm)	2.81 ± 0.08	2.82 ± 0.10	2.67 ± 0.12
LVIDs (mm)	1.49 ± 0.06	1.48 ± 0.06	1.47 ± 0.07
IVS (mm)	0.77 ± 0.04	0.73 ± 0.04	0.80 ± 0.03
LVPW (mm)	0.65 ± 0.03	0.68 ± 0.02	0.67 ± 0.02
IVS/LVPW	1.20 ± 0.04	1.1 ± 0.1	1.2 ± 0.1
EDV (μL)	60.1 ± 4.5	60.9 ± 6.0	54.6 ± 6.9
ESV (μL)	9.7 ± 1.1	9.5 ± 4.9	9.7 ± 1.4
LV mass (mg)	645 ± 3	645 ± 3	644 ± 3
FS (%)	47 ± 1	47 ± 1	45 ± 1
EF (%)	84 ± 1	85 ± 1	82 ± 1

BP, blood pressure; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; FS, fractional shortening; HF, heart failure; IVS, interventricular septum; LV, left ventricle; LVIDd, left ventricle internal diameter diastole; LVIDs, left ventricle internal diameter systole; LVPW, left ventricle posterior wall.

Data are means ± SEMs.

Table 2. Morphometric, hemodynamic and functional change after three weeks follow-up

Parameters	Sham (n = 18)	HF-only (n = 18)	HF-aliskiren (n = 18)
Body weight (g)	25.1 ± 0.4	25.9 ± 0.2	25.4 ± 0.3
Heart/weight (%)	0.48 ± 0.01	0.54 ± 0.01*	0.50 ± 0.01 [†]
LV/weight (%)	0.34 ± 0.00	0.38 ± 0.01*	0.36 ± 0.01 [†]
Lung/weight (%)	0.53 ± 0.01	0.57 ± 0.02	0.57 ± 0.02
Systolic BP (mmHg)	104 ± 2	110 ± 2	106 ± 2
Diastolic BP (mmHg)	84 ± 2	86 ± 2	77 ± 2 [†]
Mean BP (mmHg)	94 ± 2	98 ± 2	92 ± 2
Heart rate (bpm)	534 ± 12	576 ± 12	572 ± 13
LVIDd (mm)	2.63 ± 0.13	3.49 ± 0.09*	3.02 ± 0.12 [†]
LVIDs (mm)	1.39 ± 0.07	2.16 ± 0.07*	1.67 ± 0.08* [†]
IVSd (mm)	0.78 ± 0.02	0.80 ± 0.04	0.73 ± 0.03
LVPWd (mm)	0.81 ± 0.03	0.80 ± 0.04	0.69 ± 0.03*
IVS/LVPW	0.99 ± 0.06	1.04 ± 0.06	1.08 ± 0.05
EDV (μL)	53.3 ± 6.9	111.7 ± 9.0*	76.8 ± 8.1 [†]
ESV (μL)	8.1 ± 1.0	28.6 ± 2.8*	14.1 ± 1.8 [†]
LV mass (mg)	649 ± 4	674 ± 4*	650 ± 3 [†]
FS (%)	47 ± 1	38 ± 1*	45 ± 1 [†]
EF (%)	85 ± 1	72 ± 1*	82 ± 1 [†]

BP, blood pressure; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; FS, fractional shortening; HF, heart failure; IVS, interventricular septum; LV, left ventricle; LVIDd, left ventricle internal diameter diastole; LVIDs, left ventricle internal diameter systole; LVPW, left ventricle posterior wall.

* $p < 0.05$ vs. sham, [†] $p < 0.05$ vs. HF-only.

Data are means ± SEMs.

function as expressed by fractional shortening and ejection fraction was decreased in the HF-only group compared to the sham group and HF-aliskiren group (Table 2).

Myocardial interstitial characteristics

In the HF group, AT1 receptor levels were significantly increased as compared to the sham group. Whereas aliskiren treatment reduced AT1 receptor significantly and the same phenomenon was observed in MMP-2 and MMP-9 levels, which were increased in HF and decreased after direct renin inhibitor treatment (Figure 2).

DISCUSSION

The results of our study show that in mice, the direct renin inhibitor, aliskiren, attenuates LV remodeling

after experimental myocardial ischemia-induced HF.

Postinfarction left ventricular remodeling

In patients with myocardial infarction, the RAAS is activated and plays an important role in the development of cardiac hypertrophy, apoptosis, and LV dilatation leading to HF and increased morbidity and mortality. Post-infarction injuries are related to up-regulated local neurohormonal factors, including pro-inflammatory cytokines and reactive oxygen species with some anti-oxidant agents attenuating this process.¹³ Post-infarction remodeling has been arbitrarily divided into an early phase (within 72 hours) and a late phase (beyond 72 hours). The early phase involves expansion of the infarct zone¹⁴ which may result in early ventricular rupture or aneurysm formation. Late remodeling involves the LV globally and is associated with time-

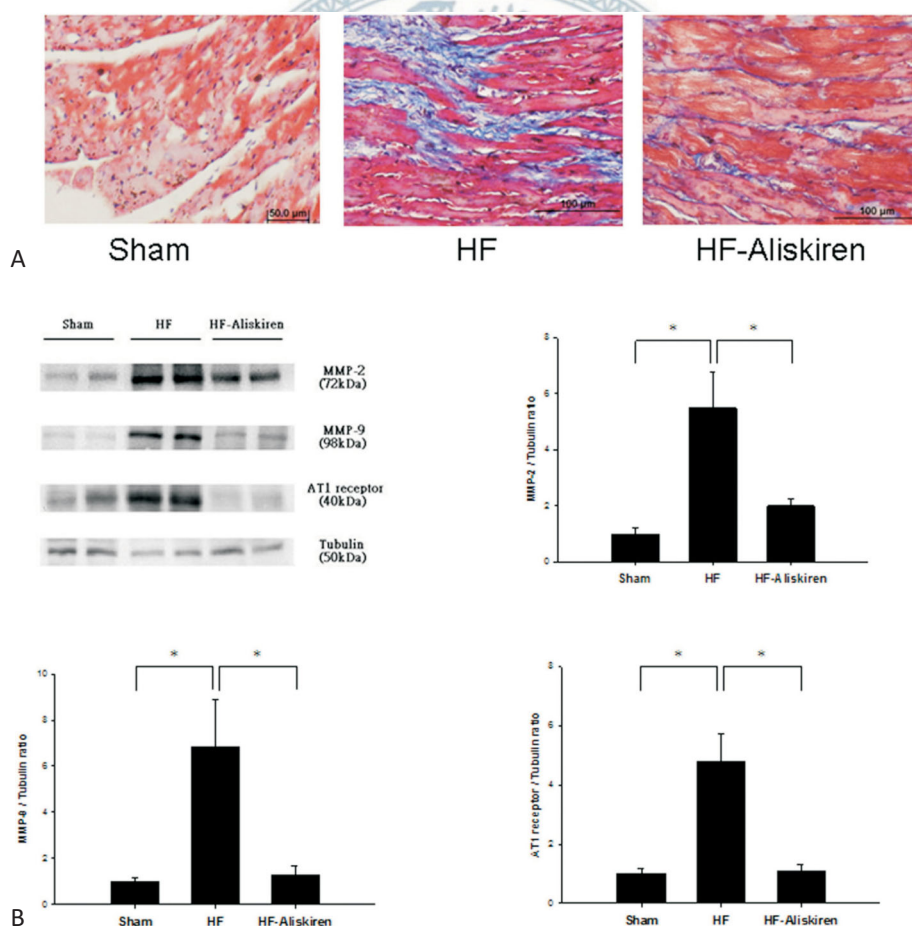


Figure 2. Direct renin inhibitor aliskiren attenuates left ventricular remodeling at the interstitial level. (A) Less peri-infarcted left ventricular tissue collagen content in the heart failure (HF)-aliskiren group compared to the HF group. (B) Western blot analysis (20 µg per lane) showing reduced anti-angiotensin II type 1 (AT1) receptor, matrix metalloproteinase (MMP)-2 and MMP-9 expression after direct renin inhibitor aliskiren treatment compared to the HF group. Data are represented as mean ± SEM. * $p < 0.05$ vs. HF-only group.

dependent dilatation, the distortion of ventricular shape, and mural hypertrophy. Prevention of the cardiac remodeling post myocardial infarction is of utmost importance. It has already been established that ACEI and ARBs are effective in attenuating the progress of cardiovascular remodeling by inhibiting the increased RAAS activity. However loss of the negative feedback inhibition of renin release during chronic treatment with an ACEI or ARB leads to a compensatory rise in renin secretion and downstream components of the RAAS cascade. Therefore direct renin inhibitors have been thought to be more efficient RAAS inhibitors as they block an enzyme with only one known substrate (angiotensinogen), inhibiting the rate limiting step in the RAAS cascade, and reducing synthesis of all subsequent components of the cascade. It has been shown that Aliskiren can effectively lower blood pressure in patients with arterial hypertension.¹⁵ However, in patients with HF, the effect seems limited to favorable neuro-humoral improvements, with no apparent clinical benefit.⁴ In the ASPIRE study, aliskiren added to a standard therapy, including an inhibitor of the RAAS, did not further attenuate LV remodeling in patients with LV ejection fraction $\leq 45\%$ post acute myocardial infarction.⁵ These findings may be attributed to the fact that patients were also treated with β -blockers, which are known to inhibit renin.¹⁶ We adopted the C57BL/6L mice model which has an active RAAS pathway as compared to rats. For synergistic activation of the RAAS, a combined coronary artery ligation and 2K1C surgery was performed. Previous studies have shown that aliskiren attenuates myocardial apoptosis and oxidative stress in the chronic murine model of cardiomyopathy.⁶ In our study, we have shown that LV end-diastolic volume and end-systolic volume increased significantly in the HF model as compared to the sham-operated mice. After 3 weeks of aliskiren treatment, no significant increase in LV volume was seen, showing that direct renin inhibition could ameliorate the progression of LV remodeling. LV systolic function as presented both by LV fraction shortening and ejection fraction was reduced in the HF mice, whereas an improvement was seen with aliskiren treatment.

Remodeling and hypertrophy

Hypertrophy is an adaptive response during post-

infarction remodeling that offsets increased load, attenuates progressive dilatation, and stabilizes contractile function.¹⁷ Myocyte hypertrophy is initiated by neurohormonal activation, myocardial stretch, the activation of local tissue RAAS and paracrine/autocrine factors. Serine proteases activate the local RAAS in the noninfarcted myocardium, leading to the up-regulation of angiotensinogen gene expression and increased local ACE activity. These changes enhance local angiotensin II production, which is the likely stimulus for hypertrophy in noninfarcted myocardium.¹⁸ In our study, LV mass increased in HF mice with no significant increase in those receiving aliskiren treatment. These results suggest that direct renin inhibitor protects the serial adverse effect of RAAS in HF by preventing the inappropriate growth and hypertrophy stimulated by angiotensin II and other growth factors. These findings are similar to the Aliskiren in Left Ventricular Hypertrophy (ALLAY) Trial, where a combination of aliskiren and losartan given to diabetics showed greater LV mass regression than those treated with losartan alone.¹⁹ In the present study, no difference was seen in mean blood pressure and heart rate among these 3 groups during follow-up, suggesting that the structural attenuation seen with aliskiren treatment was not a result of any hemodynamic change. These findings are in keeping with Westermann's study, in which aliskiren was able to improve cardiac function independent of its blood pressure-lowering effects.⁷

Collagen degradation

The triple-helical structure of collagen renders it resistant to proteolytic degradation, except by MMPs, which are secreted into the extracellular matrix in their latent proenzyme form.²⁰ In our study, Western blotting showed that MMP-2 and MMP-9 were increased in heart failure mice. Both of them were reduced in the HF-aliskiren treatment group. Attenuated MMP-9 activity might induce the accumulation of extracellular matrix in the resistance arteries. As a type of gelatinase B, MMP-9 might digest gelatin and type IV and V collagen.²¹ Another mechanism leading to HF after myocardial infarction is subsequent cardiac dilatation and infarct thinning which may lead to development of aneurysm and LV rupture. It has been shown that MMP-9 contributes to this type of cardiac dilatation.²²

Triggers for tissue repair

Myocardial repair is triggered by cytokines released from injured myocytes. The cytokine transforming growth factor beta 1 increases early in the infarct zone, stimulating macrophage and fibroblast chemotaxis and fibroblast proliferation. Activated macrophages are genetically transformed to express ACE, which provides a local source of angiotensin II that is regulated independently of plasma angiotensin II, but plays a pivotal role in reparative fibrosis.²³ Synthesis of collagen types I and III by myofibroblasts is modulated by several factors, including angiotensin II-related mechanical deformation, fibroblast growth factor, platelet-derived growth factor, atrial natriuretic peptide, and bradykinin mediated prostaglandin E2 and nitric oxide release.²⁴ Deposition of type III and type I collagen occurs predominantly in the infarct zone; however, it also occurs in noninfarcted myocardium when intercellular signaling is potentiated by extensive myocyte necrosis.

Some limitations of our study—were that when evaluating the hearts of mice, due to the small heart size, it was difficult to perform analysis of regional wall motion. In addition, we did not measure renin levels in our mice.

CONCLUSION

The results of our study showed that the direct renin inhibitor-aliskiren improved myocardial function in the myocardial ischemia-induced HF mouse model. The improvement seen was present not only in myocardial mass and LV systolic function, but also at the myocardial interstitial tissue level.

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