Original Article
Angiotensin II receptor blocker reverses heart failure by attenuating local oxidative stress and preserving resident stem cells in rats with myocardial infarction

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Abstract: The present study aimed to test whether angiotensin receptor blockers (ARBs) are cardioprotective after myocardial infarction (MI) by preventing augmented local renin-angiotensin-system (RAS)-induced oxidative stress injury and senescence, preserving resident stem cells, and restoring the insulin-like growth factor (IGF-1)/IGF-1 receptor (IGF-R) pathway. Sprague-Dawley rats with ligated or unligated coronary arteries were treated with losartan (20 mg/kg/d) or vehicle for 3 or 9 weeks. Heart function and molecular and histological changes were assessed. It was found MI induced left ventricular dysfunction and remodeling, increased levels of the oxidative stress marker 8-hydroxy-2'-deoxyguanosine and cell senescence marker p16^{ink4a}, and downregulated the IGF-1/IGF-1R/Akt pathway after both 3 and 9 weeks post-MI. MI induced an increase in stem cells identified by immunostaining for c-kit and Wilms' tumor-1 predominantly after 3 weeks. Losartan significantly inhibited local cardiac RAS activation and improved left ventricular function and remodeling at both timepoints. Losartan also preserved c-kit- and Wilms' tumor-1-positive cells (particularly at 3 weeks), attenuated 8-hydroxy-2'-deoxyguanosine- and p16^{ink4a}-positive cardiomyocytes, and restored the IGF-1/IGF-1R/Akt pathway at both 3 and 9 weeks. In conclusion, ARBs aided cardiac repair post-MI through short-term preservation of stem cells and persistent anti-oxidative stress and anti-senescence effects, partially by attenuating activation of cardiac RAS and restoring the local IGF-1/IGF-1R/Akt pathway.

Keywords: Renin-angiotensin system, myocardial infarction, stem cell, losartan, oxidative stress, IGF-1/IGF-1R/Akt pathway

Introduction

Myocardial infarction (MI) with resultant chronic heart failure (CHF) is a leading cause of mortality and morbidity worldwide. MI induces progressive inappropriate activation of the renin-angiotensin-system (RAS), which may be an important contributor to the pathogenesis of left ventricular remodeling, hypertrophy, and dysfunction. Inhibition of the RAS using angiotensin receptor blockers (ARBs) post-MI only partially reverses these adverse effects [1, 2]. Therefore, it is of paramount importance to characterize the mechanisms relevant for ARB efficacy when treating MI with subsequent CHF.

Growing evidence suggests undue activation of the local cardiac RAS plays a pivotal role in the abrupt deterioration and failure of the heart post-MI. MI significantly enhances activation of the local cardiac RAS as indicated by an increase in RAS components. Additionally, enhanced activation of the local RAS contributes to progressive heart dysfunction pathogenesis following induced MI, while treatment with the ARB losartan has cardioprotective effects after MI [3]. Activation of the local cardiac RAS components after MI may be associated with the homing and engraftment of circulating and/or resident stem cells to cardiac tissue, resulting in regeneration of myocardial tissue [4]. MI could also induce cell injury through accelerated senescence and increased oxidative stress, impairing the recovery of cardiac function and remodeling [5]. Therapies designed to maintain the number and activity of resident stem cells and interfere with oxidative stress and senescent injuries might aid in preventing cardiac damage post-MI.

Table 1. PCR primer sequences and conditions

Genes	Sequence	Cycles	Length (bp)
AT-1R		26	173
Sense	5'-GACCATTCACCCTGCCTCAG-3'		
Anti-sense	5'-CCAGACCCACCAATCCATCC-3'		
AT-2R		28	156
Sense	5'-GGCAGATAAGCATTTGGAAGC-3'		
Anti-sense	5'-AAGTCAGCCACAGCCAGATTG-3'		
Renin		26	112
Sense	5'-GGTGCTAAAGGAGGAAGTGTTT-3'		
Anti-sense	5'-GTGAAAGTTGCCCTGGTAATG-3'		
Angiotensinogen		25	244
Sense	5'-TTGGGTGCTGAGGCAAATCT-3'		
Anti-sense	5'-CCACATTTTGGGGGTTATCC-3'		
IGF-1R		25	101
Sense	5'-CGAGCAAGTTCTTCGTTTCGT-3'		
Anti-sense	5'-TGTACTGCCAGCACATGCG-3'		
β-actin		20	229
Sense	5'-CGTAAAGACCTCTATGCCAACA-3'		
Anti-sense	5'-CGGACTCATCGTACTCCTGCT-3'		

a soft tube in the cavity in order to allow air and blood to escape. After ventilation with room air for approximately 5 min, the animal was gradually weaned from the respirator once spontaneous respiration had resumed and was observed until completely conscious. Sham-operated animals underwent the same surgical procedures as described above with the exception of the ligations.

Ethics statement

Animals used in these experiments were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH PublicationNo. 85-23, revised 19-96) and study protocols were approved by the Animal Ethics Committee of Sun Yat-sen University.

However, it remains unclear whether MI-triggered activation of the local cardiac RAS axis plays an important role in the maintenance of c-kit- and WT-1-positive cell numbers and attenuation of oxidative stress and cell senescence. Therefore, the present study aimed to characterize changes in local cardiac RAS components, the number of c-kit- and WT-1-positive cells, and biomarkers of oxidative stress and senescence in rats with post-MI heart failure that were left untreated or treated with losartan, as well as explore the potential mechanisms underlying these changes.

Materials and methods

Animals and experimental MI

Experimental MI was induced in rats by left coronary artery ligation as described in our previous studies [6, 7]. Briefly, male Sprague-Dawley rats weighing 280-310 g were anesthetized with sodium pentobarbital (intraperitoneal, 30 mg/kg). The rats were intubated and then ventilated with a rodent respirator. A left thoracotomy was performed via the left fourth intercostal space and the heart exposed. After the pericardium was opened, the left anterior descending coronary artery was ligated with a 6-0 silk suture. The chest was then closed with

Experimental groups and treatments

Forty-eight hours after surgery, surviving MI animals were randomly allocated into losartan- and vehicle-treatment groups. Male rats in this study were randomly divided into 4 groups: normal (n=8 and 6 for 3 and 9 weeks, respectively), sham-operated (sham; n=8 and 6 for 3 and 9 weeks, respectively), MI (n=14 and 10 for 3 and 9 weeks, respectively), and MI plus losartan (MI+los; n=22 and 16 for 3 and 9 weeks, respectively). Drugs and double-distilled water as the vehicle were administered via gastric gavage daily for 3 or 9 weeks.

Echocardiographic measurements

Echocardiographic measurements were performed 3 and 9 weeks post-treatment using a high-resolution echocardiographic imaging system equipped with a 16 MHz transducer (Vevo2100, Visualsonics, Canada). The rats were anesthetized with 3% isoflurane mixed with oxygen and a two-dimensional short-axis view of the left ventricle was obtained at the midpapillary level to record M-mode tracings. Systolic and diastolic anatomic parameters were obtained.

Sample collection

Trunk blood was collected and supernatants collected and stored at -80°C for further analysis. Immediately after euthanasia using an overdose of sodium pentobarbital (intraperitoneal, 100 mg/kg), the heart was excised and residual blood flushed out with phosphate buffer. Cardiac tissue samples of border zone regions of infarct, normal, and sham rats were obtained. Equally sized samples were homogenized in 0.1 mol/l acetic acid (10%, wt/vol) containing EDTA and protease inhibitors. Supernatants were obtained after centrifugation and AngII levels measured. Other equally sized cardiac samples were homogenized in cold PBS, supernatants were obtained, and insulin-like growth factor-1 (IGF-1) measured. Cardiac tissue samples were cut and fixed in 4% formalin and embedded in paraffin. The other cardiac samples were snap-frozen in liquid nitrogen and stored at -80°C for RNA and protein measurements.

Reverse transcription polymerase chain reaction

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to measure changes in cardiac expression of AngII type 1 receptor (AT-1R), AT-2R, rennin, angiotensinogen, and IGF-1 receptor (IGF-1R) mRNA as described in our previous studies [6, 7]. Total RNA was isolated from heart tissues using Trizol reagent (GIBCO Invitrogen) and RNA was quantified by spectrophotometry. Equal amounts of RNA were used for complementary DNA synthesis with Moloney murine leukemia virus reverse transcriptase with the primers indicated in **Table 1**, where primers for β-actin were included in each reaction as an internal control (**Table 1**). Reverse transcription was performed at 42°C for 1 h, followed by a denaturation at 94°C for 5 min. The PCR cycle conditions were as follows: denaturation for 30 sec at 94°C, annealing for 30 sec at 60°C (AT-1R: 58°C; AT-2R: 58°C; renin: 57°C; angiotensinogen: 58°C; IGF-1R: 57°C; and β-actin: 57°C), and extension for 45 sec at 72°C. Different numbers of cycles were performed for amplification of different genes as indicated in **Table 1**. The products of each PCR reaction were separated using 2% agarose gel electrophoresis. After quantifying the band intensities by densitometry, relative steady-state levels of mRNA were calculated after normalizing to β-actin.

Western blotting

Western blotting was performed as previously described in our studies [6, 7]. Total protein was extracted from heart tissue with modified radioimmunoprecipitation assay (RIPA) buffer. Equal protein lysates were separated on a 10% polyacrylamide gel, transferred to polyvinylidene difluoride membranes, and then immune blotted with anti-AT-1R (Abcam, UK), anti-AT-2R (Abcam, UK), anti-IGF-1R (Abcam, UK), anti-phosphorylated (*p*)-Akt (CST, USA), anti-Akt (CST, USA), and anti-GAPDH antibodies (CST, USA) at 4°C overnight. The membranes were washed, incubated with horseradish peroxidase-conjugated secondary antibodies, and visualized using the ECL chemiluminescence system. Densitometry analysis was performed using the Bio-Rad image detection system and Quantity One software (Bio-Rad, CA, USA). After quantifying the band intensities by densitometry, relative steady-state protein levels were calculated after normalizing to GAPDH.

Histological and immunohistochemical examinations

Heart sections (4 μm) were stained with hematoxylin and eosin (HE) for routine histological examination of scar tissue and viable myocardium. Heart sections (4 μm) were processed for immunohistochemical staining according to our previously published protocol [6, 7]. Heart section slides were deparaffinized in xylene, rehydrated using graded alcohol, and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity and antigens were retrieved by pressure cooking in citrate buffer (pH=6). After blocking of nonspecific binding, the slides were incubated with anti-c-kit (Santa Cruz, CA), anti-WT-1 (Santa Cruz, CA), anti-p16[ink4a] (Santa Cruz, CA), or anti-8-hydroxy-2'-deoxyguanosine (8-OHdG) antibodies (Japan Institute for the control of Aging, Japan) and stored overnight at 4°C. The slides were sequentially incubated with a secondary antibody (Dako, Denmark) and stained with 3,3-diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. For the negative control, the primary antibody was replaced with a normal murine or rab-
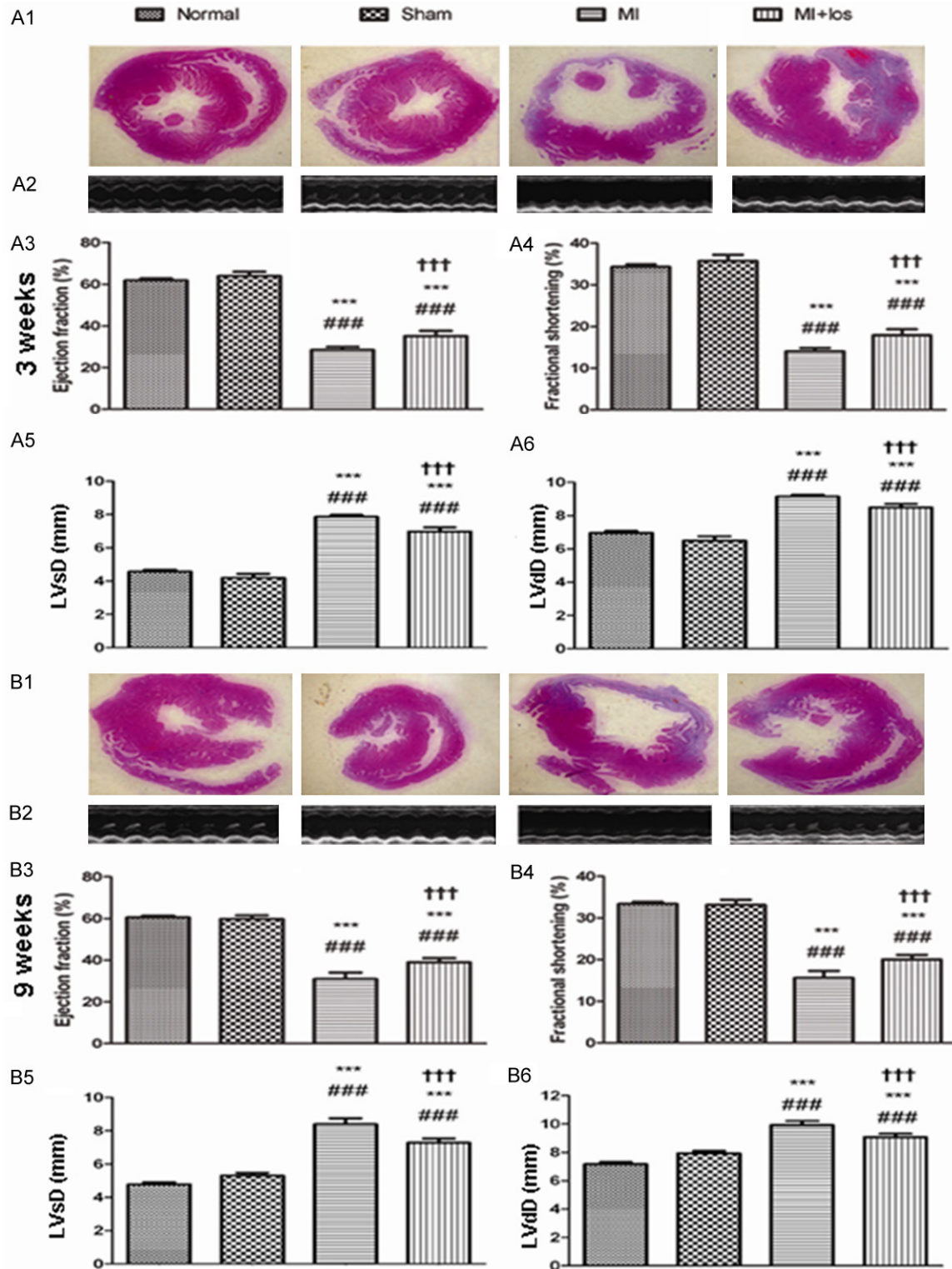
Figure 1. Ventricular function and remodeling in normal, sham, MI, and MI+los rats as evaluated based on morphometric changes and echocardiographic measurements. (A1) Scar tissue and viable myocardium in myocardial sections from 3 weeks post-MI are blue and red, respectively, due to hematoxylin and eosin staining (original magnification, ×7). (A2) Representative M-mode echocardiographic images, (A3) ejection fraction, (A4) fractional shortening, (A5) LVsD, and (A6) LVdD at 3 weeks post-MI. (B1) Scar tissue and viable myocardium in myocardial sections from 9 weeks post-MI are blue and red, respectively, due to hematoxylin and eosin staining (original magnification, ×7). (B2) Representative M-mode echocardiographic images, (B3) ejection fraction, (B4) fractional shortening, (B5)

LVsD, and (B6) LVdD at 9 weeks post-MI. Data are presented as mean ± SD. ###P < 0.008 vs. normal; ***P < 0.008 vs. sham; †††P < 0.008 vs. MI. MI, myocardial infarction; los, losartan. LVsD, left ventricular systolic diameter; LVdD, left ventricular diastolic diameter.

Table 2. Gross measurements

	Normal	Sham	MI	MI+los	P
3 week					
Heart weight (g)	0.74±0.04	0.79±0.05	0.90±0.06[a,b]	0.82±0.10[a,c]	< 0.001
Heart weight/body weight (*10[-3])	2.63±0.15	2.67±0.08	2.86±0. 24[a,b]	2.49±0. 28[c]	0.001
9 week					
Heart weight (g)	0.81±0.03	0.82±0.06	0.96±0.07[a,b]	0.80±0.06[c]	< 0.001
Heart weight/body weight (*10[-3])	2.34±0.04	2.35±0.20	2.76±0.31[a,b]	2.35±0.15[c]	< 0.001

Data are presented as means ± SD. P-value based on one-way ANOVA followed by LSD test. [a]P < 0.008 vs. normal group; [b]P < 0.008 vs. sham group; [c]P < 0.008 vs. MI alone. MI, myocardial infarction; los, losartan; LV, left ventricular.

bit IgG. In each section, c-kit- and WT-1-positive cells and p16[ink4a]- and 8-OHdG-positive cardiomyocytes per 100 mm^2 were quantified using Image-Pro Plus software (Media Cybernetics, MD). A total of 5 consecutive 100 mm^2 were examined per section by a pathological expert in a blind manner.

Enzyme-linked immunosorbent assay

AngII and IGF-1 content was measured in tissue homogenate supernatant and blood serum samples collected at 3 and 9 weeks post-MI. Enzyme-linked immunosorbent assays were performed using AngII (SPI-BIO, France) and IGF-1 commercial kits (MG100, R&D Systems) according to the manufacturers' instructions.

Statistical analysis

All quantitative data are presented as mean ± SD. According to normality test results, data were compared using one-way ANOVA followed by LSD *post-hoc* tests. Two-tailed P-values < 0.05 were considered statistically significant and adjusted P-values were used to compare subgroups. All statistical analyses were performed with the software package SPSS 22.0 (IBM, USA) for Windows.

Results

*Left ventricular function and ventricular remodeling*

Compared to normal and sham-operated animals, animals that underwent coronary artery ligation had significant decreases in the heart function parameters of ejection fraction and fractional shortening at both 3 and 9 weeks post-MI (**Figure 1**). Administration of losartan significantly improved the ejection fraction and fractional shortening at both timepoints. Scar tissue indicative of MI was identified by HE staining. Left ventricular systolic and diastolic diameters in MI rats were significantly increased at both timepoints and losartan treatment caused a decrease in both. However, losartan failed to induce full recovery of left ventricular systolic diastolic diameter to the size observed in normal and sham animals. The increased heart weights and ratios of heart weight to body weight post-MI were significantly improved by losartan treatment (**Table 2**).

Changes in local RAS components

MI animals had more AngII in both the serum and border zone regions of infarct hearts than normal or sham animals at 3 and 9 weeks (**Figure 2**). Losartan significantly decreased local levels of AngII in the infarct hearts, but serum AngII levels further increased after losartan treatment. At 3 weeks, compared to normal animals, the sham operation had induced a significant increase in mRNA levels of local AT-1R, AT-2R, renin, and angiotensinogen, and MI further increased these levels. Losartan administration decreased mRNA expression of all but AT-1R. At 3 weeks post-MI, sham rats had higher levels of both AT-1R and AT-2R protein than normal rats and the MI group had the highest levels of these two proteins. Increased local AT-1R and AT-2R protein levels were inhibited by losartan treatment. At 9 weeks post-MI,
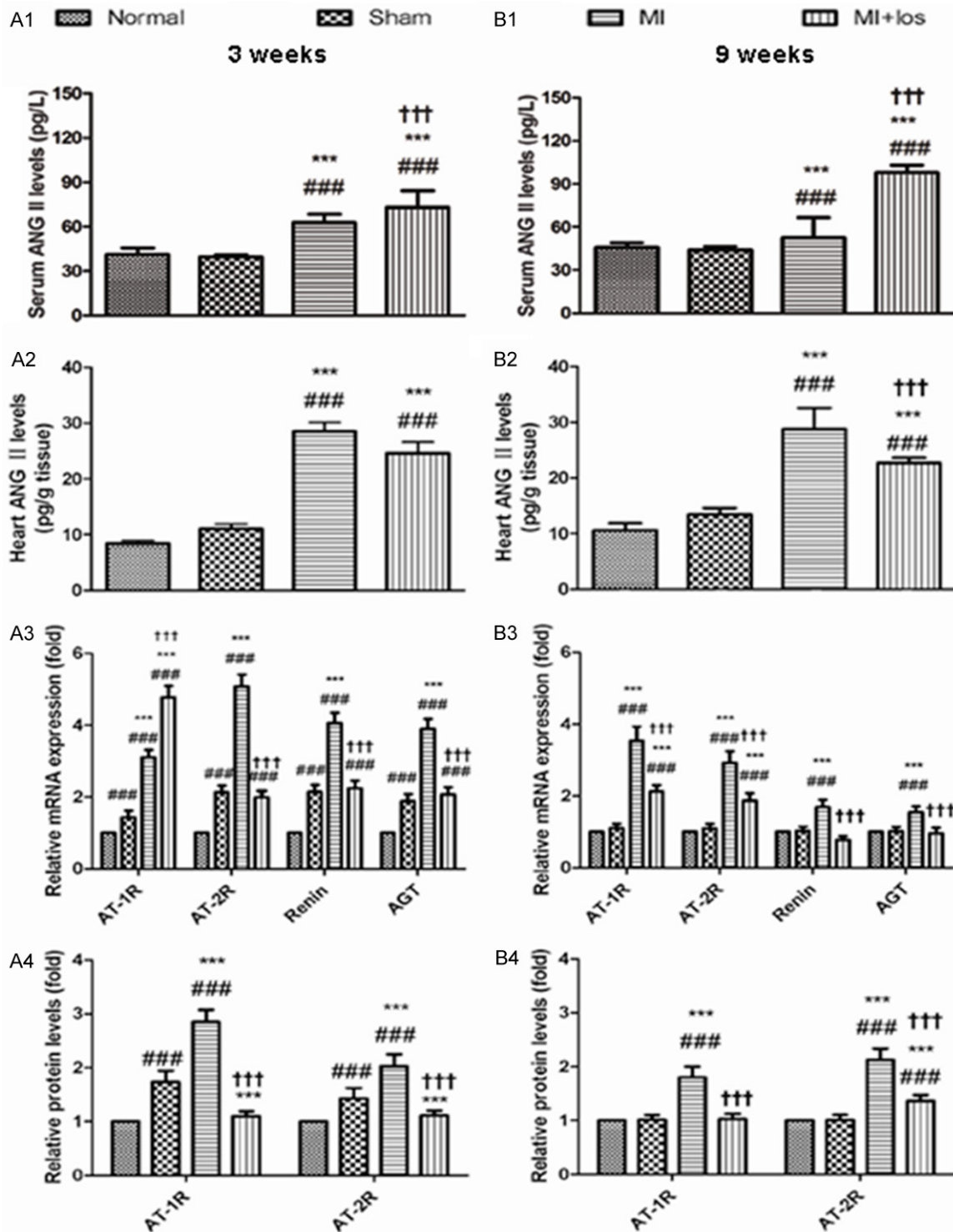
Figure 2. Local changes in renin-angiotensin-system components and serum angiotensin II in normal, sham, MI, and MI+los rats at 3 and 9 weeks post-MI. (A1) Serum ANG II, (A2) cardiac ANG II, (A3) cardiac AT-1R, AT-2R, renin and AGT mRNA, and (A4) cardiac AT-1R and AT-2R protein levels at 3 weeks post-MI. (B1) Serum ANG II, (B2) cardiac ANG II, (B3) cardiac AT-1R, AT-2R, renin, and AGT mRNA, and (B4) cardiac AT-1R and AT-2R protein levels at 9 week post-MI. ###$P < 0.008$ vs. normal; ***$P < 0.008$ vs. sham; †††$P < 0.008$ vs. MI. MI, myocardial infarction; los, losartan; ANG II, angiotensin II; AT-1/2R, angiotensin II type 1/2 receptor; AGT, angiotensinogen.

MI, but not sham, animals still had higher levels of local RAS component mRNA and protein compared to normal animals. Treatment with losartan prevented augmentation of border
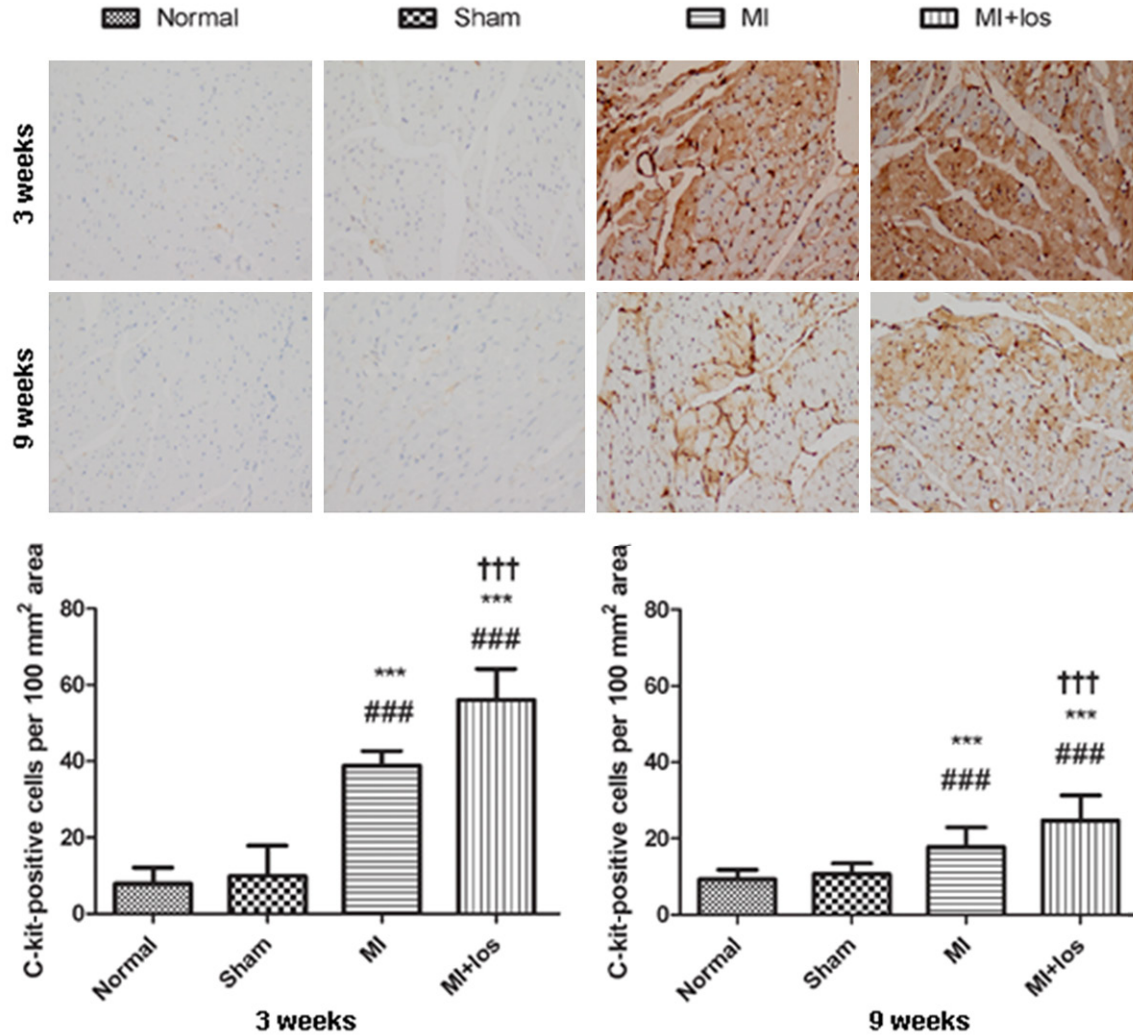
Figure 3. Representative immunohistochemical staining of c-kit-positive cells (original magnification, ×400) and the average number of c-kit-positive cells per 100 mm^2 at 3 and 9 weeks post-MI. ###$P < 0.008$ vs. normal; ***$P < 0.008$ vs. sham; †††$P < 0.008$ vs. MI. MI, myocardial infarction; los, losartan.

zone region AngII and local RAS component mRNA and protein levels.

Populations of c-kit- and WT-1-positive cells

Compared to the normal and sham group, the MI-induced group had more c-kit-positive cells in the border zone regions and losartan further increased the number of these c-kit-positive cells at both timepoints (**Figure 3**). However, no significance differences were found between normal and sham rats and rats in both the MI and MI+los groups had more c-kit-positive cells at 3 weeks than at 9 weeks post-MI ($P < 0.001$, respectively). Compared to normal animals, MI, but not sham, animals had more WT-1-positive cells in the border zone regions and losartan

treatment caused a further increase in the number of these cells (**Figure 4**). However, significant differences in WT-1-positive cell population size were only found after the shorter time period of 3 weeks, where no significance differences were found in WT-1-positive cells among the four groups at 9 weeks post-MI.

Oxidative stress injury and senescence of cardiomyocytes

In the present study, we examined a marker of oxidative stress, 8-OHdG, by immunostaining cardiomyocytes in the border zone regions and found the MI group had more 8-OHdG-positive cardiomyocytes than the normal and sham groups at 3 and 9 weeks post-MI (**Figure 5**). The
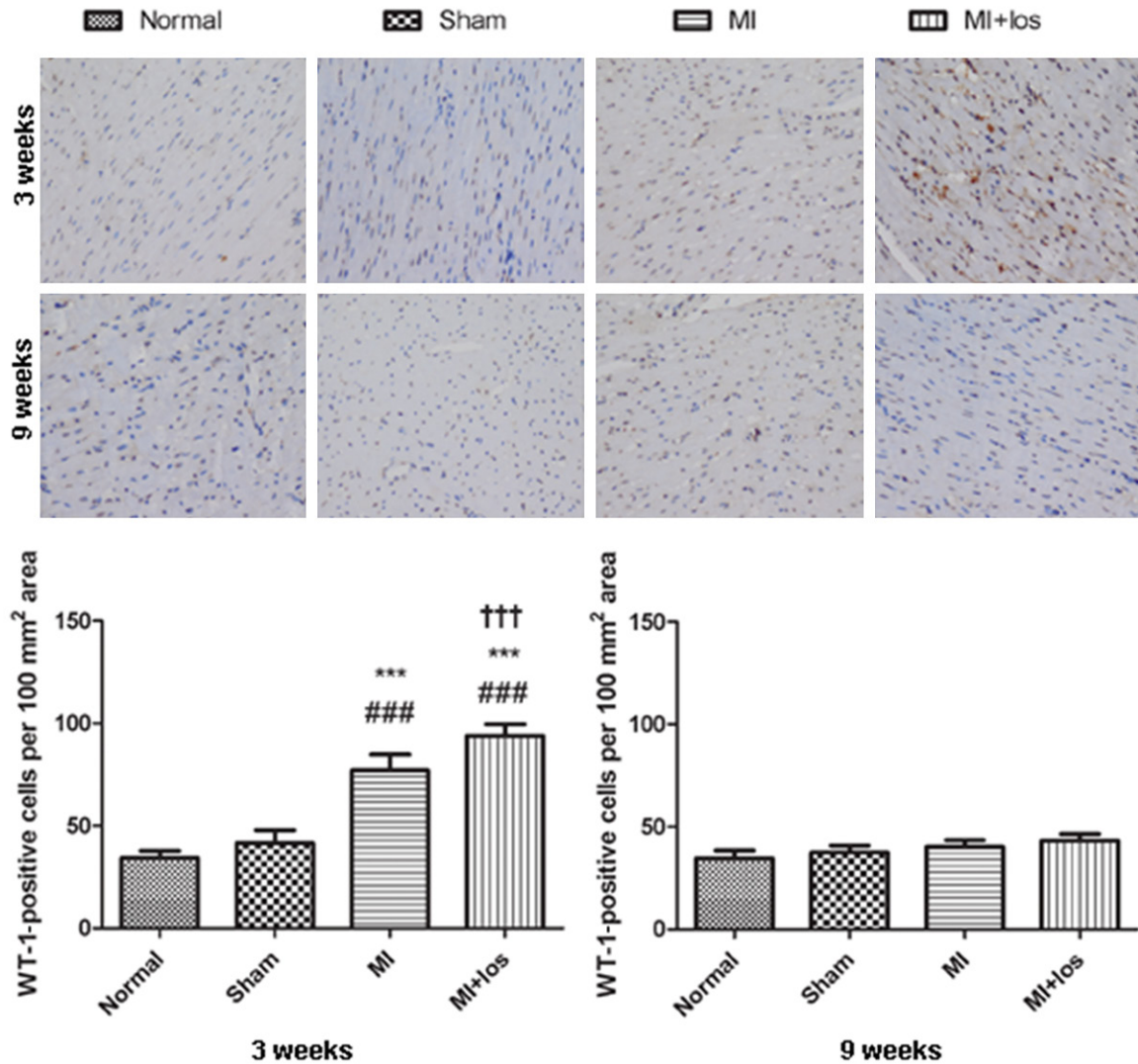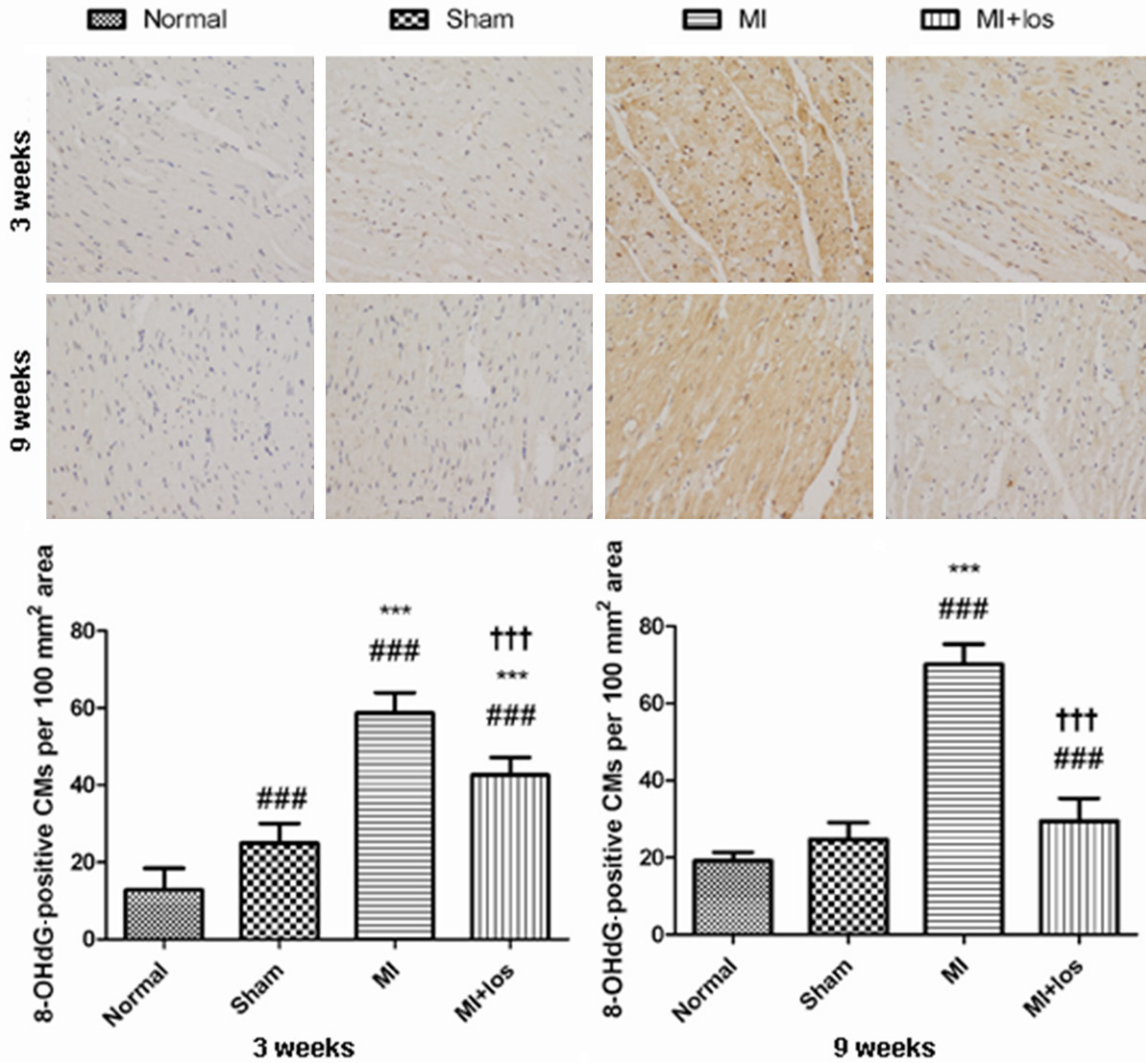
Figure 4. Representative immunohistochemical staining of WT-1-positive cells (original magnification, ×400) and the average number of WT-1-positive cells per 100 mm^2 at 3 and 9 weeks post-MI. ###$P < 0.008$ vs. normal; ***$P < 0.008$ vs. sham; †††$P < 0.008$ vs. MI. MI, myocardial infarction; los, losartan; WT-1, Wilms' tumor-1.

sham group also had more 8-OHdG-positive cardiomyocytes than the normal rats at 3 weeks post-MI. Losartan treatment significantly inhibited the increase in 8-OHdG-positive cardiomyocytes in the border zone regions of MI animals. There were significantly more senescent cardiomyocytes, as tested by the p16^{ink4a} assay, in cardiac tissues in MI animals than in animals that were not operated on or underwent a sham operation (**Figure 6**). However, losartan-treated rats had significantly fewer p16^{ink4a}-positive cardiomyocytes at the end of both time periods. Additionally, there was a trend of more 8-OHdG- and p16^{ink4a}-positive cardiomyocytes at 9 weeks than 3 weeks post-MI ($P < 0.001$, respectively).

Potential mechanisms involving IGF-1/IGF-1R/ Akt pathway

The authors also determined whether the IGF-1/IGF-1R/Akt pathway was involved in losartan-mediated cardioprotection in MI animals. IGF-1 levels were decreased in both the serum and cardiac tissues of rats 3 and 9 weeks post-MI (**Figure 7**). MI rats also had significantly lower IGF-1R mRNA and protein levels, as well as decreased cardiac *p*-Akt protein levels at both timepoints. Additionally, losartan treatment significantly restored downregulated IGF-1, IGF-1R mRNA and protein, and *p*-Akt protein levels at the end of both timepoints in MI animals.
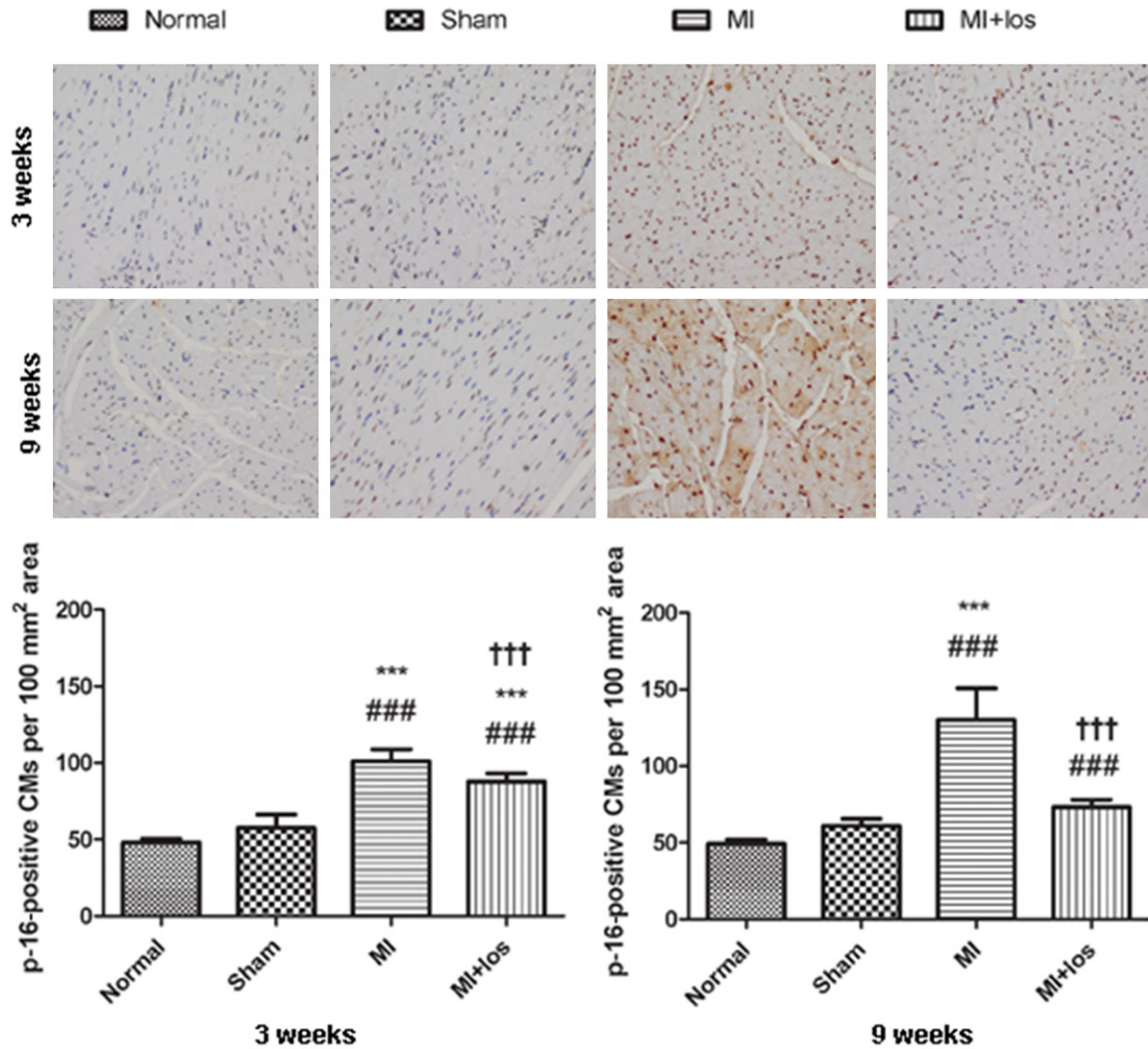
Figure 5. Representative immunohistochemical staining for the marker of oxidative stress 8-OHdG (original magnification, ×400) and the average number of 8-OHdG-positive cardiomyocytes per 100 mm^2 at 3 and 9 weeks post-MI. ###$P < 0.008$ vs. normal; ***$P < 0.008$ vs. sham; †††$P < 0.008$ vs. MI. MI, myocardial infarction; los, losartan; 8-OhdG, 8-hydroxy-2'-deoxyguanosine; CMs, cardiomyocytes.

Discussion

The findings in the present study were consistent with prior work showing progressive ventricular dysfunction and remodeling in MI models could be improved by ARBs, such as losartan [3]. However, as reported by other authors [1, 2], the present study revealed losartan could only partially reverse left ventricular function and remodeling post-MI. The local cardiac RAS plays a pivotal role in the pathophysiologic functions of cardiomyocyte survival, renewal, and growth, as well as cardiac remodeling [4]. MI increases expression of local cardiac RAS components, including renin, AngII, and AngII receptors [8, 9] and angiotensinogen mRNA levels are elevated in CHF hearts [10]. Consistent with these findings, the authors found that all RAS components were increased in local myocardium after MI with resultant CHF in this study. In addition, both serum and cardiac AngII levels were increased post-MI at both timepoints and increased renin and angiotensinogen levels might contribute to increased generation of AngII in local myocardium. Treatment with losartan to prevent an increase in RAS activation improved ventricular function and remodeling in this study. However, another study has also demonstrated that circulating AngII levels do not reflect the status of RAS axis

Figure 7. Evaluation of local changes in IGF-1/IGF-1R/AKT pathway and serum IGF-1 in normal, sham, MI, and MI+los rats at 3 and 9 weeks post-MI. (A1) Serum IGF-1, (A2) cardiac IGF-1, (A3) cardiac IGF-1R mRNA, and (A4) cardiac IGF-1R,AKT and *p*-AKT protein levels at 3 week post-MI. (B1) Serum IGF-1, (B2) cardiac IGF-1, (B3) cardiac IGF-1R mRNA, and (B4) cardiac IGF-1R, AKT and *p*-AKT protein levels at 9 weeks post-MI. ###$P < 0.008$ vs. normal; ***$P < 0.008$ vs. sham; †††$P < 0.008$ vs. MI. MI, myocardial infarction; los, losartan; IGF-1/R, insulin-like growth factor-1/receptor.

myogenic phenotype is subject to temporal limitations [17]. Other authors have also demonstrated that these c-kit-positive cells do not acquire a cardiomyogenic phenotype other than adopting a vascular fate within the infarct myocardium [18]. Whether these c-kit-positive cells become myogenic progenitors or cardiomyocytes, it is consistent that these cells could be induced to enhance endogenous repair following MI. Our findings demonstrate that local cardiac RAS activation is associated with homing and engraftment of c-kit-positive cells, where losartan treatment preserves the size of the population of these induced c-kit-positive cells, although there was still a decrease in cell number over time. ARB potentially offers translational options for improving the regenerative potential of these cells. However, activating these cells is not sufficient to prevent the infarct heart from CHF. Therefore, delineation of the mechanisms underlying the increase in these presumably cardiac resident cells may help with developing promising strategies for endogenous repair post-MI.

Increasing evidence suggests WT-1-positive cells mainly residing in the epicardium may be another cell class important in the increase in and homing of cells to the border zone of the infarct heart at different timepoints [19, 20]. However, the present study showed MI caused a significant increase in WT-1-positive cells at 3 weeks, but not 9 weeks, post-MI, which is inconsistent with findings from another study [20]. Other findings have demonstrated these WT-1-positive cells have cardiogenic potential and differentiate into not only fully functional cardiomyocytes, but also vascular endothelial cells and coronary artery smooth muscle cells in the adult heart [21]. Furthermore, cardiac resident c-kit-positive cells also express stem cell antigen WT-1 post-MI [22], suggesting these two cell categories share a similar origin or one may be the progenitor of the other. This study also revealed that inhibition of local cardiac RAS activation using losartan kept WT-1-positive cells within the border zone regions of the infarct heart, especially in the short term. All these findings presume the WT-1-positive cell class specifically can be used as a resource when repairing failing hearts post-MI. Developing methods of attenuating the adverse features of the local myocardial environment, such as excessive activation of the local RAS cascade, may improve the success of endogenous cardiac regenerative processes and, thus, permit therapeutic myocardial repair without cell delivery *per se.*

Our findings also indicate local activation of the RAS may stimulate local oxidative stress, which is a major player in the pathobiology of cardiovascular disease. Levels of 8-OHdG, a biomarker of oxidative DNA, were significantly increased in rats with MI in this study and there were significantly more 8-OHdG-positive cardiomyocytes in MI animals than in sham animals. The present study demonstrates increased 8-OHdG expression may be a better biomarker of oxidative stress and is associated with left ventricular dysfunction and remodeling, which is supported by work of other authors [5]. Similar to our previous findings [6], oxidative stress may contribute to cell senescence post-MI. A significant increase in p16^{ink4a} expression is accompanied by more p16^{ink4a}-positive cardiomyocytes in the border zone regions of infarct rats. The gradual increase over time post-MI in p16^{ink4a}-positive senescent c-kit-expressing cells also contributes to the loss of functionally competent cells in chronic ischemic cardiomyopathy and underlies progressive functional deterioration and the onset of terminal failure [23]. However, this study did not assess these p16^{ink4a}-positive senescent c-kit- and WT-1-positive cells. The present study also found a gradual increase in 8-OHdG- and p16^{ink4a}-positive cardiomyocytes post-MI over time and losartan prevented cardiomyocyte oxidative stress injury and senescence. These findings suggest that losartan suppressed the local RAS and resultant cardiac oxidative stress cycle, leading to cardioprotection as indicated by an inhibition of oxidative stress and cell senescence.

The present study indicates enhanced oxidative stress and cell senescence were associated with downregulation of the IGF-1/IGF-1R/Akt pathway, while losartan restored IGF-1/IGF-1R/Akt pathway activation and prevented associated abnormalities. Losartan also preserved the increase in the number of c-kit- and WT-1-positive cells, which may also be associated with the observed reversion of this pathway. Both serum and local cardiac IGF-1 levels had decreased at both timepoints assessed post-MI and losartan restored these levels in both

the serum and local myocardium in this study. Circulating levels of IGF-1 are pivotal for preserving cardiac structure and function and predicting the evolution of ischemic cardiomyopathy and prognosis post-MI [24]. Serum IGF-1 levels are significantly decreased in infarct rats and increasing its levels could improve cardiac function in failing hearts [25]. Local cardiac IGF-1 expression, which is decreased significantly in animals with post-MI heart failure, contributes to the improvement of cardiac function by enhancing angiogenesis [26]. In addition, increased IGF-1 levels may contribute to the upregulation of c-kit and WT-1 in adult mouse hearts post-MI [22]. Cardiac AngII acts via AT-1R to enhance inflammation, oxidative stress, and cell death, most likely via downregulation of PI3-kinase and Akt [27]. IGF-1 activation of its receptor IGF-1R may counteract AngII-mediated cellular senescence and oxidative stress in an AT-1R-dependent manner [28]. Increased IGF-1 levels in the heart could also suppress p16^{ink4a} protein expression in cardiac stem cells by enhancing nuclear p-Akt in post-infarct rats [25]. Influencing RAS with captopril increases IGF-1R mRNA and protein expression in the myocardium post-MI [29]. These data suggest that the beneficial attenuation of cardiac remodeling and functioning by RAS inhibitors may be mediated in part through increased expression of IGF-1R, which sensitizes the myocardium to the positive effects of endogenous IGF-1. Our previous studies have shown IGF-1R/PI3K/Akt signaling is involved in every aspect of stem cell fate in cardiac repair post-MI [15]. However, future experiments should investigate sequential activation of the IGF-1/IGF-1R/Akt signaling pathway during MI-induced oxidative stress, senescence, and homing and engraftment of c-kit- and WT-1-positive stem cells.

The local RAS and potential mechanisms of losartan action were tested only in the border zone regions of infarct hearts, which is an important limitation of the present study. In addition, the properties of c-kit- and WT-1-positive cells and the effects of IGF-1/IGF-1R on the homing and engraftment of these cells were not further evaluated in this study. Therefore, genetically modified animal models with c-kit-and/or WT-1 deletions or overexpressing AngII receptors or IGF-1R should be used in conjunction with relevant pharmacological ag-

ents to further test the hypotheses put forth in this work in future studies.

Disclosure of conflict of interest

None.

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