Research Article

Knockdown of Salusin-β Improves Cardiovascular Function in Myocardial Infarction-Induced Chronic Heart Failure Rats

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Salusin-β is a biologically active peptide with 20 amino acids that exerts several cardiovascular activity-regulating effects, such as regulating vascular endothelial function and the proliferation of vascular smooth muscle cells. However, the regulatory effects of salusin-β in myocardial infarction-induced chronic heart failure (CHF) are still unknown. The current study is aimed at investigating the effects of silencing salusin-β on endothelial function, cardiac function, vascular and myocardial remodeling, and its underlying signaling pathways in CHF rats induced by coronary artery ligation. CHF and sham-operated (Sham) rats were subjected to tail vein injection of adenoviral vectors encoding salusin-β shRNA or a control-shRNA. The coronary artery (CA), pulmonary artery (PA), and mesenteric artery (MA) were isolated from rats, and isometric tension measurements of arteries were performed. Compared with Sham rats, the plasma salusin-β, leptin and visfatin levels and the salusin-β protein expression levels of CA, PA, and MA were increased, while the acetylcholine- (ACh-) induced endothelium-dependent vascular relaxation of CA, PA, and MA was attenuated significantly in CHF rats and was improved significantly by salusin-β gene knockdown. Salusin-β knockdown also improved cardiac function and vascular and myocardial remodeling, increased endothelial nitric oxide synthase (eNOS) activity and nitric oxide (NO) levels, and decreased NAD(P)H oxidase activity, NOX-2 and NOX-4 expression, and reactive oxygen species (ROS) levels in arteries in CHF rats. The effects of salusin-β knockdown in CHF rats were attenuated significantly by pretreatment with the NOS inhibitor L-NAME. These results indicate that silencing salusin-β contributes to the improvement of endothelial function, cardiac function, and cardiovascular remodeling in CHF by inhibiting NAD(P)H oxidase-ROS generation and activating eNOS-NO production.

1. Introduction

Chronic heart failure (CHF) is the terminal stage of a variety of cardiovascular diseases and is characterized by progressive left ventricular dysfunction and declining ejection fraction (EF), usually accompanied by vascular endothelial dysfunction, neuroendocrine activation, and ventricular remodeling [1–3]. Among them, vascular endothelial dysfunction usually occurs at the early stages of heart failure and is closely related to the development of CHF and is also a predictor of adverse events, such as cardiovascular remodeling in CHF patients [4, 5]. Normally, vascular endothelial cells (VECs) can release vasoconstrictor factors, such as endothelin-1, and vasodilatation factors, such as nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostaglandin (PGI2), to control vascular tone. When endothelial dysfunction occurs, the release of vasoconstrictor factors and vasodilatation factors from VECs becomes imbalanced. Among these, the decrease in NO bioavailability plays the most important role in CHF [6–8], which results in the decline of vascular relaxation and the increase in vascular contraction function [9, 10]. Attenuated vascular relaxation caused by endothelial
dysfunction in the coronary artery (CA) in CHF decreases vascular compliance, reduces the blood supply to the heart, and subsequently causes myocardial ischemia and angina and further deterioration of cardiac function [11, 12]. Attenuated pulmonary artery (PA) relaxation in CHF increases pulmonary vascular resistance and might contribute to the high incidence of pulmonary hypertension due to left heart disease [13, 14]. Due to the enormous quantity of mesenteric artery (MA), attenuated MA relaxation increases peripheral resistance and cardiac afterload, leading to further cardiac dysfunction. It is necessary to find therapeutic methods or interventions to correct the endothelial dysfunction of heart failure and then improve cardiac function and prevent the progression of CHF.
were then calculated. Next, the catheter was pushed into the left ventricle from the right carotid artery to record LV pressure. The LV peak systolic pressure (LVSP), LVEDP, LV developed pressure (LVDP), and LV +dP/dt max were calculated.

2.5. Isometric Tension Measurements in Arteries. Isometric tension of arteries was measured to evaluate vascular endothelial function as described in our previous report [39]. Briefly, after hemodynamic parameters were measured, the heart, lung, and mesentry were isolated from rats. Heart weight and infarct size were measured. Then, the third-order CA, PA, and MA were isolated and cut into 1-1.2 mm segments. Arterial rings (1 arterial ring/artery/rat was used) were mounted in a four-chambered myograph (620 M, DMT, Denmark) with 20 μm wires and set at a resting tension of 0.1 g. After arterial ring contraction induced by prostaglandin F2α (PGF 2α), six doses of ACh (10^{-9} - 10^{-4} mol/L) were administered in a dose-dependent manner to induce vascular relaxation. The degree of relaxation is shown as a percentage of PGF 2α-induced contraction.

2.6. Treatment with L-NAME in CHF Rats. The nitric oxide synthase (NOS) inhibitor L-NAME (50 mg/kg/day) was given to rats by gavage for four weeks. The control group was given saline. At the start of the third week of L-NAME application, knockdown of salusin-β was performed.

2.7. Measurement of Protein Expression in Arteries by Western Blot. Third-order CA, PA, or MA samples were isolated from rats, flash-frozen in liquid nitrogen, and stored at -70°C. Then, the artery tissues were homogenized and centrifuged. The tissue homogenate from the artery was subjected to Western blot analysis for determination of protein levels. The protein concentration was measured using a protein assay kit (BCA, Pierce, USA), loaded onto an SDS-PAGE gel, and separated by electrophoresis. The protein bands were visualized by staining with Coomassie blue. The bands were excised, digested with trypsin, and analyzed by mass spectrometry.

2.8. Measurement of Salusin-β, Leptin, Visfatin, eNOS, NO Levels, and eNOS Activity by ELISA. Commercial ELISA kits were used for the measurement of the plasma salusin-β (Cloud-clone corp, Wuhan, China), leptin (Hui Jia Biotechnology, Xiamen, China), visfatin (Hui Jia Biotechnology, Xiamen, China), and eNOS levels of arteries (Yi Fei Xue Biotechnology, Nanjing, China) according to the manufacturer’s descriptions. A nitrate/nitrite colorimetric assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) was used to evaluate NO production in arteries based on the detection of the concentration of its stable metabolites nitrate and nitrite. The measurement of eNOS activity by ELISA was performed using a Nitric Oxide Synthase Assay Kit (Beyotime Biotech Inc., Nanjing, China) by assessing the ability of conversion of L-arginine to NO.

2.9. Measurement of NAD(P)H Oxidase Activity and Reactive Oxygen Species (ROS) Levels. NAD(P)H oxidase activity and ROS levels were measured using the enhanced lucigenin-derived chemiluminescence method as we previously reported [27, 40–42]. Briefly, the light emissions produced by the reactions between lucigenin (5 μM) and the ROS in tissue homogenate supernatant were measured by a luminometer (20/20n, Turner, CA, USA) once every minute for 10 minutes to measure ROS levels. To measure NAD(P)H oxidase activity, NAD(P)H (100 μM) was first added to the medium as a substrate to react with NAD(P)H oxidase to generate ROS before the reactions between lucigenin and ROS were detected by a luminometer. The values representing NAD(P)H oxidase activity and ROS levels are expressed as the mean light unit (MLU) per minute per milligram of protein.

2.10. Evaluation of Vascular Remodeling. Third-order CA, PA, and MA isolated from rats were embedded in paraffin, cut into 5-μm thick cross-sections, and stained with hematoxylin-eosin (HE). The structural changes of these arteries were observed with a light microscope. The media thickness, lumen diameter, and media thickness/lumen diameter were measured and used as indexes of vascular remodeling [27].

2.11. Evaluation of Left Ventricular Remodeling and Microvascular Density. Perivascular fibrosis in the intramural arteries and arterioles and myocardium fibrosis were evaluated in Masson’s trichrome-stained sections under high and low power microscope as previously reported [27, 43]. Myocyte cross-sectional area was determined in the left ventricular lateral-midfree wall, including epicardial and endocardial portions, in HE-stained sections [44]. Immunohistochemistry staining of cardiomyocytes with dystrophin antibody (1:200, Proteintech Inc., Wuhan, China) was also performed to observe the change in cardiomyocyte morphology of rats. Paraffin sections of the myocardial infarct border and remote zone (apex of heart) were immunohistochemically stained with an endothelial marker CD31 antibody (1:500, Servicebio Inc., Wuhan, China) to show capillary and arteriolar density in the myocardial tissue [45].

2.12. Cell Experiments. Human pulmonary arterial endothelial cells (HPAECs) (ScienCell Research Laboratories, Carlsbad, CA, USA) were cultured in DMEM containing 10% FBS, 1% penicillin and streptomycin, and 1% growth factor at 37°C in an incubator containing 95% air and 5% CO₂. Tumor necrosis factor-α (TNF-α) (10 μg/mL) was added to DMEM for 24 h to stimulate cells to mimic the similar inflammatory responses produced in heart failure. Then,
Hence, we used the normal distribution of values. Statistical comparisons were made. The Bonferroni test for posthoc analysis when multiple comparisons were made between two groups.

2.13. Chemicals. Salusin-β was obtained from Bachem (Bubendorf, Switzerland). Prostaglandin F2α (PGF 2α), acetylcholine (ACH), N′-nitro-L-arginine-methyl ester hydrochloride (L-NAME), and TNF-α were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals were dissolved in normal saline.

2.14. Statistical Analysis. Data are expressed as the mean ± S.E. The Kolmogorov-Smirnov test and Shapiro-Wilk test were used to measure the normal distribution of values. Student’s unpaired t-test was used for comparisons between two groups. One-way or two-way ANOVA was used, followed by the Bonferroni test for posthoc analysis when multiple comparisons were made. P < 0.05 was considered statistically significant.

3. Results

3.1. Effects of Salusin-β Knockdown on Anatomy and Hemodynamics. The body weight (BW), heart weight (HW), infarct area, and baseline hemodynamic arguments were measured after two weeks of cont-shRNA or salusin-β-shRNA application in CHF and Sham rats. Although the BW was not significantly different between CHF and Sham rats, the HW and the HW/BW were increased in CHF rats, which suggested the occurrence of myocardial hypertrophy. Knockdown of salusin-β decreased the infarct size of the LV in CHF rats. Compared with the Sham rats, the SAP, PP, LVSP, LVDP, and LV +dP/dt max decreased, while LVEDP increased significantly in CHF rats, which was consistent with our previous study findings [46]. These abnormal hemodynamic parameters in CHF were improved by salusin-β knockdown (Table 1).

3.2. Effects of Salusin-β Knockdown on Echocardiography. Compared to Sham rats, the LVEDD, LVESD, LV mass, and LV mass/BW were obviously increased, while EF, FS, and LV PWVs were significantly decreased in CHF rats. More importantly, these abnormal parameters, except LV mass and LV mass/BW, of CHF rats were improved by salusin-β knockdown (Table 2).

3.3. Effects of Salusin-β Knockdown on Salusin-β Levels in Plasma and Protein Expression in Arteries. The content of salusin-β in the plasma (Figure 1(a)) and salusin-β protein expression in the CA, PA, and MA (Figure 1(b)) of CHF rats were significantly higher than those of Sham rats. After tail intravenous injection of adenoviral vectors encoding salusin-β shRNA to knockdown salusin-β, the salusin-β levels in the plasma and salusin-β protein expression in arteries were decreased considerably in both CHF and Sham rats.

3.4. Effects of Salusin-β Knockdown on Plasma Leptin and Visfatin Levels. Both the leptin (Figure 1(c)) and visfatin levels (Figure 1(d)) in plasma were increased in CHF rats compared with Sham rats and were restored by salusin-β-shRNA intervention in CHF rats.

3.5. Effects of Salusin-β Knockdown on Endothelium-Dependent Vascular Relaxation. ACh-induced dose-dependent relaxations in CA, PA, and MA were attenuated

Table 1: BW, HW, infarct area, and baseline hemodynamics in a representative group of CHF and Sham rats after two weeks of Cont-shRNA or Salusin-β-shRNA application.

|            | Cont (mean ± SE) | Salusin-β (mean ± SE) | CHF (mean ± SE) | Salusin-β (mean ± SE) |
|------------|------------------|-----------------------|-----------------|-----------------------|
| BW (g)     | 424 ± 5.9        | 408 ± 21.1            | 415 ± 7.3       | 418 ± 21.6            |
| HW (g)     | 1.3 ± 0.1        | 1.2 ± 0.1             | 1.9 ± 0.0 †     | 1.8 ± 0.1 †           |
| HW/BW (g/kg)| 3.0 ± 0.1       | 2.9 ± 0.1             | 4.6 ± 0.1 †     | 4.3 ± 0.2 †           |
| Infarct size (% LV area) | 0       | 0                     | 43.1 ± 1.0 †    | 30.4 ± 1.0 †          |
| SAP (mmHg) | 142.5 ± 4.2      | 139.8 ± 3.5           | 112.5 ± 3.2 †   | 122.4 ± 3.6 †         |
| DAP (mmHg) | 103.5 ± 5.1      | 102.5 ± 6.7           | 95.6 ± 7.2      | 86.7 ± 6.2            |
| PP (mmHg)  | 39.0 ± 7.2       | 37.2 ± 6.9            | 17.0 ± 2.9 †    | 35.7 ± 3.0 †          |
| MAP (mmHg) | 119.5 ± 6.7      | 116.2 ± 6.4           | 102.3 ± 5.2 †   | 97.5 ± 6.1 †          |
| HR (beats/min) | 386.9 ± 26.2 | 335.9 ± 20.6          | 358.7 ± 38.2    | 375.9 ± 26.1          |
| LVSP (mmHg)| 137.9 ± 6.1      | 129.7 ± 7.3           | 97.9 ± 3.3 †    | 109.8 ± 4.6 †         |
| LVEDP (mmHg)| 2.5 ± 1.7        | 1.9 ± 1.4             | 13.5 ± 0.7 †    | 10.3 ± 0.5 †          |
| LVDP (mmHg)| 135.3 ± 6.7      | 127.7 ± 7.0           | 84.5 ± 5.2 †    | 99.5 ± 4.5 †          |
| LV +dP/dt max (mmHg/sec) | 3669.8 ± 396.0 | 3725.9 ± 423.0       | 1981.7 ± 133.3 †| 3389.6 ± 333.9 †      |

BW: body weight; HW: heart weight; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; PP: pulse pressure; MAP: mean arterial pressure; HR: heart rate; LV: left ventricle; LVSP: left ventricle peak systolic pressure; LVEDP: left ventricle end-diastolic pressure; LVDP: left ventricle developed pressure; LV +dP/dt max: maximum of the first derivative of left ventricle pressure. Data are given as mean ± SE. *P < 0.05 vs. Cont-shRNA. †P < 0.05 vs. Sham. n = 6 for each group.
TABLE 2: Echocardiographic data of the left ventricle in a representative group of Sham and CHF rats after two weeks of Cont-shRNA or salusin-β-shRNA application.

| Parameter          | Cont   | Salusin-β | Cont   | Salusin-β |
|--------------------|--------|-----------|--------|-----------|
| LVEDD, mm          | 7.12 ± 0.23 | 6.76 ± 0.43 | 11.36 ± 0.21† | 9.24 ± 0.28† |
| LVESD, mm          | 4.65 ± 0.13 | 3.49 ± 0.30 | 9.27 ± 0.40† | 7.85 ± 0.25† |
| IVSd, mm           | 1.79 ± 0.05 | 1.70 ± 0.10 | 1.28 ± 0.13 | 1.40 ± 0.19 |
| IVSs, mm           | 2.86 ± 0.13 | 2.93 ± 0.14 | 1.86 ± 0.26 | 1.76 ± 0.26 |
| LVPWd, mm          | 1.86 ± 0.03 | 1.97 ± 0.12 | 1.63 ± 0.10 | 2.02 ± 0.07 |
| LVPWs, mm          | 3.21 ± 0.11 | 3.08 ± 0.11 | 2.46 ± 0.30† | 2.97 ± 0.08* |
| FS, %              | 43.41 ± 1.24 | 48.54 ± 1.90 | 17.84 ± 1.02† | 25.52 ± 2.01† |
| EF, %              | 72.60 ± 1.33 | 78.59 ± 1.83 | 34.73 ± 1.78† | 47.55 ± 3.30† |
| LV mass (g)        | 1.19 ± 0.08 | 1.03 ± 0.17 | 1.55 ± 0.10† | 1.53 ± 0.08† |
| LV mass/BW (mg/g)  | 2.81 ± 0.33 | 2.52 ± 0.47 | 3.73 ± 0.47† | 3.66 ± 0.32† |

LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVSd: interventricular septal thickness in diastole; IVSs: interventricular septal thickness in systole; LVPWd: left ventricular posterior wall thickness in diastole; LVPWs: left ventricular posterior wall thickness in systole; FS: fractional shortening; EF: ejection fraction; LV: left ventricular; BW: body weight. Values are mean ± SD. *P < 0.05 vs. Sham. †P < 0.05 vs. Cont-shRNA.
rats (Figure 6(g)), which might suggest the occurrence of neovascularization in the infarcted area of CHF rats after salusin-β knockdown. However, there was no significant difference in microvascular density in the noninfarcted area (apex of heart) (Figure 6(h)) between Sham and CHF rats treated with either Cont-shRNA or salusin-β-shRNA.
3.13. Effects of Salusin-β Knockdown on NAD(P)H Oxidase Activity and ROS and NO Levels in Cardiac Tissues. We found that there was no significant difference of salusin-β protein expression in cardiac tissues between Sham and CHF rats. Compared with Sham rats, the NAD(P)H oxidase activity and ROS level in cardiac tissues in CHF rats were increased, and NO levels were decreased. However, they were not influenced by tail intravenous injection of salusin-β shRNA (Supplemental Figure S1). These results indicated that salusin-β in cardiomyocytes does not play a direct role in regulating the NO and ROS signaling pathways in rats, which excluded the direct effect of salusin-β knockdown on cardiomyocytes.

3.14. The Salusin-α Level in Sham and CHF Rats. There were no significant differences in the plasma level of salusin-α or its protein expression in the three arteries, MA, CA, and PA, or cardiac tissues between Sham and CHF rats (Supplemental Figure S2). We speculated that salusin-α might not be involved in the pathogenesis of CHF.

4. Discussion

Endothelium-dependent diastolic dysfunction is closely related to the occurrence and development of CHF and could trigger deteriorating events, such as cardiovascular remodeling, in CHF patients. The CA, PA, and MA are three essential arteries related to endothelial dysfunction in CHF. The major new findings of the present study were (1) The levels of salusin-β, leptin, and visfatin in plasma and salusin-β protein expressions of CA, PA, and MA of CHF were much higher than that of Sham rats, which were decreased significantly by salusin-β knockdown; (2) endothelium-dependent vascular relaxation was attenuated in CHF which was improved by salusin-β knockdown; (3) the eNOS activity and protein expressions as well as NO level in the three arteries of CHF were lower than those of Sham rats, while NAD(P)H oxidase activity, NOX-2 and NOX-4 protein expressions, and ROS level of arteries were higher than those of the Sham rats. After salusin-β knockdown, these abnormalities were substantially improved; (4) the improved effects of salusin-β knockdown on ACh-induced vascular relaxation, NO level, NAD(P)H oxidase activity, and ROS level of arteries of CHF were inhibited by L-NAME pretreatment; (5) the interference of salusin-β gene expression in CHF rats improved cardiac function, vascular remodeling, and left ventricular remodeling and promotes the angiogenesis in infarct zone of heart; (6) silencing of the salusin-β gene expression also reversed the depressed eNOS activity and NO level as well as the increased NAD(P)H oxidase activity and the ROS level of HPAECs stimulated by TNF-α. These results indicated that salusin-β is closely related to endothelial dysfunction and vascular and ventricular remodeling in chronic heart failure. Knockdown of salusin-β contributes to the improvement of endothelial dysfunction, cardiac function, and cardiovascular remodeling in CHF by inhibiting vascular NAD(P)H oxidase-derived ROS generation, activating eNOS and increasing NO production.
Figure 3: The protein expression of p-eNOS (a, b), T-eNOS (a, c), and p-eNOS/T-eNOS (d), as well as NO level (e) of CA, PA, and MA in Sham and CHF rats. Values are mean ± SE. *P < 0.05 compared with Cont-shRNA, †P < 0.05 compared with Sham. n = 6 for each group.
Figure 4: NAD(P)H oxidase activity (a), NOX-2 (b, c), and NOX-4 protein expression (b, d), as well as ROS levels (e), of CA, PA, and MA in Sham and CHF rats. Values are mean ± SE. *P < 0.05 compared with Cont-shRNA, †P < 0.05 compared with Sham. n = 6 for each group.
Salusins are vasoactive peptides, including salusin-α (28 amino acids) and salusin-β (20 amino acids) [18]. Salusin-β is more closely related to cardiovascular diseases than salusin-α [32, 47]. Salusin-β is expressed in the hypothalamus, posterior pituitary gland, gastrointestinal tract, immune system, endocrine system, and peripheral vascular tissue, and it has rich expression in VECs and VSMCs [48-50]. The plasma salusin-β levels in patients with diabetes mellitus [47], coronary artery disease [51, 52], and hypertension [36, 53] are distinctly higher than those in healthy controls. It has been reported that salusin-β regulates blood pressure [27], activates sympathetic outflow [28], and promotes the proliferation, migration, and foam cell formation of VSMCs [29–31]. More importantly, salusin-β is closely related to vascular endothelial function [29, 32]. Salusin-β causes endothelial injury and dysfunction in diabetes mellitus [47, 54, 55] and promotes the inflammatory response of human umbilical vein endothelial cells through NF-κB signaling [47, 56]. However, whether the salusin-β level in myocardial infarction-induced CHF is also increased and whether salusin-β is involved in endothelial dysfunction and impaired cardiovascular function in CHF is still unknown. The present study found that both the plasma salusin-β level and its protein expressions in the CA, PA, and MA of CHF rats were much higher than those of the Sham rats, which indicated that the activity of salusin-β in CHF was increased. However, there were no significant differences in the salusin-α plasma level and its protein expressions in MA, CA, and PA and cardiac tissues between Sham and CHF rats, which suggested that salusin-α might not be involved in the pathogenesis of CHF.

It has been reported that plasma leptin and visfatin levels were increased in patients with myocardial infarction or other cardiovascular diseases [57, 58]. High levels of leptin increase oxidative stress in endothelial cells, reduce vasodilation, and contribute to obesity-related hypertension [59]. Visfatin causes vascular endothelial dysfunction, inhibits the production of NO and vasodilatation, and promotes the proliferation of vascular smooth muscle cells [60]. Leptin and visfatin have been proposed as clinical markers of atherosclerosis, endothelial dysfunction, and vascular injury in cardiovascular disease. In this study, the leptin and visfatin levels in plasma were increased, while ACh-induced endothelium-dependent vascular relaxation was significantly attenuated in CHF, which suggested the occurrence of endothelial dysfunction in CHF rats. Salusin-β knockdown by shRNA improved endothelium-dependent vascular relaxation and decreased the plasma leptin and visfatin levels in CHF. These results indicated that salusin-β is a critical regulator of endothelial function in CHF, and the elevated activity of salusin-β in the circulatory system in CHF might be an important cause of endothelial dysfunction in CHF.

It has been reported that salusin-β stimulates the production of NAD(P)H oxidase-derived ROS in human umbilical vein endothelial cells [61–64]. Through the oxidative stress-related signaling pathway, salusin-β stimulates the migration of VSMCs and intimal hyperplasia after vascular injury [47, 65]. Salusin-β promotes the formation of foam cells and monocyte adhesion in atherosclerosis by stimulating the production of ROS [30, 31]. Salusin-β enhances oxidative stress and inflammation in diabetic cardiomyopathy, and knockdown of salusin-β improves cardiac dysfunction and decreases oxidative stress and inflammation in diabetic cardiomyopathy [47]. In this study, it was found that both NAD(P)H oxidase activity and the protein expression of its subunits NOX-2 and NOX-4 in the CA, PA, and MA of CHF rats were increased. In addition, the ROS levels in the arteries of CHF rats were much higher than those of Sham rats. Silencing salusin-β decreased the elevated NAD(P)H oxidase activity, NOX-2 and NOX-4 protein expression, and ROS levels in the arteries of CHF rats, which suggested that the activation of NAD(P)H oxidase and elevated ROS production played important roles in mediating the effects of salusin-β. In addition, we also found that the eNOS
Figure 5: The media thickness (a, b), lumen diameter (a, c), and media thickness/lumen diameter (d) of CA, PA, and MA in Sham and CHF rats. Values are mean ± SE. * $P < 0.05$ compared with Cont-shRNA, † $P < 0.05$ compared with Sham. $n = 6$ for each group.
Figure 6: Effects of salusin-β knockdown on left ventricular remodeling and microvascular density. Sections of myocardium with Masson’s stain under low- (a) and high-power microscope (c) showing fibrosis. Sections of myocardium with HE staining under low- (b) and high-power microscopy (d) and dystrophin staining (f) showing the size of cardiomyocytes. Bar graph showing the quantitative analysis of the cross-sectional area of cardiomyocytes (e). Sections of myocardial infarct border (g) and remote zone (apex of heart) (h) with endothelial marker CD31 immunohistochemistry staining showing the microvascular density. Values are mean ± SE. *P < 0.05 compared with Cont-shRNA, †P < 0.05 compared with Sham. n = 6 for each group.
activity and protein expression and NO level in the three arteries of CHF rats were lower than those of Sham rats and were substantially improved by knockdown of salusin-β. The effects of salusin-β knockdown on ACh-induced vascular relaxation, NO levels, NAD(P)H oxidase activity, and ROS levels in CHF arteries were markedly inhibited by pretreatment with the NOS inhibitor L-NAME. Although the relationship between salusin-β and NO is rarely reported at present, the above results suggested that lowering salusin-β levels in circulation alleviated oxidative stress and improved damaged eNOS-NO production in CHF, which might be the major reason for the improvement of endothelial dysfunction by silencing salusin-β expression.

CHF is a chronic inflammatory response, and TNF-α plays an important role in its pathogenesis [66]. Studies have found that the levels of TNF-α in the peripheral circulation or heart tissues of patients with CHF are significantly increased [67, 68]. In the present study, we used TNF-α to stimulate HPAECs to simulate the injury of endothelial cells suffering from chronic heart failure and found that the eNOS activity and NO levels of the cells were decreased, while NAD(P)H oxidase activity and ROS levels were increased, which were also reversed by cell transfection with salusin-β shRNA. Therefore, our in vitro cell experiments again verified the results found in animal experiments.

In this study, echocardiography showed that the EF and FS were markedly decreased, while the LVEDD, LVESD, LV mass, and LV mass/BW were obviously increased in CHF rats, suggesting the significant reduction of cardiac function and the occurrence of left ventricular compensatory myocardial hypertrophy in CHF rats. Masson’s stain, HE staining, and dystrophin staining further revealed severe perivascular and myocardial fibrosis and cardiomyocyte hypertrophy in the LV myocardium of CHF. In addition, the lumen diameter of the CA, PA, and MA in CHF decreased, while the media thickness and media thickness/lumen diameter were significantly increased, suggesting the occurrence of vascular remodeling in CHF. The interference of salusin-β gene expression in CHF rats improved cardiac function, vascular remodeling, and left ventricular remodeling and simultaneously induced neovascularization in the infarcted region of CHF rats, which might be the important reason for the decrease in the infarcted area. The mechanisms of these improvements in CHF are still not clear, but some studies have indicated that NO released from endothelial cells might be an inhibitor of vascular remodeling [69,
Therefore, we speculated that the improvement in vascular remodeling might be due to the improvement in endothelial function and the increase in eNOS-NO production in arteries induced by salusin-β silencing. Then, improved vascular relaxation of CA in CHF increases the blood supply to the heart and relieves myocardial ischemia; improved MA relaxation decreases total peripheral resistance and, more importantly, decreases cardiac afterload, which all contribute to the subsequent improvement of left ventricular remodeling and cardiac function. In addition, improved PA relaxation in CHF decreased pulmonary vascular resistance and the incidence of pulmonary hypertension due to left heart disease.

In addition, we found that there were no significant differences in salusin-β protein expression in cardiac tissues between Sham and CHF rats. Compared with Sham rats, the NAD(P)H oxidase activity and ROS level in cardiac tissues in CHF rats were increased, and NO level were decreased, which suggested the increased oxidative stress and impaired NO bioavailability also occurred in hearts of CHF rats. However, NAD(P)H activity, ROS, and NO levels were not influenced by tail intravenous injection of salusin-β shRNA. These results indicated that salusin-β in cardiomyocytes might not play direct roles in regulating the NO and ROS signaling pathways in CHF rats, which excluded the direct effect of salusin-β knockdown on cardiomyocytes.

In conclusion, the present study indicates that salusin-β in the circulatory system is closely related to endothelial dysfunction, cardiovascular remodeling, and cardiac dysfunction in CHF. Silinging salusin-β contributes to the improvement of endothelial function, cardiac function, and cardiovascular remodeling in CHF by inhibiting NAD(P)H oxidase-derived ROS generation and activating eNOS and NO production, which provides a new strategy and target for the treatment of chronic heart failure in the future.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

No competing financial interests exist.

Authors’ Contributions

All authors contributed to the work in this paper. Y.H. conceived and designed the experiments. Y.X., Y.P., X.X.W., and A.D.C. performed the experiments and collected data. Y.X., X.Y.T., and X.X.L. analyzed the data. Y.H. wrote the manuscript.

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Supplementary Materials

Supplemental figures associated with this article can be found in the Supplemental file. (Supplementary Materials)

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