Divergent Effects of Losartan and Metoprolol on Cardiac Remodeling, C-kit⁺ Cells, Proliferation and Apoptosis in the Left Ventricle after Myocardial Infarction

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Abstract

There is strong evidence for the use of angiotensin converting enzyme inhibitors and beta-blockers to reduce morbidity and mortality in patients with myocardial infarction (MI), whereas the effect of angiotensin receptor blockers is less clear. We evaluated the effects of an angiotensin receptor blocker losartan and a beta-blocker metoprolol on left ventricular (LV) remodeling, c-kit⁺ cells, proliferation, fibrosis, apoptosis, and angiogenesis using a model of coronary ligation in rats. Metoprolol treatment for 2 weeks improved LV systolic function. In contrast, losartan triggered deleterious structural remodeling and functional deterioration of LV systolic function, ejection fraction being 41% and fractional shortening 47% lower in losartan group than in controls 2 weeks after MI. The number of c-kit⁺ cells as well as expression of Ki-67 was increased by metoprolol. Losartan-induced thinning of the anterior wall and ventricular dilation were associated with increased apoptosis and fibrosis, while losartan had no effect on the expression of c-kit or Ki-67. Metoprolol or losartan had no effect on microvessel density. These results demonstrate that beta-blocker treatment attenuated adverse remodeling via c-kit⁺ cells and proliferation, whereas angiotensin receptor blocker-induced worsening of LV systolic function was associated with increased apoptosis and fibrosis in the peri-infarct region.

Keywords: myocardial infarction, heart failure, remodeling, cells

Introduction

The activation of the renin–angiotensin–aldosterone system and sympathetic hyperactivation play central roles in pathogenesis of postinfarction left ventricular (LV) remodeling and heart failure. This understanding has provided strong therapeutic rationale for using angiotensin converting enzyme (ACE) inhibitors and beta-blockers in patients with myocardial infarction (MI).^{1,2} However, data supporting the use of angiotensin receptor blockers (ARBs) for the primary prevention of myocardial infarction are generally weak and, in some cases, negative.^{3–5} In the VALUE trial in hypertensive patients, the ARB valsartan-based regimen had a significantly higher (19%, $p < 0.02$) incidence of myocardial infarction than the calcium channel blocker amlodipine-based regimen.⁶ In the recently published ONTARGET trial in patients with vascular disease or high-risk diabetes, myocardial infarction occurred in 440 patients (5.2%) in the ARB telmisartan group and in 413 patients (4.8%) in the ACE inhibitor ramipril group (relative risk, 1.07, 0.94–1.22), telmisartan being equivalent to ramipril for the prespecified primary outcome of death from cardiovascular causes, myocardial infarction, stroke, or hospitalization for heart failure.⁷ As compared with a beta-blocker in the LIFE study, myocardial infarction occurred in 198 ARB losartan-treated and 188 beta-blocker atenolol-treated hypertensive patients (relative risk, 1.07, 0.88–1.31), while the stroke outcome was highly in favor of losartan, showing a 24.9% relative risk reduction compared with atenolol.⁸

In view that the randomized, controlled trials have convincingly demonstrated that beta-blockers reduce rates of myocardial infarction and improve LV structure and function after MI,^{1,2,5} we evaluated the effects of a beta-blocker, metoprolol, and an ARB, losartan, on the progression of LV remodeling and function in rats subjected to MI by ligating the left anterior descending artery (LAD). Since these experiments revealed that

early treatment with metoprolol improved and losartan worsened LV systolic function, we tested the hypothesis that metoprolol and losartan may divergently affect apoptosis, proliferation, fibrosis, angiogenesis, or the number of cardiac stem cells (CSCs) in the LV after MI. Our results demonstrate that the impairment of cardiac function and structure with the ARB was associated with increased apoptosis and fibrosis whereas beta-blocker treatment attenuated adverse LV remodeling via c-kit⁺ cells and cellular proliferation.

Methods

Myocardial infarction, drug treatments with osmotic minipumps and echocardiography

MI was produced by ligation of the LAD.⁹ Beta-blocker metoprolol (1.5 mg/kg/h) or angiotensin II type 1 (AT₁) receptor antagonist losartan (400 µg/kg/h) was administered via osmotic minipumps. Transthoracic echocardiography was performed 1 day, 2 weeks or 4 weeks after operation using Acuson Ultrasound System (Sequoia™ 512) and a 15-MHz linear transducer (15L8) (Acuson, Mountain View, CA). A group of rats was sacrificed at each time point, hearts were removed and the chambers separated. LV tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 5 µm sections and mounted on slides. Methods are described in detail in the online-only Supporting Information.

Results

Metoprolol but not losartan improves LV function and remodeling after MI

To compare the effect of metoprolol and losartan on postinfarction remodeling, the LAD was ligated in rats. MI progressively

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decreased systolic function and caused dilatation of the left ventricle, as assessed by echocardiography during the 4-week follow-up period (*Supporting Table 1*). The LV dilatation, thinning of the anterior wall, and hypertrophy of the posterior wall were also seen in hematoxylin-eosin stained myocardial sections (*Supporting Figure 1A*). The size of the infarcted area, measured from histological sections stained with Masson's trichrome as area of fibrosis of LV circumference (*Supporting Figure 1B*), was $32 \pm 9\%$ ($n = 3$), $40 \pm 2\%$ ($n = 5$) and $40 \pm 4\%$ ($n = 5$) 1 day, 2 weeks and 4 weeks after MI, respectively. These functional and morphological changes were accompanied by an increase in A-type and B-type natriuretic peptide gene expression in the left ventricle (data not shown).

Metoprolol treatment for 2 weeks improved LV systolic function (*Figure 1A-C*). LV ejection fraction and fractional shortening increased 32% ($p < 0.05$) and 47% ($p < 0.05$), respectively, in metoprolol-treated rats compared with the vehicle-treated animals. In contrast, treatment with losartan resulted in a significant functional deterioration of LV systolic function, ejection fraction being 41% ($p < 0.01$) and fractional shortening 47% ($p < 0.05$) lower in losartan group than in controls 2 weeks after MI (*Figure 1A-C*). Consistently with the opposite effects on LV systolic function, losartan treatment intensified the dilatation of the left ventricle ($p < 0.05$), whereas metoprolol treatment attenuated ($p < 0.05$) it, as assessed by hematoxylin-eosin stained myocardial sections (*Figure 2A*) or echocardiography (*Figure 2B*). Furthermore, the posterior wall was significantly thinner in losartan-treated rats compared with metoprolol-treated rats 2 weeks after MI ($p < 0.05$, *Figure 2C*). Metoprolol and losartan treatments had no significant effect on infarct size, the area of fibrosis of LV circumference being $43 \pm 3\%$ ($n = 6$), $44 \pm 3\%$ ($n = 5$) and $40 \pm 2\%$ ($n = 5$) in metoprolol, losartan and vehicle groups, respectively (*Figure 2D*). Interestingly, the percentage of fibrotic area measured from infarct border zone was 2.3-fold higher ($p < 0.05$) in the losartan-treated animals than in metoprolol-treated animals (*Figure 2E*).

Increased apoptosis in infarcted hearts with losartan treatment

Because losartan treatment augmented the deterioration of cardiac function, we analysed apoptosis in response to metoprolol and losartan treatments. By TUNEL analysis, the number of apoptotic cells and bodies in the LV increased 1.5-fold at 1 day, 4.5-fold ($p < 0.05$) at 2 weeks and 5.7-fold ($p < 0.01$) at 4 weeks after MI (*Figure 3A-B*). The results of TUNEL

were supported by immunostaining for activated caspase-3, which showed a similar increase in the number of apoptotic cells (*Figure 3C-D*). A significant additional increase ($p < 0.05$) in apoptosis was observed with 2 weeks losartan treatment in infarcted hearts, as assessed by TUNEL (*Figure 4A-C*). Apoptotic cells were not positive for cardiomyocyte marker alpha-actinin (*Figure 4C*). There was also a tendency for the increase of activated caspase-3 in the LV by losartan treatment, however, this change was not statistically significant (*Figure 4D-E*). On the contrary, metoprolol treatment for 2 weeks had no effect on apoptosis (*Figure 4*).

Metoprolol increases the number of c-kit positive cells after MI

We next tested the hypothesis that metoprolol and losartan treatments may have divergent effects on c-kit⁺ cells during

Figure 1. Metoprolol but not losartan improves LV systolic function after MI. Effects of drug treatments on cardiac function were examined by echocardiography 2 weeks after MI. (A) Representative M-mode images from each experimental group are shown. (B) LV ejection fraction and (C) fractional shortening were significantly improved by metoprolol treatment. Results are mean $\pm$ SEM ($n = 6$ /group). Data were analyzed by one-way ANOVA followed with LSD post hoc test. AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan. *** $p < 0.001$ versus sham; † $p < 0.05$, †† $p < 0.01$ versus AMI; ††† $p < 0.001$ versus AMI + METO.

Figure 2. Metoprolol attenuates and losartan augments LV remodeling after MI. (A) LV sections stained with hematoxylin-eosin. (B) LV diastolic diameter was significantly greater and (C) interventricular septum diastolic diameter smaller in losartan-treated rats compared to vehicle-treated rats. (D) Masson's trichrome technique was used to measure the size of MI and (E) define the area of fibrosis by analyzing the extent of positive staining. Results are mean $\pm$ SEM ($n = 6$ /group). Data were analyzed by one-way ANOVA followed with LSD *post hoc* test. AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus sham; † $p < 0.05$ versus AMI, ‡ $p < 0.05$ versus AMI + METO.

postinfarction LV remodeling. The number of c-kit⁺ cells increased in the LV 3.5-fold ($p < 0.01$) at 1 day, 4.2-fold ($p < 0.01$) at 2 weeks and 13.3-fold ($p < 0.05$) at 4 weeks after MI (Figure 5A-C). Interestingly, 2 weeks metoprolol treatment further increased the number of c-kit⁺ cells in the LV (1.9-fold, $p < 0.01$), whereas it did not differ in infarcted hearts between losartan- and vehicle-treated rats (Figure 5D-G). Immunohistochemistry using c-kit-specific antibody showed that the number of c-kit⁺ cells increased particularly in the border zone of the infarction by metoprolol treatment (Figure 5H).

To characterize further the potential mechanism by which metoprolol may influence LV remodeling and function, we

performed immunohistochemical staining of the infarcted region against leukocyte common antigen CD45 and Ki-67 antigen. The number of CD45⁺ cells was significantly increased at 2 and 4 weeks after MI (Supporting Table 2). Similarly to c-kit⁺ cells, metoprolol but not losartan treatment for 2 weeks further increased the number of CD45⁺ cells in the LV compared with control rats (2.5-fold, $p < 0.01$, Figure 6A-B). Moreover, Ki-67 immunostaining showed a higher number of Ki-67⁺ nuclei in the LV wall of the metoprolol-treated hearts compared with vehicle-treated hearts (2.1-fold, $p < 0.01$), while losartan treatment had no effect on the number of Ki-67⁺ cells (Figure 6C-D). Staining of Ki-67⁺ proliferative cells was not different between control and infarcted hearts 1 day, 2 weeks or 4 weeks after MI (Supporting Table 2).

Increase in the expression of IL1 β by metoprolol treatment after MI

Metoprolol treatment for 2 weeks significantly increased the expression of interleukin-1 β (IL1 β) in infarcted hearts (2.6-fold, $p < 0.01$), whereas losartan treatment had no effect (Figure 6E-F). The levels of stromal cell-derived factor-1 α (SDF1 α) and SDF1 β as well as tumor necrosis factor- α (TNF α) were not significantly altered by drug treatments after MI (Supporting Figure 2).

Angiogenesis in the peri-infarct region of metoprolol- and losartan-treated hearts

Finally, we counted the capillary density of the peri-infarct area in order to evaluate the degree of angiogenesis in the LV in response to drug treatments. Capillary density in the peri-infarct region decreased within 2 weeks after MI (111 ± 2 /field, $n = 6$ vs. 73 ± 7 /field, $n = 6$, $p < 0.001$), as shown earlier.⁹ Treatment with metoprolol or losartan for 2 weeks had no effect on microvessel density, as assessed by immunostaining

of endothelial cells with primary antibodies for Pecam-1 and von Willebrand factor (Supporting Figure 3A-B). Metoprolol and losartan treatments for 2 weeks had no effect on hypoxia-inducible factor-1 α (HIF1 α) protein expression. Perfusion of rat hearts for 4 hours, starting with 30 minutes ischemia, was used as a positive control for induction of HIF1 α expression (Supporting Figure 3C).

Discussion

Left ventricular remodeling remains an important treatment target in patients after myocardial infarction and chronic heart failure.¹⁰ Accumulating evidence has supported the concept

Figure 3. Apoptosis was assessed by TUNEL (A) and immunostaining for cleaved caspase-3 (C) 1 day, 2 weeks and 4 weeks after MI. (B) Number of TUNEL⁺ cells/high power field. (D) Number of cleaved caspase-3⁺ cells/high power field. White bars indicate sham and gray bars AMI group. Results are mean $\pm$ SEM ($n = 5-6$ /group). AMI = acute myocardial infarction. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ versus AMI.**

that beneficial effects of current pharmacological treatments, such as beta-blockers, are, at least in part, due to their effects on LV remodeling and dysfunction.^{1,2} A key finding of our present study using rat model of coronary ligation is that beta-blocker metoprolol elicited beneficial structural remodeling and subsequent improvement of LV systolic function, whereas ARB losartan had adverse effects on LV remodeling during development of post-infarction heart failure. Both treatments had no effect on the scar area, a hallmark of late postinfarction remodeling, as shown earlier by others.^{11,12} We evaluated several potential mechanisms triggering the divergent changes in LV function and structure by metoprolol and losartan after MI. These studies showed that metoprolol treatment attenuated adverse remodeling via c-kit⁺ cells and proliferation, whereas losartan-induced worsening of LV systolic function was associated with increased apoptosis and fibrosis in the peri-infarct region.

Cardiac stem and progenitor cells, expressing characteristic surface antigens such as c-kit, have been identified in adult myocardium.^{13,14} Mobilization of bone marrow cells and transfusion of progenitor cells reduce infarct size and improve LV function after MI^{15,16} and intramyocardial injection of c-kit⁺ CSCs can result in reconstitution of the infarcted heart.¹⁷ Several clinical studies suggest that cell therapy reduces the infarct size and improves cardiac contractile function.¹⁸ In this study, we observed an increase in the number of c-kit⁺ cells in LV after MI, in agreement with previous studies.^{19,20} The number of c-kit⁺ cells remained elevated for the entire duration of the study (4 weeks). Previously, the level of c-kit⁺ cells have been shown to decline to essentially undetectable by 8 weeks after MI.²¹

A major finding of our study is that metoprolol but not losartan treatment increased the number of c-kit⁺ cells, in addition to Ki-67⁺ and CD45⁺ cells, in infarcted LV. The cell surface glycoprotein CD45 is expressed on almost all hemopoietic lineage cells, also on hematopoietic stem cells,²² while Ki-67 is considered as a marker of cellular proliferation.²³ The precise mechanism(s) for the increase in number of c-kit⁺ cells as well as proliferative Ki-67⁺ cells and hematopoietic CD45⁺ cells by metoprolol treatment may be due to the tissue's diminished need for oxygen. Beta-blockers reduce heart rate, lower blood pressure and cardiac contractility²⁴ leading to diminished oxygen consumption of the surrounding tissue, likely resulting in more intense proliferation. The proliferative changes in the peri-infarct area may in turn elicit the metoprolol-induced improvement in

function and structure. This is in agreement with the notion of worsening in cardiac dilatation and function in c-kit-deficient mice.²⁵ To our knowledge the effect of metoprolol or losartan on CSCs after MI has not been studied earlier. The number of c-kit⁺ cells, however, was limited considering the extent of myocardial damage by infarction, and thus the changes in c-kit⁺ cells may be an indication of an overall phenotypic change caused by beta-blocker treatment in addition to or instead of a distinct regenerative process.

The observed effect of beta-blocker treatment on LV remodeling and proliferation, on the other hand, could also be explained by factors involved in mobilization of cells and engraftment into the heart. Recent studies suggest a requirement for cooperation between a combination of factors that regulate homing (chemokines, selectins, and integrins) and engraftment (matrix metalloproteases and cathepsins) to determine the specific cell types recruited to ischemic tissue.²⁶

Figure 4. Losartan treatment increases apoptosis after MI. Apoptosis was assessed by TUNEL (A, B, C) and immunostaining for cleaved caspase-3 (D, E) 2 weeks after MI. The apoptotic cells and bodies were counted in 5 high power fields (40× objective) from the peri-infarct regions choosing hot spot areas in each sample. Peri-infarct zone is an ischemic area adjacent to infarct and hot spot areas are areas where the number of apoptotic cells appeared maximal. (C) Immunofluorescence staining showing that apoptotic cells are not cardiomyocytes (green; TUNEL, blue; DAPI, red; alpha-actinin). AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan. Results are mean ± SEM ($n = 6$ /group). * $p < 0.05$ versus AMI.

It is well established that stress signals such as tissue injury or inflammation cause upregulation of SDF1 α , which promotes the recruitment of stem cells into heart.²⁷ The fact that the expression of SDF1 α and SDF1 β remained unaltered with metoprolol treatment suggests that the increase in c-kit⁺ and Ki-67⁺ cells was a local phenomenon, rather than an indication of mobilization of cells from bone marrow. However, the increase in the number of CD45⁺ cells suggests that cells could be derived

from the circulation and thus the true nature of the c-kit⁺ cells requires further studies. Despite the origin of c-kit⁺ cells, they clearly are associated with better cardiac performance in post-MI rats. IL1 β has previously been shown to reinitiate myocyte DNA synthesis²⁸ and we observed a simultaneous induction of IL1 β and proliferation with metoprolol treatment. Interestingly, IL1 β plays a protective role for the heart in the acute phase of MI,²⁹ suggesting that IL1 β could contribute to the increased proliferation and protection of the post-MI heart by metoprolol treatment.

Myocardial infarction results in hypertrophy of the viable myocardial tissue bordering the infarct zone. Because angiogenesis within the infarcted tissue is unable to maintain tissue growth and support the tissue with required oxygen and nutrients, the viable myocardium undergoes apoptosis.¹⁶ Mobilization of bone-marrow-derived angioblasts has been shown to prevent cardiomyocyte apoptosis, reduce remodeling and improve cardiac function in a rat MI model.¹⁶ On the other hand, a phase of apoptosis a few days after pressure overload³⁰ and upregulation of apoptosis-related genes as a response to MI³¹ implies that apoptosis is rather a signal indicating the phenotypic changes involved in the initiation of cardiac hypertrophy and remodeling. Since losartan treatment after LAD-ligation caused adverse effects on LV remodeling, we tested the hypothesis that losartan may increase apoptosis in the peri-infarct region. In previous studies, AT₁ receptor blockade by losartan has been connected to decreased apoptosis in cultured ventricular myocytes³² and in MI model 1 day post-MI.³³ It is important to note that in the present study, losartan treatment was started simultaneously with the ligation of LAD and continued for 2 weeks. Increased apoptosis and fibrosis with the thinning of the anterior wall as well as the posterior wall of the LV was observed. TUNEL positive cells did not express alpha-actinin, suggesting that they were not cardiomyocytes.

However, this cannot be excluded, because cardiomyocytes may not necessarily express cytoskeletal proteins 2 or more weeks after the onset of the apoptotic program. The dilatation of LV was increased with losartan treatment, similarly to a study, in which rabbits were administered losartan 3 hours after MI for 35 days.³⁴ Thus, losartan treatment at the time or immediately after MI seems to be detrimental for subsequent LV remodeling process at least partly via apoptotic effect.

Figure 5. Metoprolol treatment for 2 weeks increases the number of c-kit⁺ cells in the border zone of the infarction. (A) C-kit⁺ cells in rat sternum (positive control). (B) Single c-kit⁺ cell in peri-infarct area of the left ventricle. (C) Number of c-kit⁺ cells in the anterior wall of LV of sham-operated and AMI groups during the 4-week follow-up period. The area of counted section was determined by computerized methods and a number of positively staining cells was related to the area (cells/25 mm²). White bars indicate sham and gray bars AMI. **p* < 0.05, ***p* < 0.01 vs. sham. Section of the left ventricle 2 weeks after AMI (D), AMI with metoprolol (E) and AMI with losartan (F). (G) Metoprolol treatment further increased the number of c-kit⁺ cells in the LV. ***p* < 0.01 versus AMI, ##*p* < 0.01 versus AMI + METO. (H) Section of LV 2 weeks after AMI with metoprolol treatment, yellow dots representing localization of c-kit⁺ cells in the infarct border zone. Results are mean ± SEM (*n* = 5–6/group). AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan.

In addition to stem cell recruitment, apoptosis, and fibrosis, also angiogenesis in the ischemic border zone of the infarct may affect the remodeling process.¹⁰ Angiogenesis is induced by bradycardia, and reduction of heart rate by beta-blockers has been shown to promote growth of arterioles in post-MI heart.³⁵ ARBs, on the other hand, have no such effect on heart rate and this could explain the worsening of LV systolic function

after losartan treatment. By using a rat model of coronary ligation, we observed, however, that metoprolol and losartan treatments did not affect angiogenesis in the peri-infarct region. In agreement with this, metoprolol treatment after MI did not influence the density of vessels in the peri-infarct area in nude rats.³⁶ On the other hand, several reports indicate that angiotensin II can induce neovascularization in experimental systems due to the expression of different growth factors, nitric oxide synthase, metalloproteinases, and inflammation.^{37,38} In agreement with this, a significant decrease in vascular density in rat glioma after losartan treatment was observed,³⁹ but also a stimulatory effect with losartan on angiogenesis in rat hearts⁴⁰ and in cerebral cortex⁴¹ have been reported. Taken together, ARBs may have angiogenic or antiangiogenic effects, or no effect on the number of capillaries depending on experimental conditions.

One reason for the detrimental effects of losartan treatment on the remodeling process might be the activation of AT₂-receptors, because blockade of AT₁-receptors results in increased plasma angiotensin II levels.⁴² Stimulation of AT₂-receptors has beneficial, but also—especially in chronic stimulation—deleterious effects including antiangiogenesis and stimulation of apoptosis.^{42,43} Although the precise role of AT₂-receptors in myocardial remodeling is not clear, they could, in part, explain the differences in the effects of ACE inhibitors and ARBs.⁴⁴ Furthermore, some progenitor cells, e.g., endothelial progenitor cells, express both AT₁- and AT₂-receptors⁴⁵ and CSCs are known to express AT₁-receptor subtype.⁴⁶ In the present study, AT₁-receptor blockade by losartan had no effect on the number of c-kit⁺ cells. ARBs and beta-blockers have different effect on peripheral resistance,^{5,24} but this difference unlikely contributes to their divergent effects on LV remodeling, because one would expect losartan by decreasing peripheral resistance and ventricular afterload to attenuate adverse remodeling better than metoprolol.

The size of the infarcted area and the extent of postinfarction LV remodeling are major factors that determine the prognosis of patients after MI.⁴⁷ Activation of the renin–angiotensin–aldosterone system and sympathetic hyperactivation, which occur early after myocardial injury, play central roles in the pathogenesis of cardiac structural and functional abnormalities.^{1,2,10} Consistent with this, a substudy of the CAPRICORN trial suggests a beneficial effect of beta-blocker carvedilol on LV remodeling.⁴⁸ Yet, while beta-blocker and

Figure 6. Treatment with metoprolol for 2 weeks after MI increases the number of hematopoietic and proliferative cells, and the expression of IL1 β in the LV. Sections of LV were stained for CD45⁺ cells (A), Ki-67⁺ cells (C) and IL1 β (E). The number of CD45⁺ cells/high power field (B) and Ki-67⁺ cells/high power field (D), and IL1 β staining ($\mu\text{m}^2/\text{field}$) (F) in each experimental group. Results are mean $\pm$ SEM ($n = 5-6/\text{group}$). AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan. ** $p < 0.01$ versus AMI.

ACE inhibitor trials have found that these treatments significantly reduce the risk of myocardial infarction and cardiovascular death, several meta-analyses have found no reduction in the risk of myocardial infarction with ARBs.³⁻⁵ Our results on the effects of losartan on postinfarction LV remodeling suggest that the use of ARBs immediately after or at the time of myocardial infarction in primary prevention might be disadvantageous. Furthermore, the proliferative changes including the increase in number of c-kit⁺ cells could contribute to the positive outcome of beta-blockers in primary prevention.

In the present study, we specifically aimed to mimic the design of VALUE,⁶ LIFE,⁸ and ONTARGET⁷ trials in which patients were on ARB treatment at the time when the first myocardial infarction occurs, i.e., during the very early phase of remodeling process.

VALUE, LIFE and ONTARGET trials enrolled patients with hypertension and/or high-risk individuals and diabetics, but without previous heart failure and myocardial infarction within the previous 6 months or post uncomplicated MI.⁶⁻⁸ In contrast, VALIANT⁴⁹ and OPTIMAAL⁵⁰ studies enrolled patients with evidence of heart failure or LV dysfunction, including patients with MI who developed signs of heart failure or evidence of LV dysfunction within 10 days of hospitalization. It is also possible that the potential adverse effects of the ARBs observed in the present study may be blunted by concomitant beta-blockade, since the majority of patients with heart failure are treated with both agents. However, this hypothesis is not supported by OPTIMAAL,⁵⁰ VALIANT,⁴⁹ and ONTARGET trials⁷ reporting that the results for major outcomes were consistent in analyses that were adjusted for the patients' use of various concomitant drugs including beta-blockers. Moreover, in LIFE study,⁸ patients with no previous or concomitant beta-blocker treatment were enrolled whereas patients receiving antihypertensive treatment discontinued taking previous drugs in VALUE trial before valsartan or amlodipine treatments were started.⁶

Conclusion

In summary, the major finding of the present study is that angiotensin receptor blocker losartan had adverse effects on LV remodeling during development of postinfarction heart failure. Our rat model of coronary ligation demonstrates that losartan triggered deleterious structural remodeling characterized by ventricular dilation, infarct wall thinning and corresponding impairment of contractile function. It is likely that the pronounced apoptotic and profibrotic effects of losartan disturbed healing of the infarcted area, whereas the beneficial effects of beta-blocker metoprolol on the

LV structure and function were associated with the increase in number of c-kit⁺ cells and cellular proliferation (Figure 7). These results suggest that combining drugs that stimulate CSCs to promote myocardial repair with stem-cell therapies may further enhance the efficacy of cell therapy. Furthermore, the present studies also warrant further analysis on the effects of early treatment of angiotensin receptor blockers on left ventricular remodeling.

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Figure 7. A schematic diagram summarizing the effects of metoprolol and losartan treatments on LV function and remodeling after MI. Metoprolol treatment attenuated adverse remodeling via c-kit⁺ and CD45⁺ cells and proliferation, whereas losartan-induced worsening of LV systolic function was associated with the increased apoptosis in the peri-infarct region.

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Supporting Information

The following Supporting Information is available for this article online:

Figure S1. Effect of coronary ligation on LV remodeling. Rats were killed 1 day ($n = 3$) and 2 ($n = 5$) and 4 ($n = 5$) weeks after myocardial infarction, and paraffin-embedded histological sections were cut. LV sections were consequently stained with hematoxylin-eosin (A) and Masson's trichrome (B) to reveal fibrosis. AMI = acute myocardial infarction.

Figure S2. Effect of metoprolol and losartan treatments for 2 weeks after MI on the the expression of stromal cell-derived factor (SDF)-1 and tumor necrosis factor- α (TNF α) in the left ventricle. Sections of the left ventricle were stained for SDF-1 α , SDF-1 β and TNF α . (A) SDF1 α staining ($\mu\text{m}^2/\text{field}$), (B) SDF1 β staining ($\mu\text{m}^2/\text{field}$) and (C) TNF α staining ($\mu\text{m}^2/\text{field}$) in each experimental group. Results are mean $\pm$ SEM ($n = 6/\text{group}$). AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan.

Figure S3. Effects of metoprolol and losartan treatments on markers of myocardial angiogenesis. (A) Pecam-1 stained sections of the ischemic border zone of infarcted LV area 2 weeks after MI in each experimental group. (B) Number of capillaries per high power (40 $\times$) field. Results are mean $\pm$ SEM ($n = 6/\text{group}$). $***, \uparrow\uparrow, \uparrow\uparrow\uparrow p < 0.001$ versus SHAM. (C) Sections stained for HIF1 α . Perfusion of rat hearts by the Langendorff's technique for 4 hours with 30 minutes ischemia in the beginning was used as a positive control for HIF1 α induction. AMI = acute myocardial infarction; METO = metoprolol; LOS = losartan.

Table S1. Effects of ligating the left anterior descending artery (LAD) on left ventricular structure and systolic function, as analyzed by echocardiography.

Table S2. Number of CD45 $^{+}$ and Ki-67 $^{+}$ cells in left ventricle after AMI measured by immunohistochemistry.

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