Int J Clin Exp Med 2020;13(4):2095-2103
www.ijcem.com /ISSN:1940-5901/IJCEM0106548

Review Article

Losartan combined with simvastatin can enhance the inhibition of myocardial necrosis and fibrosis and improve the expression of apoptosis-related genes in rats with heart failure

Dongxia Zhang[1], Shijie Wang[2], Hualong Zhang[2], Yanming Fan[2], Yuyan Wan[3], Xiaogang Wang[2]

[1]Emergency of Xingtai People's Hospital, Xingtai 054001, Hebei Province, China; [2]Cardiology of Xingtai People's Hospital, Xingtai 054001, Hebei Province, China; [3]Cardiology of Fengnan District Hospital, Tangshan 063000, Hebei Province, China

Received December 17, 2019; Accepted February 11, 2020; Epub April 15, 2020; Published April 30, 2020

Abstract: Objective: To investigate the effect of losartan combined with simvastatin on rats with heart failure. Method: Rats were randomly divided into four groups of A, B, C and D. Group A (GA) was model group without treatment. Group B (GB) was normal rats group. Group C (GC) was sham operation group. Group D (GD) was treatment group with drug therapy. There were 15 rats in each group. The rat model was established and no drug intervention was given to rats in GA, GB and GC. In GD, rats was given drug therapy 5 days after operation for 35 consecutive days, while rats in GA, GB and GC was given distilled water of equal volume. ELISA was used to detect the relevant indexes in serum. The cardiac function indexes were detected by cardiac ultrasound instrument. The hemodynamic indexes of rats were observed by physiological recorder. The cardiac mass index of rats was observed. SP staining was used to detect Bax, Bcl-2 and apoptosis rate. Result: The related indexes in serum of rats in GA were significantly higher than those in GB and GC ($P<0.05$). The related indexes in serum of rats in GD were significantly lower than those in GA ($P<0.05$). The cardiac function index, hemodynamics index, cardiac mass index and myocardial cell apoptosis of rats in GD were better than those in GA ($P<0.05$). Conclusion: Losartan combined with simvastatin can effectively improve heart failure in rats.

Keywords: Losartan, simvastatin, rats, heart failure

Introduction

Heart failure is a shared chronic stage of heart function damage secondary to many causes, with an estimated prevalence rate of over 37.7 million worldwide [1]. Patients with heart failure generally suffer from many symptoms affecting their quality of life, such as exercise intolerance, physical decline and weakness permanence [2]. Despite significant advances in treatment and prevention, mortality and morbidity are still high and the quality of life is poor [3]. Some studies have identified that 2-7% of patients with heart failure die when they are first admitted to hospital in the world. The mortality rate is 17-45% within one year of admission and more than 50% within five years [4].

Losartan is a cardiovascular drug with potential to treat cardiovascular diseases [5]. Losartan can reduce the adhesion of platelets to fibrous myocardial collagen [6], thus improving the left ventricular rigidity of hypertension patients [7]. Simvastatin is a prodrug, which can be rapidly absorbed in human body and form multi-active metabolites [8]. Simvastatin is also an HMG-CoA reductase inhibitor, which has shown beneficial effects on chronic heart failure. It can reduce the incidence of new heart failure, improve adverse prognosis [9] and improve the survival rate of patients [10]. This study aimed to explore the effect of losartan combined with simvastatin on heart failure rats.

Materials and methods

Materials

There were 60 SPF healthy male rats (Changzhou Cavens Experimental Animal Co., Ltd.),

losartan (SFDA approval No. H20030654, Hangzhou Mercadon Pharmaceutical Co., Ltd.), simvastatin (SFDA approval No. H20066736, Zhejiang Pharmaceutical Co., Ltd. Xinchang Pharmaceutical Factory), centrifuge (Sichuan Shuke Instrument Co., Ltd.), ELISA Kit (Shanghai Xitang Biotechnology Co., Ltd.), cardiac ultrasound instrument (Xuzhou Hengda Electronics Co., Ltd.), RM-6000 Multi-channel Physiological Recorder (Shanghai Tongge Medical Devices Co., Ltd.), TUNEL reaction liquid (Wuhan Hualianke Biotechnology Co., Ltd.), anti-fluorescein antibody (Shanghai Rongweida Industry Co., Ltd.), DAB developer (Beijing Fubo Biotechnology Co., Ltd.), hematoxylin developer (Shanghai Gantu Biotechnology Co., Ltd.), mouse anti-rat Bax monoclonal antibody and mouse anti-rat Bcl-2 monoclonal antibody (Emmett Technology Co., Ltd.).

Establishment of heart failure rat model

The rats were grouped before the model was established. Rats were randomly divided into four groups of A, B, C and D. GA was model group without treatment. GB was normal group. GC was sham operation group. GD was treatment group with drug therapy. There were 15 rats in each group. In GA and GD, rats were injected with appropriate dose of anesthetics according to their body weight. The abdominal cavity of the rats was opened to expose the abdominal aorta and separate it from the bilateral renal artery branches. After the abdominal aortic stenosis was formed manually, the abdomen was closed. Antibiotics were injected intramuscularly after the operation. 5 mg/kg of losartan plus 2 mg/kg of simvastatin was administered to rats by gavage in GD 5 days after operation for 35 days. In GA, GB and GC, rats were given distilled water of equal volume.

Monitoring inflammatory factors in rats

5 ml of venous blood of rats was extracted in each group, left standing for 20 min, centrifuged at 3000 r/min for 10 min to quickly separate the freeze serum with liquid nitrogen, and stored at -80°C for later use. According to the instructions, serum IL-6 (interleukin-6), TNF-α (tumor necrosis factor) and BNP (B-type natriuretic peptide) were detected by ELISA.

Observation of the changes of cardiac function in mice

The left ventricular end-diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), left ventricular posterior wall thickness (LVPWT) and left ventricular fractional shortening (LVFS) of rats on the 5th and 20th day of administration were observed by using a cardiac ultrasound instrument equipped with an 11-MHZ probe.

Monitoring hemodynamic indexes of rats

Rats were weighed, anesthetized by intraperitoneal injection. Then systemic arterial systolic pressure (SAP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular maximum ascending velocity (+dp/dtmax) and left ventricular pressure maximum descending velocity (-dp/dtmax) were observed by physiological recorder.

Observation of the cardiac mass index of rats

After observing the corresponding indexes, the rats were killed and the hearts of the rats were quickly taken out. Then hearts were washed with distilled water and dried with filter paper, and its quality was weighed. Except the left ventricle, all other parts were cut off, and the left ventricle was weighed. The ratio of the left ventricle to the body weight was left ventricle mass index.

Detection of AI (apoptosis index)

Some heart specimens were taken out, fixed and embed into slices. They were sealed off with hydrogen peroxide after dewaxing. Their membrane protein and nucleoprotein were digested, and then TUNEL reaction solution was added. The anti-fluorescein antibody was added and DAB was used for color development. Counterstaining was conducted by hematoxylin. Dehydration was performed and slices were sealed. The apoptotic cells and the total number of cells in different fields under high power lens were randomly observed and AI was calculated.

Monitoring Bax and Bcl-2 by SP immunochemistry staining

Conventional paraffin sections were dewaxed. Mouse anti-rat Bax monoclonal antibody and mouse anti-rat Bcl-2 monoclonal antibody were diluted at 1:50. PBS was used as negative control instead of primary antibody. DAB was used for color development. Hematoxylin was used

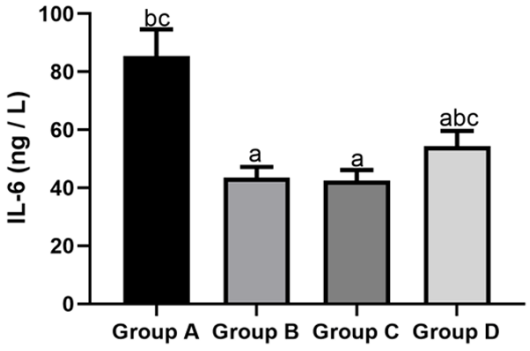

Figure 1. Comparison of IL-6 content in serum of rats in each group. The content of IL-6 in serum of rats in GA was significantly higher than that in GB and GC (P<0.05). The content of IL-6 in serum of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

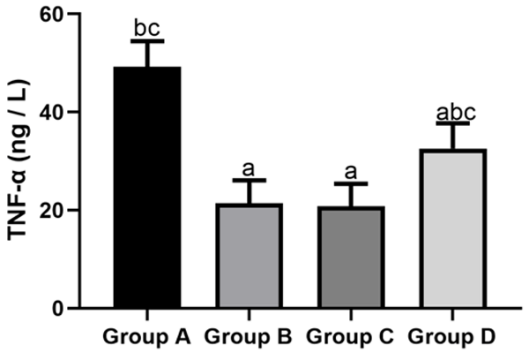

Figure 2. Comparison of TNF-α content in serum of rats in each group. The content of TNF-α in serum of rats in GA was significantly higher than that in GB and GC (P<0.05). The content of TNF-α in serum of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

to stain lightly. Neutral gum seal was used for microscopic examination. Positive cells and total number of cells in different fields were randomly observed and averaged.

Statistical methods

Differences were verified by SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). Measurement data were expressed as mean number ± standard deviation (x±sd). One-way ANOVA was used for the comparison between the two groups. F test was used among groups. The difference was statistically significant with P<0.05.

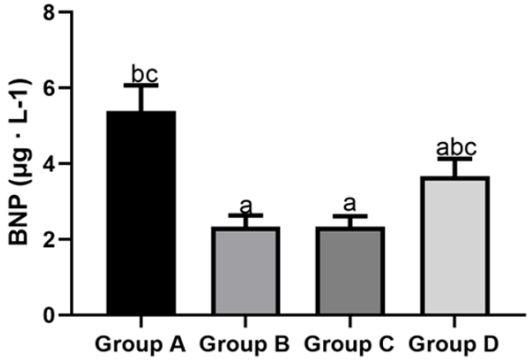

Figure 3. Comparison of BNP content in serum of rats in each group. The content of BNP in serum of rats in GA was significantly higher than that in GB and GC (P<0.05). The content of BNP in serum of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

Results

Comparison of inflammatory factors in each group

The expression levels of IL-6 in serum of rats in GA, GB, GC and GD were 85.38±9.24 ng/L, 43.51±3.69 ng/L, 42.45±3.66 ng/L and 54.38±5.24 ng/L, respectively. The expression levels of BNP in serum of rats in GA, GB, GC and GD were 5.39±0.68 μg L^{-1}, 2.34±0.29 μg L^{-1}, 2.33±0.28 μg·L^{-1} and 3.67±0.46 μg L^{-1}, respectively. The expression levels of TNF-α in serum of rats in GA, GB, GC and GD were 49.24±5.24 ng/L, 21.45±4.61 ng/L, 20.78±4.60 ng/L and 32.57±5.13 ng/L, respectively. The expression level of related factors in serum of rats in GD was significantly lower than that in GA (P<0.05). More details are shown in **Figures 1**-**3**.

Cardiac function indexes of rats in each group

Intra-group comparison: Compared with the 5th day, LVEDD, LVESD and LVPWT indexes of rats in GA and GD increased on the 20th day (P<0.05), while there was no change in GB and GC (P>0.05). Inter-group comparison: On the 5th day, there was no difference in LVEDD, LVESD and LVPWT indexes of rats in each

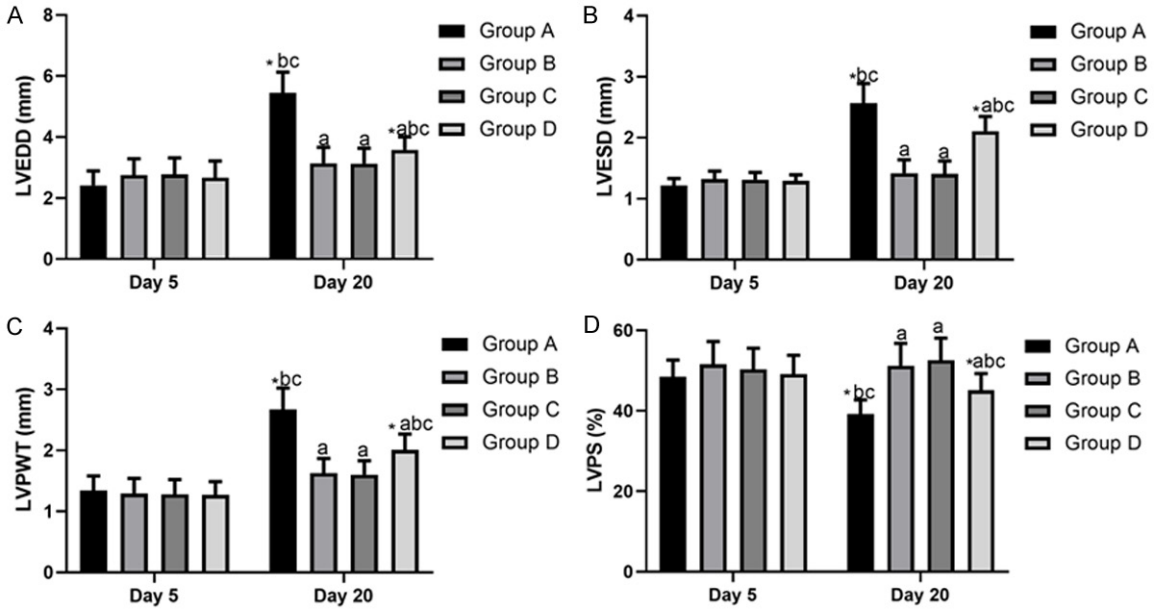

Figure 4. A. LVEDD changes of rats in each group. Intra-group comparison: compared with the 5th day, LVEDD of rats in GA and GD increased significantly on the 20th day (P<0.05). Inter-group comparison: on the 5th day, there was no difference in each group (P>0.05). On the 20th day, LVEDD of rats in GA was significantly higher than that in GB and GC (P<0.05). LVEDD of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: Intra-group comparison: *indicates the 5th day (P<0.05); Inter-group comparison: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). B. LVESD changes of rats in each group. Intra-group comparison: compared with the 5th day, LVESD of rats in GA and GD increased significantly on the 20th day (P<0.05). Inter-group comparison: on the 5th day, there was no difference in each group (P>0.05). On the 20th day, LVESD of rats in GA was significantly higher than that in GB and GC (P<0.05). LVESD of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: Intra-group comparison: *indicates the 5th day (P<0.05); Inter-group comparison: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). C. LVPWT changes of rats in each group. Intra-group comparison: compared with the 5th day, LVPWT of rats in GA and GD increased significantly on the 20th day (P<0.05). Inter-group comparison: on the 5th day, there was no difference in each group (P>0.05). On the 20th day, LVPWT of rats in GA was significantly higher than that in GB and GC (P<0.05). LVPWT of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: Intra-group comparison: *indicates the 5th day (P<0.05); Inter-group comparison: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). D. LVPS changes of rats in each group. Intra-group comparison: compared with the 5th day, LVPS of rats in GA and GD decreased significantly on the 20th day (P<0.05). Inter-group comparison: on the 5th day, there was no difference in each group (P>0.05). On the 20th day, LVPS of rats in GA was significantly lower than that in GB and GC (P<0.05). LVPS of rats in GD was significantly higher than that in GA and lower than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: Intra-group comparison: *indicates the 5th day (P<0.05); Inter-group comparison: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

groups (P>0.05). On the 20th day, LVEDD indexes of rats in GA were higher than those of the remaining groups (P<0.05). Intra-group comparison: Compared with the 5th day, LVPS index of rats in GA decreased on the 20th day (P<0.05), but there was no change in GB, GC and GD (P>0.05). Inter-group comparison: On the 5th day, there was no difference in LVPS indexes of rats in each groups (0>0.05). On the 20th day, LVPS indexes of rats in GA were lower than those of the remaining groups (P<0.05). More details are shown in **Figure 4**.

Comparison of hemodynamic parameters of rats in each group

Compared with GB and GC, the LVEDP in GA increased (P<0.05), while SAP, LVSP, +dp/dtmax and -dp/dtmax decreased (P<0.05). SAP, LVSP and LVEDP in GD were lower than those in

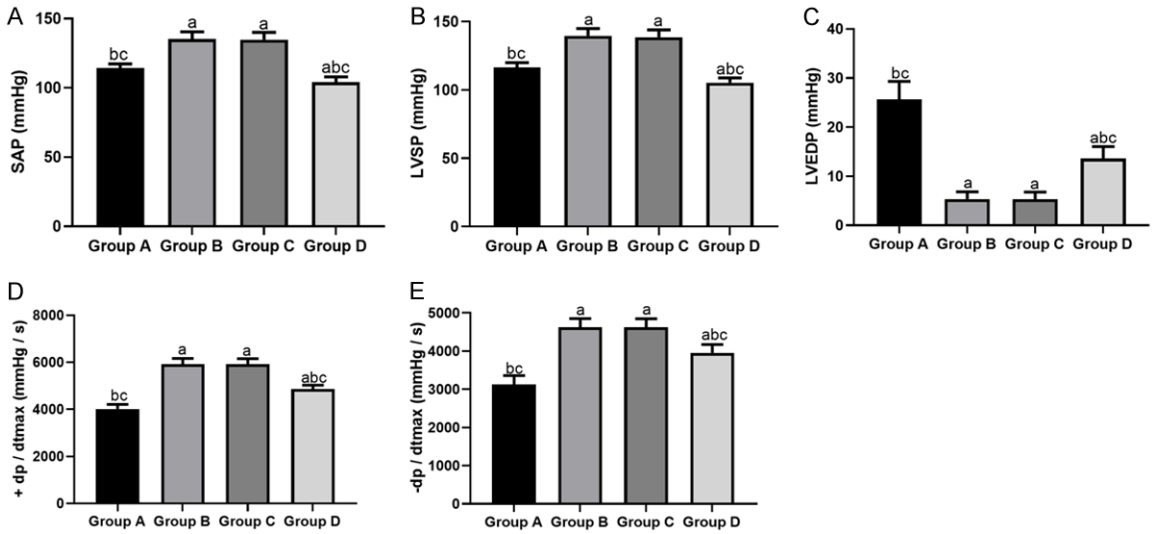

Figure 5. A. Comparison of SAP of rats in each group. SAP of rats in GA was significantly lower than that of GB and GC (P<0.05); SAP of rats in GD was significantly lower than that of the other three groups (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). B. Comparison of LVSP of rats in each group. LVSP of rats in GA was significantly lower than that of GB and GC (P<0.05); LVSP of rats in GD was significantly lower than that of the other three groups (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). C. Comparison of LVEDP of rats in each group. LVEDP of rats in GA was significantly higher than that of GB and GC (P<0.05); LVEDP of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). D. Comparison of +dp/dtmax of rats in each group. +dp/dtmax of rats in GA was significantly lower than that of GB and GC (P<0.05); +dp/dtmax of rats in GD was significantly higher than that in GA and lower than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). E. Comparison of -dp/dtmax of rats in each group. +dp/dtmax of rats in GA was significantly lower than that of GB and GC (P<0.05); -dp/dtmax of rats in GD was significantly higher than that in GA and lower than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

GA (P<0.05), but +dp/dtmax and -dp/dtmax were higher than those in GA (P<0.05). It indicated that the ventricular systolic and diastolic function of rats in GD was better than that in GA. More details are shown in **Figure 5**.

Comparison of cardiac mass index of rats in each group

The body weight of rats in GA was lower than that in GB and GC (P<0.05), and the cardiac mass index and left ventricular mass index were higher than those in GB and GC (P<0.05). However, the weight of rats in GD was higher than that in GA (P<0.05), and the cardiac mass index and left ventricular mass index were lower than those in GA (P<0.05). More details are shown in **Figure 6**.

Comparison of apoptosis myocardial cells of rats in each group

The apoptosis myocardial cell rates AI of rats in GA, GB, GC and GD were 17.32±2.54%, 3.52±0.14%, 3.53±0.15% and 9.84±2.34%, respectively. The results showed that the apoptosis myocardial cell rate of rats in GA were higher than that in GB and GC (P<0.05), but the apoptosis myocardial cell rate of rats in GD were lower than that in GA (P<0.05). More details are shown in **Figure 7**.

Comparison of myocardial related apoptosis proteins Bax and Bcl-2 in each group

Bax of rats in GA, GB, GC and GD were 26.24±1.35%, 18.32±1.23%, 18.33±1.22%

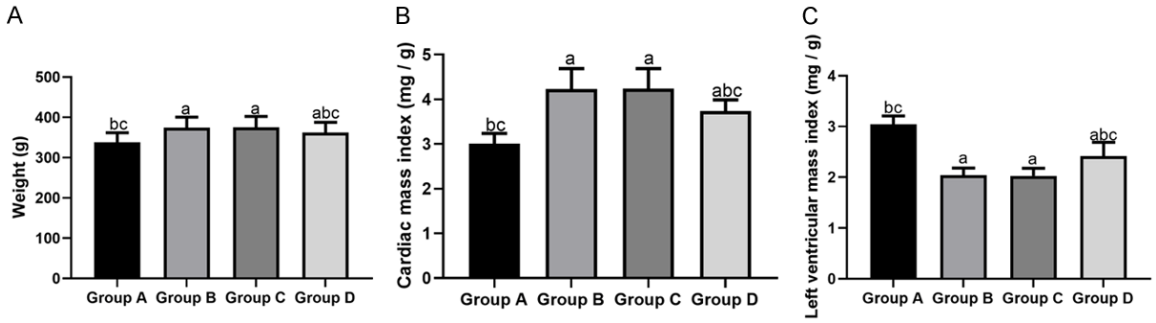

Figure 6. A. Comparison of weight of rats in each group. The weight of rats in GA was significantly lower than that in GB and GC (P<0.05); The weight of rats in GD was significantly higher than that in GA and lower than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). B. Comparison of cardiac mass index of rats in each group. The cardiac mass index of rats in GA was significantly lower than that in GB and GC (P<0.05). The cardiac mass index of rats in GD was significantly higher than that in GA and lower than that in GB and GC (P<0.05). There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05). C. Comparison of cardiac mass index of rats in each group. Cardiac mass index of rats in GA was significantly higher than that of GB and GC (P<0.05); Cardiac mass index of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

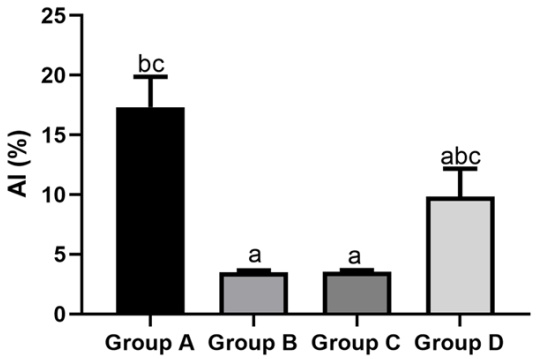

Figure 7. Comparison of AI of rats in each group. AI index of rats in GA was significantly higher than that of GB and GC (P<0.05); AI index of rats in GD was significantly lower than that in GA and higher than that in GB and GC (P<0.05); There was no difference between GB and GC (P>0.05). Note: a represents the comparison with GA (P<0.05); b represents the comparison with GB (P<0.05); c represents the comparison with GC (P<0.05).

and 16.53±1.34%, respectively. Bax of rats in GA was higher than that in GB and GC (P<0.05), while Bax of rats in GD was lower than that in GA (P<0.05). Bcl-2 of rats in GA, GB, GC and GD were 21.42±1.45%, 37.14±1.65%, 37.15±1.66% and 32.66±2.15%, respectively. Bcl-2 of rats in GA was lower than that in GB and GC (P<0.05), while Bcl-2 of rats in GD was higher than that in GA (P<0.05). More details are shown in **Table 1**, **Figures 8**, **9**.

Table 1. Comparison of myocardial related apoptosis proteins Bax and Bcl-2 in each group ($\bar{x}$±sd)

Grouping	Bax (%)	Bcl-2 (%)
GA (n=15)	26.24±1.35[b,c]	21.42±1.45[b,c]
GB	18.32±1.23[a]	37.14±1.65[a]
GC	18.33±1.22[a]	37.15±1.66[a]
GD	16.53±1.34[a,b,c]	32.66±2.15[a,b,c]
F	170.70	270.90
P	<0.001	<0.001

Note: [a]represents the comparison with GA (P<0.05); [b]represents the comparison with GB (P<0.05); [c]represents the comparison with GC (P<0.05).

Discussion

Inflammation is related to the pathogenesis of heart failure [11]. The increase of proinflammatory cytokines and other biomarkers levels in plasma of patients with heart failure has adverse effects on the prognosis of heart failure [12]. IL-6 is a pleiotropic cytokine produced in response to steady-state disturbances. It has cardiac protective effect in acute myocardial injury, but its long-term elevation may lead to chronic inflammation and fibrotic diseases [13]. TNF-α is an inflammatory cytokine released by activated glial cells [14], which can be activated in asymptomatic phase and continuously increases with the deterioration of

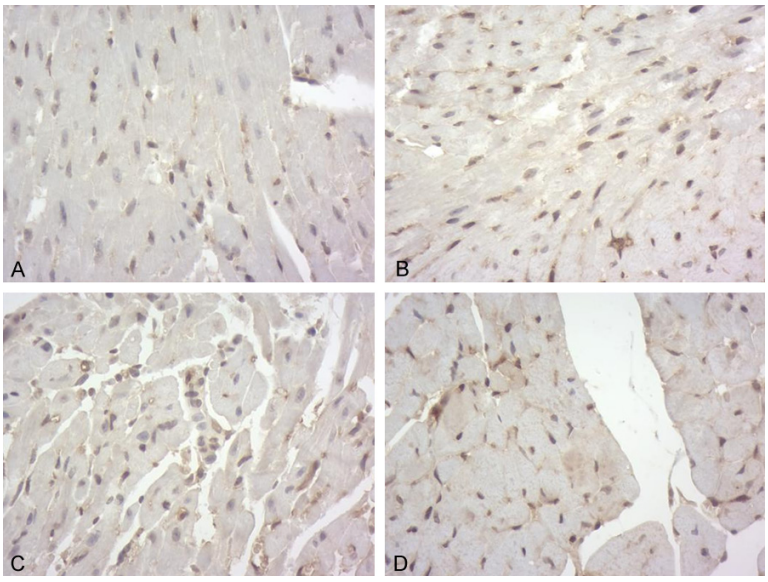

Figure 8. A. Apoptotic protein Bax in GA. B. Apoptotic protein Bax in GB. C. Apoptotic protein Bax in GC. D. Apoptotic protein Bax in GD.

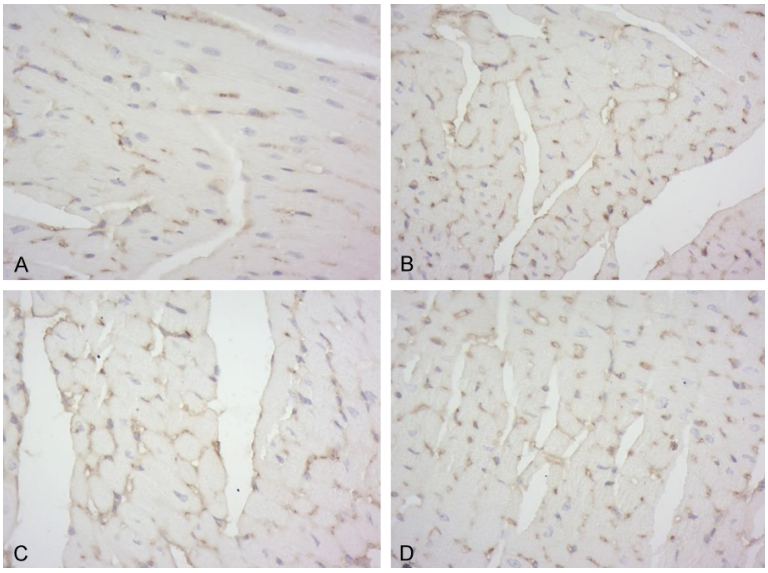

Figure 9. A. Apoptotic protein Bcl-2 in GA. B. Apoptotic protein Bcl-2 in GB. C. Apoptotic protein Bcl-2 in GC. D. Apoptotic protein Bcl-2 in GD.

heart failure [15]. BNP is one of peptide hormone families with similar structures [16]. Its synthesis and secretion are mainly from ventricular myocardium [17]. It is also a powerful experimental parameter for detecting heart failure [18]. In this study, we also observed the levels of corresponding inflammatory factors respectively. The results showed that compared with normal rats, the levels of IL-6, TNF-α and BNP in serum of rats with heart failure were significantly increased, but the relevant levels of rats after treatment were significantly lower than those of rats with heart failure. Some studies have shown that inhibition of inflammatory factors may contribute to its anti-remodeling effect [19]. Some studies also showed that simvastatin has potential therapeutic effects in treating inflammation-related relaxants [20]. Some studies showed that losartan can improve left ventricular systolic function and reduce the level of inflammatory factors [21]. This can indicate that the combination of the two drugs has a good effect on inhibiting inflammation and may have an inhibitory effect on myocardial fibrosis. In this regard, we also observed and compared the cardiac function, hemodynamic index and heart quality index of rats. The cardiac function index indicated that the ventricle was enlarged and thickened and the ventricular systolic function was decreased in untreated heart failure rats. Hemodynamic indexes showed that rats in GA had obvious heart failure. However, heart quality index indicated ventricular remodeling in GA rats. However, these conditions were obviously improved in rats after administration. All of these can indicate that the combination of the two drugs can obviously improve the hemodynamics, reduce water retention and reverse cardiac hypertrophy of rats with heart failure. Bax and Bcl-2 are pro-apoptotic proteins [22]. They are also important regulators of programmed cell death and apoptosis [23]. The pro-apoptotic effect of Bax can be stimulated by intrinsic pore-forming activity, while Bcl-2 can antagonize this activity [24]. For this reason, we also observed the myocardial cell apoptosis index of rats in each group, show-

ing that the myocardial cell apoptosis rate of heart failure rats was significantly higher than that of normal rats, but the myocardial cell apoptosis rate of heart failure rats after the combination of the two drugs was significantly lower than that of untreated heart failure rats. This can indicate that the combination of the two drugs has a good inhibitory effect on myocardial cell apoptosis. There is also evidence that the synergistic effect of simvastatin and losartan can prevent angiotensin II-induced myocardial cell apoptosis in vitro, suggesting that the synergistic effect between the two drugs may provide a new therapeutic approach to prevent cardiac remodeling [25].

In this study, there are still some shortcomings. We have not compared different doses, nor have we observed the drug alone, nor have we paid attention to the prognosis of rats. We will continue to carry out research and update it.

To sum up, losartan combined with simvastatin can enhance the inhibition of myocardial necrosis and fibrosis and improve the expression of apoptosis-related genes in rats with heart failure.

Disclosure of conflict of interest

None.

Address correspondence to: Xiaogang Wang, Cardiology of Xingtai People's Hospital, No. 16, Hongxing Street, Xingtai 054001, Hebei Province, China. E-mail: xiaopapinha6136180@126.com

References

[1] Ziaeian B and Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol 2016; 13: 368-378.

[2] Redfield MM, Anstrom KJ, Levine JA, Koepp GA, Borlaug BA, Chen HH, LeWinter MM, Joseph SM, Shah SJ, Semigran MJ, Felker GM, Cole RT, Reeves GR, Tedford RJ, Tang WH, McNulty SE, Velazquez EJ, Shah MR and Braunwald E; NHLBI Heart Failure Clinical Research Network. Isosorbide mononitrate in heart failure with preserved ejection fraction. N Engl J Med 2015; 373: 2314-2324.

[3] Savarese G and Lund LH. Global public health burden of heart failure. Card Fail Rev 2017; 3: 7-11.

[4] Dick SA and Epelman S. Chronic heart failure and inflammation: what do we really know? Circ Res 2016; 119: 159-176.

[5] Yamada Y, Tsuboi K, Hattori T, Murase T, Ohtake M, Furukawa M, Ueyama J, Nishiyama A, Murohara T and Nagata K. Mechanism underlying the efficacy of combination therapy with losartan and hydrochlorothiazide in rats with salt-sensitive hypertension. Hypertens Res 2011; 34: 809-816.

[6] Chabielska E, Pawlak R, Wollny T, Rolkowski R and Buczko W. Antithrombotic activity of losartan in two kidney, one clip hypertensive rats. A study on the mechanism of action. J Physiol Pharmacol 1999; 50: 99-109.

[7] Miguel-Carrasco JL, Beaumont J, San José G, Moreno MU, López B, González A, Zalba G, Díez J, Fortuño A and Ravassa S. Mechanisms underlying the cardiac antifibrotic effects of losartan metabolites. Sci Rep 2017; 7: 41865.

[8] Todd PA and Goa KL. Simvastatin. A review of its pharmacological properties and therapeutic potential in hypercholesterolaemia. Drugs 1990; 40: 583-607.

[9] Pliquett RU, Cornish KG, Peuler JD and Zucker IH. Simvastatin normalizes autonomic neural control in experimental heart failure. Circulation 2003; 107: 2493-2498.

[10] Horwich TB, MacLellan WR and Fonarow GC. Statin therapy is associated with improved survival in ischemic and non-ischemic heart failure. J Am Coll Cardiol 2004; 43: 642-648.

[11] Ueland T, Gullestad L, Nymo SH, Yndestad A, Aukrust P and Askevold ET. Inflammatory cytokines as biomarkers in heart failure. Clin Chim Acta 2015; 443: 71-77.

[12] Lino DOC, Freitas IA, Meneses GC, Martins AMC, Daher EF, Rocha JHC and Silva Junior GB. Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. Braz J Med Biol Res 2019; 52: e8658.

[13] Fontes JA, Rose NR and Cihakova D. The varying faces of IL-6: from cardiac protection to cardiac failure. Cytokine 2015; 74: 62-68.

[14] Lewitus GM, Konefal SC, Greenhalgh AD, Pribiag H, Augereau K and Stellwagen D. Microglial TNF-alpha suppresses cocaine-induced plasticity and behavioral sensitization. Neuron 2016; 90: 483-491.

[15] Redwine LS, Henry BL, Pung MA, Wilson K, Chinh K, Knight B, Jain S, Rutledge T, Greenberg B, Maisel A and Mills PJ. Pilot randomized study of a gratitude journaling intervention on heart rate variability and inflammatory biomarkers in patients with stage B heart failure. Psychosom Med 2016; 78: 667-676.

[16] Cowie MR, Jourdain P, Maisel A, Dahlstrom U, Follath F, Isnard R, Luchner A, McDonagh T, Mair J, Nieminen M and Francis G. Clinical applications of B-type natriuretic peptide (BNP) testing. Eur Heart J 2003; 24: 1710-1718.

[17] Weber M and Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. Heart 2006; 92: 843-849.
[18] Vogeser M and Jacob K. B-type natriuretic peptide (BNP)--validation of an immediate response assay. Clin Lab 2001; 47: 29-33.
[19] Fu H, Li G, Liu C, Li J, Wang X, Cheng L and Liu T. Probucol prevents atrial remodeling by inhibiting oxidative stress and TNF-alpha/NF-kappaB/TGF-beta signal transduction pathway in alloxan-induced diabetic rabbits. J Cardiovasc Electrophysiol 2015; 26: 211-222.
[20] Tsai CT, Wu CK, Lee JK, Chang SN, Kuo YM, Wang YC, Lai LP, Chiang FT, Hwang JJ and Lin JL. TNF-alpha down-regulates sarcoplasmic reticulum Ca(2)(+) ATPase expression and leads to left ventricular diastolic dysfunction through binding of NF-kappaB to promoter response element. Cardiovasc Res 2015; 105: 318-329.
[21] Gurlek A, Kilickap M, Dincer I, Dandachi R, Tutkak H and Oral D. Effect of losartan on circulating TNFalpha levels and left ventricular systolic performance in patients with heart failure. J Cardiovasc Risk 2001; 8: 279-282.
[22] Brady HJ and Gil-Gómez G. Bax. The pro-apoptotic Bcl-2 family member, Bax. Int J Biochem Cell Biol 1998; 30: 647-650.
[23] Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, Kitada S and Reed JC. Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 1996; 148: 1567-1576.
[24] Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod JJ, Mazzei G, Maundrell K, Gambale F, Sadoul R and Martinou JC. Inhibition of Bax channel-forming activity by Bcl-2. Science 1997; 277: 370-372.
[25] Xu J, Lu XW, Huang Y, Zhu PL and Li J. Synergism of simvastatin with losartan prevents angiotensin II-induced cardiomyocyte apoptosis in vitro. J Pharm Pharmacol 2009; 61: 503-510.