Tropical Journal of Pharmaceutical Research August 2023; 22 (8): 1567-1572
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i8.6

Original Research Article

MiR-208a reduces inflammatory responses in heart failure rats through β-catenin pathway

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Sent for review: 12 February 2023 *Revised accepted: 31 July 2023*

Abstract

Purpose: *To investigate the effect of micro-ribonucleic acid (miR)-208a on heart failure (HF) in rats through β-catenin pathway.*
Methods: *A total of 24 specific pathogen-free female Sprague-Dawley rats were enrolled and randomly divided into 3 equal groups, namely, control (normal group), model, and study group (miR-208a), with 8 rats each. Echocardiography was utilized to evaluate cardiac function, and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was applied to examine cardiomyocyte apoptosis. Finally, expression levels of interleukin (IL)-6 and IL-10 were determined using enzyme-linked immunosorbent assay (ELISA). Expression of matrix metalloproteinases (MMPs) was determined via immunohistochemistry assay, while western blotting was used to measure expression of β-catenin.*
Results: *The mRNA expression level of miR-208a was significantly lower in model group than control and study group ($p < 0.05$). Cardiac function of rats in model group was significantly better than other groups ($p < 0.05$). Cardiomyocyte apoptosis was significantly increased in model group than in other groups ($p < 0.05$). Furthermore, expression levels of MMPs, IL-6 and IL-10 in model group were elevated in comparison with those in study and control groups ($p < 0.05$).*
Conclusion: *MiR-208a reduces inflammatory response and deposition of extracellular matrix in rats with HF through inhibition of β-catenin signaling pathway, thereby restoring cardiac function.*

Keywords: *MiR-208a, Inflammatory Response, Heart failure, β-Catenin pathway*

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INTRODUCTION

Heart failure (HF) occurs in the final stage of cardiovascular diseases, and manifests as cardiac insufficiency. According to epidemiological statistics, HF carries a significant burden in terms of morbidity and mortality, imposing substantial challenges on individuals across nations. Therefore, research has intensified on understanding the basic and clinical aspect of this disease [1-4].

Micro-ribonucleic acids (miRNAs) are a class of non-coding small RNAs that are highly conserved during the evolution of higher animals [5,6]. Studies have indicated that miRNAs are closely associated with human heart diseases such as sudden myocardial infarction, ischemia,

abnormal hypertrophy, remodeling and sudden HF [7,8]. Recent research has revealed that miR-208a plays an important physiological in the cardiovascular system by participating in myocardial remodeling, cardiac conduction, and functional metabolism [8]. The β-catenin signaling pathway also plays a considerable role in various cardiovascular diseases, and its abnormal pathological regulation causes the emergence of various types of heart diseases [9-12].

However, it has not been reported whether miR-208a have significant effects in prevention and treatment of HF by regulating the β-catenin signaling pathway. Therefore, this study aims to investigate the effect of miR-208a on HF in rats by examining its activity on β-catenin pathway.

EXPERIMENTAL

Animals and grouping

A total of 24 specific pathogen-free (SPF) female Sprague-Dawley rats aged 2 months old and weighing 200 ± 20 g were enrolled and randomly divided into control (n = 8), model (n = 8) and study groups (n = 8). The rats were fed in SPF animal experiment center, and they were administrated adequate aseptic feed and water daily. This study was approved by the Animal Ethics Committee of Xingtai Medical College Animal Center (approval no. IACUC-021). The animals were handled following standard international guidelines for animal studies [13].

Animal modeling and processing

The rats were intraperitoneally anesthetized with 7 % chloral hydrate and the fur was removed from the chest. After disinfection with 75 % ethanol, the skin was cut open, and subcutaneous tissues were bluntly dissected to expose the heart. Then the left coronary artery was ligated at 3 mm away from the origin to prepare the rat model of HF. The rats in the miR-208a group were injected with miR-208a lentivirus (Shanghai Genechem Co., Ltd. Shanghai, China) after the model was established. No treatment was administered to the control group. The rats were fed with adequate nutrients and water in a ventilated environment at ambient temperature (27 ^{o}C) and relative humidity of 60 % in a 12 h light-dark cycle.

Echocardiography

Left ventricular ejection fraction of rats were determined and calculated by echocardiography to assess cardiac function after one week of treatment.

Determination of miR-208a expression via reverse transcription-PCR (RT-PCR)

Tissues were collected and ground with a grinding rod and liquid nitrogen, thereafter, total RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol and reversely transcribed into complementary deoxyribose nucleic acid (cDNA) under the following conditions: reaction at 55 °C for 5 min, denaturation at 95 °C for 10 min, maintenance for 10 s, and annealing at 60 °C for 40 s. The primer sequences are shown as follows (Table 1).

Table 1: Primer sequences used

Name	Primer sequence
MiR-208a	F: 5'-TGCGGTATAAGACGAGCAAA-3'
	R: 5'-AGGTAACGCCAGGAATTGTTGCTA-3'
U6	F: 5'-CTCGCTTCGGCAGCACA-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)

Cardiomyocyte apoptosis was examined using TUNEL kit. Five fields of view were randomly selected and photographed using the microscope. Ratio (%) of the number of apoptotic cells to the total number of cells was recorded as the apoptotic index.

Determination of the expression levels of IL-6 and IL-10

The abdominal aortic blood of each group of rats was collected after anesthesia, centrifuged and the supernatant collected. Expression levels of IL-6 and IL-10 were determined using ELISA kit (R&D Systems, Minneapolis, MN, USA) at 450 nm in a microplate reader.

Evaluation of expression of MMPs via immunohistochemistry assay

Each group was perfused with paraformaldehyde under anesthesia. The sample was collected, fixed using paraformaldehyde, dehydrated using a series of ethanol gradients and immersed in paraffin to create paraffin tissues. These tissues were sliced into sections measuring 5 μm using a microtome. Subsequently, the sections were dewaxed using xylene, dehydrated with alcohol, and placed on a glass microscopic slide. Antigen

retrieval was performed on the tissues in a water bath. The serum was blocked at room temperature, and incubated with primary and secondary antibodies. Finally, the image was developed, and the tissues were dehydrated and mounted for observation of MMPs expression.

Measurement of β-catenin expression by western blotting

Protein extraction was performed using radioimmunoprecipitation assay (RIPA) (Thermo Fisher Scientific, Waltham, MA, USA). Total protein concentration was determined by bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). The protein was loaded, separated using gel electrophoresis, and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Then the membrane was sealed by prepared skim milk power at 4 °C for 1.5 h, and incubated with rabbit anti-human β-catenin monoclonal antibody at 4 °C overnight. After washing using phosphate buffered saline-tween (PBST), goat anti-rabbit second antibody was added for reaction at room temperature for 1.5 h. Color development was visualized using an enhanced chemiluminescence (ECL) detection kit (Amersham Biosciences, Foster City, CA, USA). Finally, protein bands were exposed in the gel imaging system.

Statistical analysis

Statistic Package for Social Science (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Student *t*-test was employed for data that follows a normal distribution and has equal variance. Corrected t-test was used for data with a normal distribution but unequal variance. Non-parametric test was employed for data that did not meet the requirements of normal distribution and equal variance. The rank sum test was applied when dealing with ordinal data, while the chi-square test was used to compare two groups.

RESULTS

Echocardiography

The left ventricular ejection fraction in control (normal) group was significantly higher than study group (miR-208a) and model group ($p < 0.05$) (Figure 1).

Expression of mRNA

The results of RT-PCR displayed that the messenger RNA (mRNA) expression level of miR-208a was significantly lower in model group than control and study groups ($p < 0.05$) (Figure 2).

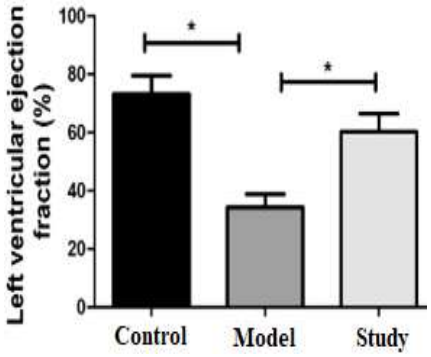

Figure 1: Left ventricular ejection fraction of each group. *$P < 0.05$ *vs.* model group

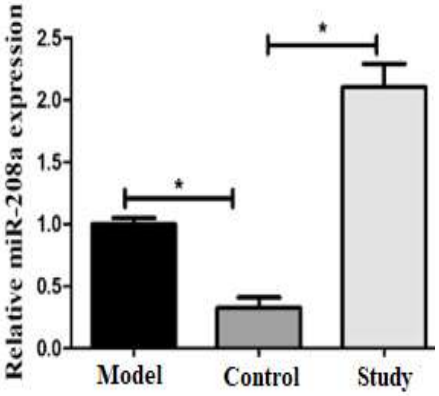

Figure 2: Expression levels of miR-208a in three groups of rats determined by RT-PCR. *$P < 0.05$ *vs.* model group

Cardiomyocyte apoptosis

The results of TUNEL staining revealed that cardiomyocyte apoptosis rate was significantly increased in model group compared with control and study groups ($p < 0.05$) (Figure 3).

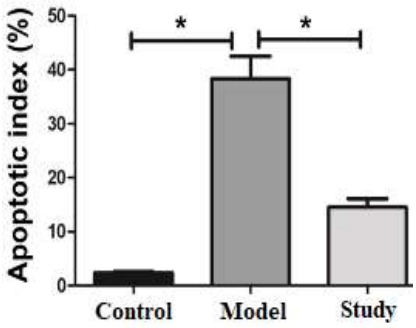

Figure 3: TUNEL results showing cardiomyocyte apoptosis rate in the model group. *$P < 0.05$ vs. miR-208a group and normal group

Expression of inflammatory factors

The results of ELISA exhibited that expression levels of IL-6 and IL-10 in serum of peripheral blood in model group were significantly increased in comparison with those in the normal group and study group ($p < 0.05$; Figure 4 A and B).

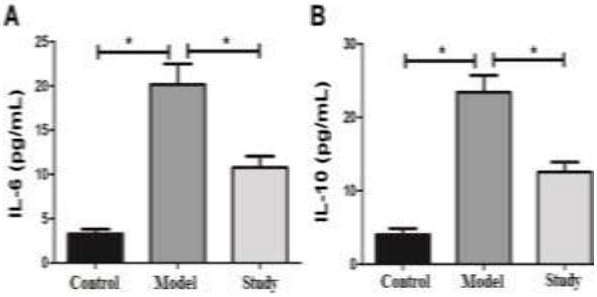

Figure 4: Expression level of IL-6, IL-10, MMPs and β-catenin were higher in the model group. (A) Expression level of IL-6 in the serum of peripheral blood in the three groups of rats. (B) expression level of IL-10 in the serum of peripheral blood in the three groups of rats. *$P < 0.05$ vs. miR-208a group and normal group

Expressions of MMPs and β-catenin

The results of immunohistochemistry revealed that MMPs were slightly distributed in the normal and study group. More MMPs were diffusely distributed in the model group, and the model group had higher expression of MMPs than the other two groups ($p < 0.05$) (Figure 5 A). The results of Western blotting demonstrated that model group exhibited significantly increased expression of β-catenin compared with the other two groups ($p < 0.05$; Figure 5 B).

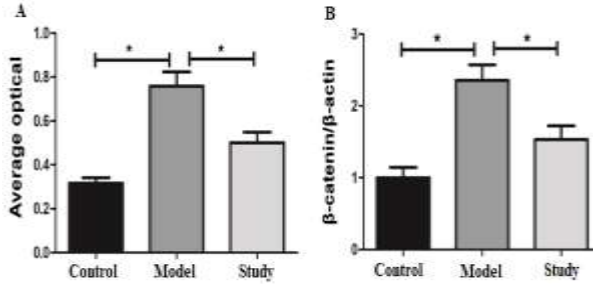

Figure 5: Expression levels of MMPs and β-catenin. (A) immunohistochemical expression results (B) Western blotting results. *$P < 0.05$ vs. control and study groups

DISCUSSION

Despite significant advancements in prevention, diagnosis, and basic research of cardiovascular diseases by clinical and scientific researchers over the years, the study of heart failure (HF) remains comparatively limited [14,15]. Incidence and mortality rate of HF remain high in both Western countries and China. Although current clinical treatment of HF achieved by reducing cardiac ejection fraction is greatly mature and effective, the survival rate of patients suffering from HF remains quite low after medication or surgery, and in recent years there has been no significant breakthrough in drug therapy for ejection fraction retention in HF and sudden acute HF [16,17].

MiRNAs are a kind of conserved RNAs with about 18-25 nt in length, which, by binding to related target genes, activate or inhibit translation of target genes, thereby controlling a series of life processes of cells, including growth, division, differentiation and even apoptosis [5-8]. Previous research was limited to body tumors and metabolic abnormalities. In recent years, research priorities have shifted to the cardiovascular system diseases with high incidence rates. It was discovered by researchers that miRNAs are closely associated with the body's irreversible myocardial injury, contractile dysfunction, cardiac remodeling, HF and infarction as well as post-ischemia perfusion. The expression profiles of miRNAs have certain specific tissue specificity, and different classes of miRNAs are expressed in different surrounding tissues. Meanwhile, such expression profiles are also disease-related, that is, a group of miRNAs are only correlated with a specific disease. Based on this specificity, it has been reported that miR-208a is abundantly expressed in the myocardium and can participate in normal metabolism and abnormal pathological behavior of the heart, which is considered as a special marker and a new therapeutic target for heart disease [18,19].

As miR-208a is continuously being discovered and explored in terms of its physiological and pathological role in the heart, researchers are gaining new insights into the complex molecular network that regulates heart development and pathophysiological processes. Previous studies have confirmed that miR-208a is involved in myocardial remodeling, the regulation of abnormal heart rhythm, and modulation of specific pathways. As scientific research deepens, there is a growing understanding of miR-208a and its implications. MiRNAs are considered special markers for myocardial damage, ventricular remodeling and HF, and these unique miRNAs are seen as potential targeted therapeutic agents, offering opportunities for clinical diagnosis and treatment [20,21].

The echocardiography of this study revealed that cardiac function of the study group (miR-208a) was better than model group, and the results of TUNEL staining displayed that cardiomyocyte apoptosis in study group was ameliorated compared with model group, indicating that miR-208a is capable of restoring the myocardial function after HF and reducing cardiomyocyte apoptosis and necrosis. The results of Western blotting exhibited that the expression of β-catenin was lower in study group model group, suggesting that miR-208a down-regulates expression of β-catenin. A study demonstrated that β-catenin is initially highly expressed on the outer membrane in the presence of acute ischemic heart injury in the body, and then migrates to cardiac fibroblasts in the injured area [10]. β-Catenin is able to induce proliferation and expression of cardiac fibroblasts, so as to promote slow myocardial fibrosis, eventually leading to cardiac damage and cardiac insufficiency. In a mouse model of myocardial infarction induced by intravenously administering β-catenin inhibitors, it was found that β-catenin inhibiting group displayed significant proliferation of cardiac progenitor cells compared with myocardial infarction group, with less cardiomyocyte apoptosis. Moreover, fibroblasts and fibroblast collagen synthesis around the myocardial infarction area were greatly reduced, thereby decreasing the area of myocardial infarction in mice, and prominently improving cardiac function. The studies have proved that β-catenin signaling pathway plays an important role in promoting secondary HF after myocardial infarction [9-11]. A similar study indicated that heart pressure is elevated in mice undergoing aortic coarctation, and β-catenin signaling pathway is activated leading to significant increase in cardiac fibrosis. As a result, necrosis occurs in cardiomyocytes, and inflammatory factors in the surrounding tissues are highly expressed [13]. Furthermore, ELISA results demonstrated that miR-208a is able to inhibit expressions of inflammatory factors (IL-6 and IL-10). Finally, immunohistochemistry results proved that miR-208a can promote decomposition of extracellular matrix and reduce production of fibrous tissues.

CONCLUSION

MiR-208a inhibits inflammatory response in rats with HF and reduces production of fibrous tissues by repressing β-catenin signaling pathway, ultimately promoting recovery of cardiac function in rats with HF. Thus, this compound has some clinical potentials for the management of HF.

DECLARATIONS

Acknowledgements

Application of Neutrophil-to-lymphocyte Ratio and BNP Detection in Heart Failure and Atrial Fibrillation. This study was financially supported by Xintai Research and Development Project (no. 2020ZC272).

Funding

None provided.

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

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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