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Original Research Article

Effect of salidroside on ventricular remodeling after acute myocardial infarction

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Abstract

Purpose: *To investigate the remodeling influence of salidroside (SAL) on the ventricles following acute myocardial infarction (AMI) in rats, and the processes involved.*
Methods: *A total of 65 Sprague Dawley (SD) rats were assigned to 5 groups: sham (n = 13), model, and low-, medium- and high-dose SAL groups given SAL at doses of 12, 34, and 36 mg/day, respectively, with 13 rats in each group. Changes in pathological structure, collagen area, ratio of collagen I/collagen III, left ventricular mass index (LVW/BW), ratio of cardiac weight to body weight (HW/BW), creatine kinase MB isoenzyme (CK-MB), lactate dehydrogenase-1 (LDH-1), endothelin (ET), laminin (LN), and hyaluronic acid (HA) were evaluated. Expression levels of dishevelled-1 (DVL-1) and β-catenin in myocardial tissues of the rats were also determined.*
Results: *The LVW/BW values were significantly higher in the low SAL and medium SAL groups than those in AMI rats, while the ratio of collagen I/III and expression levels of DVL-1 and β-catenin proteins were significantly lower than those in the model group ($p < 0.05$). The myocardial structure of rats in the sham group was normal, with no obvious lesions. The levels of CK-MB, LDH-1, ET, LN, and HA in medium and high-dose SAL groups were significantly lower than those in the model group ($p < 0.05$).*
Conclusion: *Salidroside mitigates remodeling of ventricles following AMI in rats by modulating the Wnt/β-catenin signal route.*

Keywords: *Salidroside, Wnt, β-catenin, Acute myocardial infarction*

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INTRODUCTION

Myocardial infarction can cause ventricular damage and lead to changes in the function and morphology of the ventricles, resulting in myocardial hypertrophy and apoptosis [1]. It has been reported that the destruction of myocardial cells leads to the degradation of the extracellular matrix, a disorder in myocardial arrangement, and changes in systolic function, thereby laying a foundation for the reconstruction of the extracellular matrix [2]. Myocardial interstitial remodeling leads to fibroblast hyperplasia, abnormal vascular structure, excessive deposition of extracellular matrix, and significant increases in collagen content and volume [3].

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Clinical studies have confirmed that the Wingless-type MMTV integration site family members (Wnt)/β-catenin pathway plays a key role in myocardial fibrosis [4], although the underlying mechanism is not yet fully elucidated [5]. Therefore, it is important to discover drugs that can block ventricular remodeling for use in the prevention and treatment of myocardial fibrosis in heart failure. In some studies, SAL was used to treat rats with organ fibrosis, and the results showed that the drug significantly improved angiogenesis and cell apoptosis in model rats [6]. In addition, studies have confirmed that the *Nuodikang* capsule and its crude extract affect ventricular remodeling [7,8]. However, there are limited reports on the involvement of SAL in ventricular remodeling and fibrosis. Therefore, the purpose of this study was to investigate the effect of SAL on ventricular remodeling after acute myocardial infarction in rats, and the signal route involved in this process.

EXPERIMENTAL

Animals and materials

Sixty-five male SD rats were purchased from Beijing Huizhi Taikang Pharmaceutical Technology Co. Ltd. (batch number: SCXK (Beijing): 2019-0005 (mean weight = 210 ± 10 g). All rats were kept in cages, with 5 rats in each cage, and they were allowed an unlimited provision of feed and drinking water. The study received approval from the Animal Ethics Authority of Yichang Central People's Hospital in line with the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [9].

Reagents and instruments

Reagents

Lanzhou Rongye Biotech. Co. Ltd. provided FBS, while trypsin and dimethyl sulfoxide were bought from Hong Kong JSENB International Trading Co. Ltd. Neutral formaldehyde fixative solution was the product of Beijing Biolead Biotechnol. Co. Ltd. Chloral hydrate (10 %) was obtained from Shanghai Lianshuo Biotechnology Co. Ltd, while RIPA protein lysate was supplied by Shanghai Yuanye Biotechnol. Co. Ltd. TRIzol was purchased from Harbin Xinhai Genetic Testing Co. Ltd, while GAPDH monoclonal antibody was purchased from Beijing 4A Biotech Co. Ltd. β-Catenin and DVL-1 monoclonal antibodies were products of Absin (Shanghai) Biotechnology Co. Ltd.

Instruments

The upright fluorescence microscope was purchased from Dongguan Pubiao Experimental Equipment Technology Co. Ltd. Desktop refrigerated centrifugation instrument was the product of Hangzhou Nuoding Scientific. The inverted phase contrast/fluorescence microscope was purchased from Shenzhen Yudeli Biotechnology Co. Ltd. Haier refrigerator was bought from Nanjing Beideng Medical Co. Ltd. Thermostatic water bath box was the product of Guangzhou Juneng Nano Biotechnology Co. Ltd. Tissue slicer was obtained from Guangzhou Keshi Scientific Instrument Co. Ltd, while Slide Warmers were obtained from Beijing Jiayuan Xingye Technology Co. Ltd.

Animal grouping

The sham operation group comprised 13 selected SD rats. Rats in this group were threaded without ligation. The remaining animals were ligated in the left anterior descending coronary artery to establish AMI. Thereafter, the rats were randomly divided into the model, low SAL, medium SAL and high SAL groups (n =13 each). In low-dose SAL, medium-dose SAL, and high-dose SAL groups, the rats were given SAL at doses of 12, 24 and 36 mg/day, respectively (dissolved in saline) via gavage, while those in sham and model groups received equivalent volumes of physiological saline in place of SAL. All treatments lasted for 56 days.

Hematoxylin & Eosin (H & E) staining

This was used to assess histopathological structures of the infarction border zone of rats in each group, including cell structure, cytoplasm, and nuclear morphology.

Determination of collagen area

The Masson method was used to determine the collagen area in the infarction border zone of rats in each group, and the ratio of collagen I to collagen III was calculated.

First, rats in each group were weighed and anesthetized. Then, the hearts were excised, rinsed with cold physiological saline, and blotted with filter paper. The heart samples were then weighed. Cardiac mass index (HWI), which is the ratio of cardiac weight to body weight (HW/BW), was calculated for each rat. The right ventricle was discarded, while the left ventricle was used for calculating the left ventricular mass index (LVW/BW).

Activities of serum myocardial enzymes

Abdominal venous blood was collected from each group of rats, and serum samples were obtained via centrifugation. The activities of serum myocardial enzymes i.e., creatine phosphokinase isoenzyme (CK-MB), lactate dehydrogenase (LDH-1), endothelin (ET), laminin (LN), hyaluronic acid (HA) levels in rats were assayed using the automatic biochemical analyzer.

Western blot

This was used to assay the protein expression levels of Wnt/β-catenin signaling pathway-related genes (DVL-1 and β-catenin) in the myocardial tissue of rats in each group.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Two groups were compared using ANOVA; multiple groups were compared with the LSD test, while the Kruskal-Walis method was used for comparison between groups with uneven variance. All statistical analyses were done using the SPSS 23.0 software package. Statistical significance was assumed at $p < 0.05$.

RESULTS

Influence of various doses of SAL on histopathological structures

Collagen area and collagen 1/collagen III ratios were significantly higher in the infarction border zone of model rats than in sham rats ($p < 0.05$). Collagen areas were significantly lower in the infarction border zone in low SAL and high SAL rat groups than in the model rats, while the collagen 1/collagen III ratio was significantly lower in low SAL and medium SAL rat groups than in model rats ($p < 0.05$, Table 1). The myocardial structure of rats in the sham operation group was normal, with no obvious lesions. In the model group, there were obvious pathophysiological changes in the left ventricle. Hypertrophy, elongation, swelling, degeneration, and distortion of myofibrils appeared in some cells around the infarction area, and inflammatory cell infiltration and necrosis were observed. However, these lesions were significantly mitigated in the various SAL dose groups. The results are shown in Figure 1.

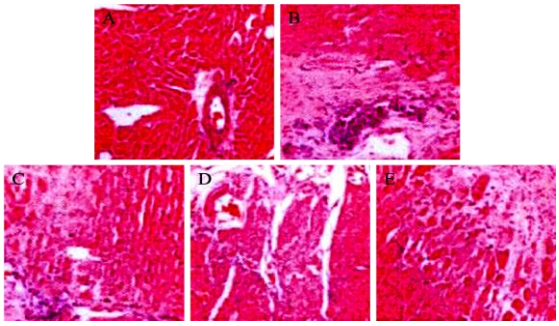

Figure 1: Photomicrograph of structure of infarction border tissue. A: Sham; B: AMI model; C: low SAL; D: medium SAL; E: high SAL

Effect of different doses of SAL on cardiac function parameters

The HW/BW ratio was significantly higher in model rats than that in sham group, while LVW/BW ratio was significantly reduced, relative to sham group ($p < 0.05$; Figure 2). However, the LVW/BW ratio was higher in rats in low SAL and medium SAL doses than in model rats.

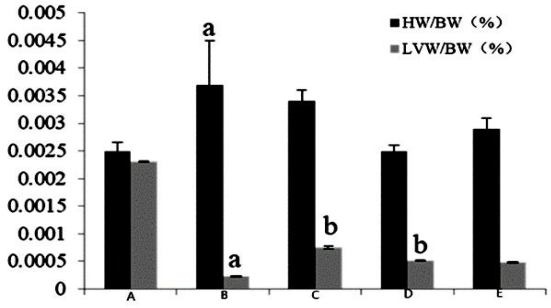

Figure 2: Comparison of cardiac function parameters of rats in each group. Values are mean ± SD**.** A: Sham operation group; B: Model; C: medium-dose SAL group; D: high-dose SAL group; E: SAL. [a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs model

Table 1: Comparison of collagen area and collagen 1/111 ratio in infarction border zones

Group	Collagen area	Collagen
Sham	8.53±9.81	0.96±0.36
AMI	44.65±11.02[a]	1.74±0.27[a]
Low SAL	36.31±11.01[b]	0.94±0.14[b]
Medium dose SAL	52.74±8.61	1.46±0.40[b]
High dose SAL	28.07±4.78[b]	1.34±0.61

[a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs AMI model

Table 2: Levels of myocardial enzyme indices in each group of rats (mean ± SD)

Group	CK-MB (×10^3 U/L)	LDH-1 (×10^3 U/L)	ET (pg/×10^3)	LN (μg/L)	HA (μg/L)
Sham operation	0.05±0.01	0.02±0.01	131.02±16.13	45.79±15.03	65.12±19.47
Model	8.13±0.19[a]	2.59±0.15[a]	191.00±17.03[a]	75.45±16.36[a]	163.46±18.45[a]
Low-dose SAL	6.99±0.05	2.25±0.09	183.16±20.18	62.10±18.46	152.36±20.18
Medium-dose SAL	4.82±0.15[b]	1.98±0.05[b]	140.12±15.06[b]	53.12±16.02[b]	123.23±11.48[b]
High-dose SAL	2.10±0.01[b]	0.82±0.01[b]	125.23±10.46[b]	42.09±13.02[b]	101.08±9.15b

[a]$P < 0.05$, compared with the sham operation group; [b]$p < 0.05$, compared with the model group

Effect of different doses of SAL on myocardial enzymes

There were significantly higher activities of LDH-1, CK-MB, ET, LN and HA in model rats than the corresponding levels in sham operation rats (Table 2). In contrast, the activities of ET, LDH-1, CK-MB, LN and HA of rats in medium and high-dose SAL groups were significantly reduced, relative to those in model rats.

Effect of different doses of SAL on protein expressions

There were higher DVL-1 and β-catenin protein levels in model rats than the corresponding levels in sham rats. However, DVL-1 and β-catenin protein levels in low-dose SAL and medium-dose SAL rat groups were significantly decreased, relative to model rats.

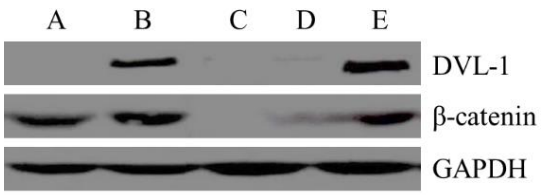

Figure 3: Impact of SAL on protein expression levels of DVL-1 and β-catenin. A: Sham; B: model; C: low SAL; D: medium SAL; E: high SAL

DISCUSSION

The pathogenesis of myocardial fibrosis is extremely complex, and it is involved in the balance between collagen formation and degradation [10]. Clinical studies have shown that excessive extracellular matrix proliferation leads to reduced cardiac compliance, which in turn leads to impaired cardiac function. Other reports have shown that pathological changes related to myocardial fibrosis exist in many types of coronary heart ailments such as rheumatic cardiac disease and diabetes-induced cardiomyopathy which are risk factors for sudden cardiac death [11,12]. Current clinical studies are focused on blocking or reversing myocardial fibrosis, and identification of the potential therapeutic targets [13]. Clinical reports have shown that depositions of type I and III collagen are important markers for the evaluation of myocardial fibrosis. When the expression levels of the two proteins are increased without coordination of their elevated ratio, left cardiac insufficiency may result [14]. Type I and III collagens are important components of the extracellular matrix of cardiomyocytes. Type I collagen, with thick fibers, strong stiffness and traction resistance, is more abundant than collagen III. Type III is composed of fine fibers with strong extensibility and elasticity which are qualities conducive for maintaining the elasticity of the chamber wall [15]. Therefore, studies on drugs and treatment methods that can effectively inhibit ventricular remodeling are important for the treatment of myocardial fibrosis in heart failure. It has been reported that traditional Chinese medicines (monomer and compound decoctions) mitigate myocardial fibrosis after ventricular remodeling in rats with acute myocardial infarction. *Rhodiola* invigorates *qi*, activates blood circulation and *dredges* venation. Clinical studies have confirmed that salidroside is one of the main bioactive components of *Rhodiola* which improve immunity, promote angiogenesis and enhance anti-apoptosis. Other reports have shown that salidroside significantly reduced atherosclerotic plaque area and alleviated impairment of vascular endothelial function caused by homocysteine [16,17]. Through the establishment of a rat model of acute myocardial infarction and intervention with salidroside, it was found that salidroside effectively relieved myocardial tissue damage dose-dependently.

In the present investigation, it was found that myocardial collagen fibers in the infarction border zone of AMI rats in each group were significantly increased, accompanied by fibrous scar formation, increased myocardial collagen area and deposition of type I and III collagens which could lead to poor ventricular wall elasticity. However, after salidroside intervention, collagen fibers and depositions of type I and type III collagens were significantly reduced, resulting in enhancement of the recovery of compensatory mobility of the ventricular wall. In addition, it was found that salidroside significantly improved

LVW/BW ratio in rats with myocardial infarction, and improved ventricular remodeling after acute myocardial infarction, all of which were beneficial to suppression of myocardial fibrosis. Salidroside produced the best effect at a low dose. It is known that ET, LN and HA are characterized by high diagnostic accuracy and reliability, and can be used to evaluate myocardial fibrosis or ventricular remodeling. Lactate dehydrogenase-1 (LDH-1) is a glycolytic enzyme. Clinical studies have confirmed that serum LDH-2 level in normal population is higher than that of LDH-1. However, during myocardial infarction, LDH-1 level is higher than that of LDH-2 due to the release of myocardial enzymes into the blood. This is used to evaluate myocardial diseases. One of the ideal markers of myocardial infarction used in the current study for experimental diagnosis is CK-MB, with a myocardial content of over 40 %. Serum CK-MB level increases significantly during the onset of acute myocardial infarction, but does not increase in liver and kidney diseases. In this study, it was found that the levels of CK-MB, LDH-1, ET, LN and HA of rats in medium-dose and high-dose SAL groups were significantly lower than those in the model group. The Wnt/β-catenin signal route which is crucial in ventricular remodeling is activated by DVL-1. Moreover, DVL-1 upregulates the expression level of β-catenin which is beneficial for promotion of the differentiation of fibroblasts, leading to myocardial fibrosis. In this research, salidroside significantly reduced translation of DVL-1 and β-catenin genes in Wnt/β-catenin signal pathway. These two are the major proteins in Wnt/β-catenin signal route, and they block the activation of ventricular fibroblasts induced by stimulation of the classical Wnt signal route. This reduces the formation and deposition of extracellular matrix proteins, alleviates pathological myocardial fibrosis and delays ventricular remodeling.

CONCLUSION

Salidroside significantly mitigates ventricular remodeling in rats with myocardial infarction through a mechanism which may involve modulation of Wnt/β-catenin signal route.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Jiahang Xu conceived and designed the study. Li Deng and Yanan Zhang collected and analysed the data. Junying Bao wrote the manuscript.

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