**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 Zhang¹, Shijie Wang², Hualong Zhang², Yanming Fan², Yuyan Wan³, Xiaogang Wang²

¹Emergency of Xingtai People’s Hospital, Xingtai 054001, Hebei Province, China; ²Cardiology of Xingtai People’s Hospital, Xingtai 054001, Hebei Province, China; ³Cardiology 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.),
Effect of losartan combined with simvastatin on rats with heart failure

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. Hematoxylin was used for counterstaining. 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 immunohistochemistry 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 for counterstaining.
Effect of losartan combined with simvastatin on rats with heart failure

2097


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.

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 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.

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
Effect of losartan combined with simvastatin on rats with heart failure

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. LVDPWT changes of rats in each group. Intra-group comparison: compared with the 5th day, LVDPWT 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, LVDPWT of rats in GA was significantly higher than that in GB and GC (P<0.05). LVDPWT 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).

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
Effect of losartan combined with simvastatin on rats with heart failure

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 Al 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%
Effect of losartan combined with simvastatin on rats with heart failure

and 16.53±1.34%, respectively. Bax of rats in GA was higher than that in GB and GC (P<0.05), while Bcl-2 of rats in GD was lower 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 (X±sd)

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Bax (%)</th>
<th>Bcl-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (n=15)</td>
<td>26.24±1.35abc</td>
<td>21.42±1.45abc</td>
</tr>
<tr>
<td>GB</td>
<td>18.32±1.23a</td>
<td>37.14±1.65a</td>
</tr>
<tr>
<td>GC</td>
<td>18.33±1.22a</td>
<td>37.15±1.66a</td>
</tr>
<tr>
<td>GD</td>
<td>16.53±1.34abc</td>
<td>32.66±2.15abc</td>
</tr>
</tbody>
</table>

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
Effect of losartan combined with simvastatin on 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-
Effect of losartan combined with simvastatin on rats with heart failure

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 indicates that the combination treatment 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

Effect of losartan combined with simvastatin on rats with heart failure


