

Effect of LLLI on Expression of Micro RNA-21 and Ventricular Remodeling in Rats after AMI

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Abstract

Objective: To assess the effects of low-level laser irradiation (LLLI) on the expression of micro RNA-21 and ventricular remodelling in the model of rat myocardial infarction.

Methods: 110 adult-female SD rats were randomly divided into sham operation (30), control (40), and treatment (40) groups. MI model was prepared by ligation of left anterior descending artery in the control and treatment groups while simply threading at the same site of MI model in sham group. LLLI treated using (635 nm, 6mW, 125s, 0.96 J/cm²) after three weeks of MI at infarct region in the treatment group. At 1 h, 24 h, 48 h, 72 h and 7 d after LLLI treatment, the expression of miR-21 at the infracted myocardial tissue were detected using qRT-PCR. At the end of 4 weeks after MI, hearts were harvested for histological analysis.

Results: MiR-21 expression in the treatment group was lower than both sham group as well as the control group (P<0.05). LVEF (%) and LVFS (%) in the treatment group before and after LLLI; ($39.37 \pm 1.35 vs. 47.62 \pm 2.75$, P<0.05; (19.23 ± 3.12) vs (24.15 ± 2.53), P<0.05). Collagen fiber content in treatment group were significantly lower than control group (28.79 ± 2.06 %) vs (69.22 ± 3.64 %), (P<0.05).

Conclusion: LLLI treatment of myocardial infarction can significantly down regulate the expression of tissue miR-21 in the infarct region, increase left ventricular function and decrease collagen fiber content suggesting the beneficial effect of LLLI on delaying myocardial fibrosis, reduce the pathological ventricular remodelling (VR) after MI.

Introduction

Myocardial infarction (MI) is a manifestation of coronary artery disease where coronary blood supply drastically reduced or interrupted. It is lethal and emergency cardiovascular disease which is one of leading causes of disability and death in clinical practice [1]. Ventricular remodeling after myocardial infarction refers to neurohormonal and genetic regulatory mechanisms activated by inflammatory cytokines causing changes in quality of cell morphology and functions of myocardial cells [2,3]. Process of Ventricular remodeling is done by developing a variety of coding and non-coding gene regulation, but a lot of gene regulation mechanism is unclear. Micro RNAs (miRNA) are a class of small RNA in cells which have regulatory role with around 22 nucleotides. Recent studies have shown that miRNA were involved in cardiac function and cardiac remodeling regulation in a variety of cardiovascular diseases [4,5]. Thum et al. [6] reported miRNA-21 being main regulator of ERK-MAP kinase signaling pathway. During myocardial infarction, miRNA-21 levels increased selectively thus controlling of miRNA-21 expression can decrease the ventricular remodeling.

LLLI is a more mature physical therapy. A large number of tissue culture and animal experiments have proved that low-energy laser irradiation (LLLI) can regulate a variety of biological processes [7,8]. Various effects and role of low-level laser irradiation (LLLI) have been found out in the treatment of myocardial infarction. However, effect of LLLI on tissue expression of miRNA-21 and its relationship with

ventricular remodeling in rats after myocardial infarction is less in current studies. Therefore, this study was designed to investigate effect of low-energy laser diode (wavelength of 635 nm) on miRNA-21 expression and ventricular remodeling along with collagen fiber content after myocardial infarction in rat.

Materials and Methods

Materials

110 adult female SD (Spragur-Dawley) rats, weighing 220 g ~ 250 g were provided by the Experimental Animal Center of Henan Province. All animal experiments were approved by the Animals Committee of Second Affiliated Hospital of Zhengzhou University.

Reagents and instrument

Biological signal acquisition and analysis system BL-420S type (Chengdu Thai Union Technology Co, Ltd.); small animal ventilator HX-100E type (Chengdu Thai Union Technology Co, Ltd.); echocardiogram machine type Sonos 5500 (Philips Netherlands company); laser treatment TY-1 type (instrument Technology Co, Ltd. Beijing-day Youke); Trizol reagent (USA invitrogen company); refrigerated centrifuge 2-16K type (Germany SIGMA company); 7500 Fast Real-Time PCR System (US Applied Biosystems company).

Grouping methods

SD rats were randomly divided into three groups: sham operation group (30), control group (40) and treatment group (40). Myocardial infarction (MI) model was prepared by ligation of the left anterior descending coronary artery in the control and treatment groups, while only threading at the same site of left anterior descending coronary artery was done without ligation in sham group,. Three weeks after MI model preparation, irradiation at infarct region in the treatment group were done after confirming by echocardiography using low level laser while the control group and the sham group were irradiating the same area but without putting LLLI power on.

Rat model of myocardial infarction (RMMI)

After anesthesia by using intra-peritoneal injection of 10% chloral hydrate (300 mg/kg), machine-assisted breathing was established by tracheotomy,. Skin incision at the left sternal border at 3rd or 4th intercostals space where the beat feels the strongest, blunt separation of muscle tissue layer by layer, retraction of ribs and fully exposed left atrial appendage and the surrounding area, the lungs pressed with a gauze pad, spray 0.05 ml of 2% lidocaine on the heart to prevent arrhythmia. At meeting point of left auricle and cone of pulmonary trunk about 3-4 mm away from the aortic root, a needle of 1-2 mm, 6-0 prolene thread is passed with depth of 1.5 mm and span of 3 mm around the LAD. Ligation of thread was performed after the heart beat became regular. MI model confirmed successfully established when left ventricular wall motions decreased, colour changes to white and ST segment elevation were observed. In the Sham group only threading without ligation into the same site of above was performed. After the surgery, rats were warmed up until regained consciousness. We used 300,000 U intramuscular antibiotics continuously for 3 days.

Screening MI model

Three weeks after prepared MI model, echocardiographies of rat heart were performed. The criteria for the standard model of myocardial infarction includes: 1. A left ventricular ejection fraction (LVEF) <60%; 2. Left ventricle fraction shortening (LVFS) <30%; 3. Ultrasound GP or judgment of a number of levels appears abnormal appearance of the anterior wall contraction. At least two of the above is required to select the MI model.

Low-energy laser irradiation process

After three weeks of prepared myocardial infarction model, low energy laser irradiation were treated in the rats with qualified echocardiography screening results. We used a gallium-arsenic (GaAs) laser diode, with 600 quartz optical fiber, continuous wavelength of 635 nm and an adjustable maximum power output of 20Mw (Figure 1). The output power was set at 6mW continuously with uninterrupted wave mode. The optical fiber tip, in all experiments, was placed 15 mm above the surface of heart to allow the laser beam diameter of 10 mm. The power density on the myocardium was 7.64 mW/cm². Thus, the laser beam could spread over most of lateral wall of left ventricle including the infarct myocardial area. The irradiation lasted for 125 sec constantly. Apparently, the energy density to the myocardium was 0.96 J/cm². All the rats in 3 groups were treated with LLLI however the rats in the sham and the control group underwent the same surgical procedure and laser irradiation, but without switching on the laser power supply.



Figure 1: LLLI treatment after 3 weeks of MI model.

Post LLLI screening of cardiac function

One week after LLLI treatment, rats in each group were performed echocardiography, evaluate left ventricular function of rats in the treatment group (n=3/group). Equipment in the second echocardiography, process, the drugs used, and evaluation methods were the same as in the first echocardiographic evaluation.

Sample collection

Samples were collected in each group after LLLI treatment at 1 h, 24 h, 48 h, 72 h, and 7 d. Rats were anesthetized; intubated and harvested heart was washed by 4^{0} C saline. Collected infarct tissue kept into refrigerator at -80⁰C

Real time quantitative PCR evaluation of microRNA-21 expression

Extraction total RNA according to the operating instructions of the Trizol kit. 1 µg total RNA was extracted from each sample, synthesis of cDNA by reverse transcription process. Conditions for PCR reaction: 95^{0} C for 5 min, then 95^{0} C for 10 sec, 60^{0} C for 30sec, running 40 cycles and then heated from 60^{0} C to 95^{0} C to obtain the corresponding melting curves. Reverse transcriptase and cDNA template were not added during the reaction as a negative control. Each reaction system set up have three sub-apertures. U6 as internal control, analyze the relative expression level of miR-214 using 2-Ct method. (Ct=Ct target gene-Ct reference gene).

Histopathological analysis of infarcted rats' myocardial tissues

All Masson tri-chrome pathological slices conducted image collection by using image acquisition Digital Imaging Analysis System as shown in figures (Figures 2-5). By colour differences of randomly selected regions in the slice, collagen fiber content of LV myocardial tissues and percentage occupied by the collagen fiber in the whole LV were determined.



Figure 2: HE staining of myocardial tissue Note: A: sham group (sham); B: the control group (control); C: the treatment group (LLLI).



Figure 3: MASSON staining of myocardial tissue; Note: A: sham group (sham); B: the control group (control); C: the treatment group (LLLI).



Figure 4: TUNEL staining of myocardial tissue. Note: A: sham group (sham); B: the control group (control); C: the treatment group (LLLI).





Statistical analysis

Statistical analyses were performed using SPSS 19.0 2.6 software (IBM, USA). Data were expressed or presented as mean \pm standard deviation (SD), the groups were compared using ANOVA, mean pairwise comparisons were done using LSD test, α =0.05 (level of significance test.), a probability (P) value of <0.05 was considered statistically significant.

Results

Evaluation of left ventricular function in rats

Comparison before and one week after LLLI processing in treatment group; LVEF (%) (39.37 \pm 1.35 vs 47.62 \pm 2.75, P<0.05), LVFS (%) (19.23 \pm 3.12 vs 24.15 \pm 2.53, P<0.05) differences showed statistical significance; comparison between the control group and the treatment group one week after the LLLI processing: LVEF (%) (39.83 \pm 1.64 vs 47.62 \pm 2.75, P<0.05), LVFS (%) (18.03 \pm 1.25 vs 24.15 \pm 2.53, P < 0.05), the difference was statistically significant.

miR-21 expression at the central infarct zone

At 1 h after LLLI treatment, miR-21 expression of the control group was significantly lower than the sham group $(15.12 \pm 1.32 \ vs \ 39.88 \pm 2.07, P<0.05)$, while miR-21 expression in the treatment group was less than sham group and higher than the control group $(37.40 \pm 1.39 \ vs \ 39.88 \pm 2.07, P<0.05; \ 37.40 \pm 1.39 \ vs \ 15.12 \pm 1.32, P<0.05)$, all showed statistically significant; Expression of miR-21 in Sham group changed a little in each time period while the expression in the control group increased slowly from the beginning 1 h and approaching maximum on 7 d, the difference was not statistically significant with the sham group (P>0.05). In the treatment group, the level of miR-21 was highest in 48 h with a slow decline on 7 d, when compared with the sham group, the difference was still statistically significant (P<0.05), as shown in Table 1 and figure 5

Group	1 h		24 h		48 h		72 h		7 d	
Sham	39.88 2.07	±	42.00 1.30	±	39.98 2.21	±	42.72 1.59	±	40.20 0.88	±
Control	15.12 1.32 *	±	20.92 0.79 *	±	35.94 2.06 *	±	37.60 2.85 *	±	38.60 3.33	±
Treatme nt	37.40 1.39 *	±	50.98 1.41 *	±	59.52 0.90 *	±	52.90 1.11 *	±	43.44 0.97 *	±
F	348.073		823.416		234.812		79.068		7.657	
Р	0		0		0		0		0.006	

Table 1: Relative comparison of miR-21 expression in central infarct area of rats heart in each group after the LLLI treatment at the respective periods of time (n=4- 6);*: Compared with the sham group, P<0.05; : Compared with the control group, P<0.05.

Changes in left ventricular myocardial tissue collagen fiber content

Left ventricular wall thickness was significantly reduced in treatment group and the control group when compared with sham group, (P<0.05); compared with control group, left ventricular wall thickness in treatment group was significantly increased (P<0.05);

infarction area in treatment group (LLLI group) was smaller than the control group (P<0.05). Collagen fiber content in Left ventricular wall were; sham group 4.17 \pm 0.65%, treatment group (LLLI group) (28.79 \pm 2.06%) and the control group (69.22 \pm 3.64%), Compared with sham group, collagen fiber content in Left ventricular wall in treatment group and the control group was significantly increased (P<0.05) while reduced significantly in the treatment group (LLLI group) than in control group, (P<0.05), all showed statistically significant difference. Figures 2-5)

Discussion

Ventricular remodeling by ischemic myocardial injury is an important cause affecting heart function and hemodynamic, which is mainly affected by myocardial infarction size and myocardial collagen remodeling in noninfarcted region. Once the ischemic myocardial injury occurs, the myocardial infarction size is mainly affected by the myocardial cell apoptosis in the border area. Overexpression of miR-21 reduced cell apoptosis in the border areas at early stage of AMI and reduced the myocardial infarct size in the rat heart [9]. Apoptosis is a major cause for cardiac infarction following ischemia in coronary artery diseases [10,11]. The inhibition of miR-21 has been shown to suppress cell growth by increasing apoptosis and decreasing cell proliferation. Yin C et al. [12] provided evidence for protective effect of miR-21 on cardiac injury by reducing expression of apoptotic genes-Bid and Bcl-10, which can bind to Bcl-2 to promote apoptosis. Previous study showed that adenoviral transfer of miR-21 in vivo decreases cell apoptosis in the border and infracted areas through its target gene, programmed cell death 4(PDCD4), and activator protein 1 (AP1) pathway [71] MiR-21 is induced by the AKT pathway and mediates an antiapoptotic effect via suppression of FasL [13]. Conversely, it activates AKT through suppression of phosphatase and tensin homolog (PTEN) through a feed forward loop [14].

MiR-21 can negatively regulate ventricular remodeling and has a positive impact on the occurrence of ventricular remodeling. Recent studies have identified expression patterns of miRNAs associated with pathological cardiac hypertrophy, heart failure, and myocardial infarction in humans and mouse models of heart disease [15]. MicroRNA-21(miR-21) that is consistently induced by cardiac stress appears to function as a regulator of cardiac growth and fetal gene activation in primary cardiomyocytes *in vitro* [16-18]. Pre-injected with heat-shock-induced miRNAs including miR-21 in the mouse hearts reduces myocardial infarct size after I/R injury. It is also confirmed the aberrantly expressed miR-21 has a protective effect on myocardial infarction and protective effect on I/R by reducing cardiac cell apoptosis via its target gene PDCD4 and AP-1 pathway.

The results of this study indicate that miRNA-21 expression raised in both central and peri-infarct zone from 1 h to the maximum at 48 h then showed gradual decline from 72 h to 7 d, increased left ventricular wall thickness, increased left ventricular function, reduce the formation of collagen tissue and myocardial infarct size after low- level laser irradiation. All these findings support the role of mi-RNA-21 in improving ventricular remodeling after myocardial infarction.

Conclusion

Ventricular remodeling after myocardial infarction is a key process of cardiovascular disease which can be associated with atrial

fibrillation; heart failure etc. miR-21 plays an important role in cardiac remodeling, and myocardial fibrosis. LLLI treatment of myocardial infarction can significantly down regulate the expression of tissue miR-21 in the infarct region, increase left ventricular function and decrease collagen fiber content suggesting the beneficial effect of LLLI on delaying myocardial fibrosis and reduce the pathological ventricular remodeling (VR) after MI. However the exact mechanism needs to be studied further.

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