MicroRNA-146a improves sepsis-induced cardiomyopathy by regulating the TLR-4/NF-κB signaling pathway

JIN XIE[1], LINA ZHANG[1], XIAOYAN FAN[2], XIAOQING DONG[1], ZHE ZHANG[1] and WENXING FAN[3]

Departments of [1]Emergency and [2]Obstetrics, Zhangqiu District People's Hospital, Jinan, Shandong 250200; [3]Department of Nephrology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650032, P.R. China

Received August 24, 2018; Accepted May 3, 2019

DOI: 10.3892/etm.2019.7657

Abstract. The aim of the current study was to investigate the regulatory effect of miR-146a on the toll-like receptor 4 (TLR-4)/NF-κB pathway and therefore inflammation in septic cardiomyopathy. A total of 60 healthy male Sprague Dawley rats were equally divided into a control, LPS, miR-146a agonist and miR-146a inhibitor group. Blood samples were collected from rats 24 h after intraperitoneal lipopolysaccharide injection and myocardial tissues were subsequently collected. After hematoxylin and eosin staining of rat myocardial tissues, the degree of inflammatory cell infiltration and myocardial damage was observed. The content of certain myocardial injury markers were also observed, including cardiac troponin I (cTnI), B-type natriuretic peptide (BNP), creatine kinase myocardial bound (CK-MB) and myoglobin (Mb). Western blot analysis was performed to detect the expression of NF-κB/TLR-4, tumor necrosis factor (TNF-α) and intercellular adhesion molecule-1 (ICAM-1) in myocardial tissues. Reverse transcription-quantitative (RT-q) PCR was used to detect the expression of miR-146a, TNF-α, interleukin (IL)-1α and IL-1β mRNA in myocardial tissues. In the LPS group, myocardial interstitial tissue edema occurred, with enlarged and loosely arranged cardiomyocytes. Compared with the sepsis model group, myocardial interstitial tissue edema was relieved in the miR-146a agonist group, but was aggravated in the miR-146a inhibition group. The serum levels of cTnI, BNP, CK-MB, Mb, NF-κB, TLR-4, TNF-α and ICAM-1 in the sepsis model group were higher than those in the control group. In the miR-146a agonist group, levels of myocardial injury markers were lower than those in the sepsis model group, but were higher in the miR-146a inhibition group. The results of RT-qPCR demonstrated that the expression of miR-146a, TNF-α, IL-1α and IL-1β in the sepsis model group were upregulated compared with the control group. In addition, miR-146a expression in the miR-146a agonist group and the miR-146a inhibition group was increased, but TNF-α, IL-1α and IL-1β mRNA was downregulated. miR-146a may regulate the TLR-4/NF-κB signaling pathway via negative feedback mechanisms, leading to the improvement of the inflammatory response and cardiac dysfunction in sepsis-induced cardiomyopathy.

Correspondence to: Dr Wenxing Fan, Department of Nephrology, The First Affiliated Hospital of Kunming Medical University, 295 Xichang Road, Kunming, Yunnan 650032, P.R. China
E-mail: fanwx1999@163.com

Key words: microRNA-146a, toll-like receptor 4/nuclear factor-κB pathway, sepsis, lipopolysaccharide

Introduction

Sepsis or systemic inflammatory response syndrome is caused by infection, which is an immune response to pathogens and immunogenic substances that induce autoimmune injury (1). Sepsis is common in cases of severe trauma, burns, shock or major surgery. The further development of sepsis may lead to septic shock and multiple organ dysfunction syndrome, making sepsis a leading cause of death in patients admitted to intensive care units (2).

Bacterial infection is the most common cause of sepsis (2). Bacterial products, including endotoxin and exotoxins, can directly or indirectly stimulate various target cells including monocytes, polymorphonuclear neutrophils or endothelial cells to initiate inflammation (1). Lipopolysaccharide (LPS) is a representative endotoxin (3). In the early stages of sepsis, the alteration of cardiovascular function is associated with patient prognosis (4). Cardiovascular complications aggravate sepsis and increase patient mortality (4,5). Sepsis that is complicated with cardiovascular dysfunction is commonly referred to as sepsis-induced cardiomyopathy (SIC). Sepsis-induced myocardial damage is closely associated with a decreased myocardial energy supply and weakened myocardial contractility (5). In addition, a large number of inflammatory factors, including tumor necrosis factor-α (TNF-α), are also involved in sepsis induced myocardial injury (6,7). In sepsis, pathogenic microorganisms transmit signals to the nucleus via cell transduction pathways, triggering a series of transcriptions and translations (7,8). Therefore, blocking the pathological process at this stage may effectively prevent sepsis induced myocardial injury (8).

MicroRNAs (miRNA or miRs) are non-coding RNAs that regulate the expression of target genes by inhibiting the translation or degredation of mRNA (9). miRNAs are involved in the regulation of metabolism, the inflammatory response,

heart-associated diseases, organ transplantation and cancer by regulating mRNA and protein expression (9,10). Among all miRNAs that regulate the progression of inflammation, the role of miR-146a is prominent. It has been demonstrated that miR-146a promotes the release of inflammatory factors by regulating the toll-like receptor 4 (TLR-4)/NF-κB signaling pathway, which is closely associated with myocardial damage (11,12). Previous studies have also revealed that miRNA-146a protects the myocardium from ischemia-reperfusion injury by suppressing interleukin-1 receptor associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6), therefore attenuating NF-κB activation and inflammatory cytokine production (13,14). Taganov *et al* (15) revealed that the stimulation of LPS, TNF-α or interleukin (IL)-1β upregulates the expression of NF-κB-dependent miR-146a in human monocyte leukemia cells. The mutational experiment and luciferase reporter gene assay performed within the aforementioned study revealed that the target genes of miR-146a were IRAK1 and TRAF6, which are adaptor protein encoding genes involved in the TLR-4/NF-κB pathway (15). Activated NF-κB induces the transcription of a large number of inflammatory genes as well as the upregulation of miR-146a. However, miR-146a negatively regulates the NF-κB pathway and inhibits the production of inflammatory factors by targeting the IRAK1 and TRAF6 signal transduction proteins upstream of the NF-κB pathway (16). A further study has revealed that miR-146a binds to the 3' untranslated region (UTR) of IRAK1 and TRAF6, inhibiting their expression at the transcriptional level and alleviating sepsis-induced cardiac dysfunction by inhibiting certain inflammatory factors, including IL-6, IL-8, IL-1β and TNF-α (17).

The TLR-4/NF-κB signaling pathway serves a crucial regulatory role in sepsis-induced cardiomyopathy. Blocking this pathway may therefore be an important method for treating SIC (18,19). The current study aimed to assess the association between the TLR-4/NF-κB pathway and levels of inflammatory cytokines by detecting changes of miR-146a expression in the myocardium of mice with SIC. The effect of the TLR-4/NF-κB pathway on sepsis-induced cardiomyopathy was also investigated.

Materials and methods

Animals. A total of 60 healthy male Sprague Dawley (SD) rats (Shandong Laboratory Animal Centre; age, 6-8 weeks; weight, 180-200 g) were housed in an specific pathogen free laboratory animal room at 20±2˚C with a 60-70% relative humidity, under a 12 h light/dark cycle. All rats recieved free access to food and water. SD rats were equally divided into four groups using a random number table, with 15 rats in each group. Groups included a control group (control), a septic model group (LPS), a miR-146a agonist group (agonist) and a miR-146a inhibitor group (inhibitor). The current study was approved by the Animal Ethics Committee of Kunming Medical University Animal Center (Kunming, China).

Rat model with septic cardiomyopathy. Rats in the LPS group received an intraperitoneal injection of 0.2 μl/g of water and a further injection of 7.5 mg/kg LPS (cat. no. L26331; Shanghai Abcone Co., Ltd.) 24 h later. At the same time point of LPS injection, in control group rats, an equal volume of water or physiological saline was injected to control rats. In the miR-146a agonist and inhibitor group, rats were injected with 0.2 μl/g miR-146a agonist (Shanghai Tuoran Biological Technology co., Ltd.) or inhibitor (Shanghai Tuoran Biological Technology co., Ltd.) in the tail vein 24 h prior to LPS injection.

Sample collection. 24 h after LPS injection, 2 ml of rat blood was collected from the internal iliac vein. Blood was maintained at room temperature for 15 min and subsequently centrifuged at 1,000 x g for 15 min at 4˚C. The upper serum was aspirated and stored at -80˚C. After blood was taken, rats were sacrificed and hearts were extracted and washed with pre-cooled physiological saline. Half of the heart tissue was frozen in a -80˚C refrigerator and the other half were soaked with 4% paraformaldehyde at room temperature for 24 h and then embedded in paraffin for pathological examination.

Hematoxylin and eosin (HE) staining. Myocardial tissues were fixed in 4% paraformaldehyde at room temperature for 24 h. Tissues were then cut into 4 μm slices after washing with PBS, dehydration with a descending alcohol series, waxing and embedding in paraffin. Slices were placed in a dish with warm water to unfold and were subsequently placed on a glass slide for patching. After holding at room temperature for 60 min, sections were observed with a light microscope (magnification, x400) after HE staining (hematoxylin, 5 min at room temperature and eosin, 3 min at room temperature).

Detection of cell apoptosis. Myocardial tissues were dehydrated, embedded and the sections were routinely dewaxed, washed, hydrated and fixed as aforementioned. The procedure followed strictly in accordance with the TUNEL Apoptosis kit manufacturers protocol (Merck KGaA). Each paraffin section was randomly selected from five fields (magnification, x400) and at least 100 cells were counted in each field of view with a light microscope (magnification, x400). The number of apoptotic cells (TUNEL positive cells) and the total number of cells were counted respectively. Apoptotic index=number of apoptotic cells/total cell number x100.

Determination of serum myocardial injury markers. Rat serum was warmed to room temperature. Cardiac troponin I (cTnI; cat. no. ab246529) and B-type natriuretic peptide (BNP; cat. no. ab108815) content were detected using an ELISA assay (Abcam). Creatine kinase myocardial bound (CK-MB) and myoglobin (Mb) content were detected using the chemiluminescence method (cat. no. E-CL-R0722c and E-CL-R0132c; Ke Lei Biological Technology Co., Ltd.).

Western blot analysis. Myocardial tissues were washed in a pre-cooled physiological saline at 4˚C. After the addition of RIPA lysate (Beyotime Institute of Biotechnology), tissues were homogenized and the supernatant was centrifuged at 5,000 x g for 10 min at 4˚C. Total protein content was determined using a BCA Assay kit (cat. no. p0011; Beyotime Institute of Biotechnology). The expression of synaptic plasticity-associated proteins including NF-κB, TLR-4, TNF-α and intercellular adhesion molecule-1 (ICAM-1) were detected via western blot analysis. Protein (10 μl) was loaded and separated by 12% SDS-PAGE electrophoresis, which was transferred to

a PVDF membrane (Roche Diagnostics). Membranes were subsequently blocked using 5% skimmed milk with TBST at 25˚C for 1 h and incubated with the following primary antibodies obtained from Abcam at 4˚C overnight: NF-κB (1:500; cat. no. ab19870), TLR-4 (1:500; cat. no. ab95562), TNF-α (1:500; cat. no. ab9755), ICAM-1 (1:500; cat. no. ab171123) and β-actin (1:500; cat. no. ab8226). The membrane was then incubated with Goat Anti-Rabbit IgG H&L secondary antibodies (1:1,000; cat. no. ab150077; Abcam) at 25˚C for 2 h. The signal on the membrane was detected using an enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.) and Quantity One (version 4.0; Bio-Rad Laboratories, Inc.) was used for quantification.

Reverse transcription-quantitative (RT-q) PCR assay. The total RNA of rat myocardial tissue was extracted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed to synthesize cDNA using the Takara PrimeScript RT Master Mix kit (Takra Bio, Inc.) according to manufacturers protocol. The sequences of miR-146a, TNF-α, IL-1α, IL-1β and GADPH mRNA were obtained from Gene Bank (https://cipotato.org/genebankcip/). Primers were designed using Primer premier 5.0 software (Primers). The following primer sequences were used: miR-146a forward, 5'-GAACTGAATTCCATGGGTTGTGT-3' and reverse, 5'-GCCCACGATGACAGAGAGATCC-3'; TNF-α forward, 5'-AGTCCGGGCAGGTCTACTTT-3' and reverse, 5'-GCACCTCAGGGAAGAGTCTG-3'; IL-1α forward, 5'-AAGTTTGTCATGAATGATTCCCTC-3' and reverse, 5'-GTCTCACTACCTGTGATGATGAGT-3'; IL-1β forward, 5'-ATAAGCCCACTCTACACCTCTGA-3' and reverse, 5'-ATTGGCCCTGAAAGGAGAGAGA-3'; GADPH forward, 5'-GGCATCCCATAGAGGCGAAGTC-3' and reverse, 5'-ACGCCGGACTTGATGAGGCGAT-3'. The average Cq values of the target gene and the reference gene were determined by RT-qPCR. qPCR was performed using SYBR® Green Master Mix (Takara Bio, Inc.) according to the manufacturer's protocol; amplification was performed under the recommended parameters: Initial denaturation at 95˚C for 5 min, followed by 40 cycles of 95˚C for 5 sec, 60˚C for 15 sec and 72˚C for 15 sec, and a final extension at 94˚C for 15 sec. The relative quantitative method was used to calculate the relative expression of the sample and the amount of the gene of the treatment group relative to the blank group was calculated using the $2^{-\Delta\Delta Cq}$ method (20).

Statistical analysis. Data analysis was performed using SPSS 17.0 statistical software (SPSS Inc.) and the results of all experimental data were expressed as mean ± standard deviation. If the experimental data was consistent with the homogeneity of variance, one-way ANOVA followed by a least significant difference test were performed to compare the differences between groups. If the experimental data variance was not with the homogeneity of variance, the Kruskal-Wallis non-parametric test and the LSD method were used to compare the differences between groups. P<0.05 was considered to indicate a statistically significant result.

Results

Pathological changes of myocardial tissue in four groups. After analyzing the myocardial pathological tissue sections, the results revealed that myocardial interstitial tissue in the LPS group were edematous, with degenerated cardiomyocytes (black arrow), loosely arranged cells (red arrow) and broken nuclei (blue arrow; Fig. 1A and B). Compared with the LPS group, cardiomyocytes in the miR-146a agonist group were neatly arranged and there was no swelling of the cells and no obvious fragmentation of the nucleus (Fig. 1B and C). However, these cardiomyocyte observations in the microRNA-146a inhibition group were aggravated compared with the LPS group (Fig. 1B-D).

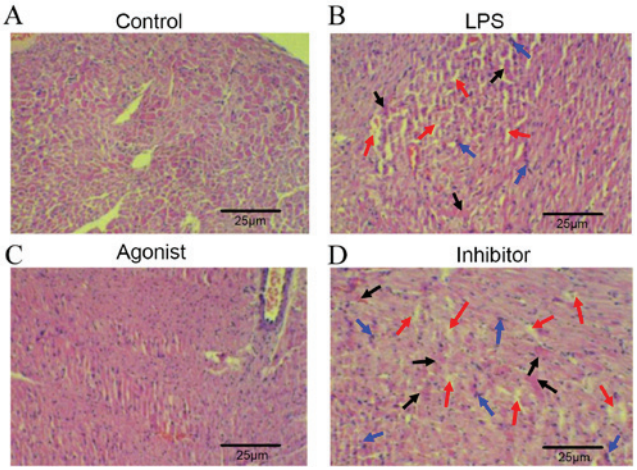

Figure 1. Morphological changes of myocardial tissue in four groups of rats. Morphological changes of rat myocardial tissues in the (A) control, (B) LPS, (C) miR-146a agonist and (D) miR-146a inhibitor group. miR, microRNA; LPS, lipopolysaccaride.

Detection of the cardiomyocyte apoptosis. Cells that exhibited brown karyon staining were deemed to be apoptotic cells. Among the four rat groups, the control group exhibited fewer apoptotic cells (Fig. 2A). The proportion of apoptosis in cardiomyocyte apoptosis in the LPS group was increased compared with the control group (Fig. 2A and B). However, in the miR-146a agonist group, the number of apoptotic cells were lower compared with the LPS group (Fig. 2B and C), while the opposite result was observed in the miR-146a inhibition group (Fig. 2B-D). Quantitative analysis revealed that the apoptotic index of the LPS group was increased significantly compared with control group. The apoptotic index was lower in miR-146a agonist group, but higher in the miR-146a inhibition group (Fig. 2E).

Expression of the serum myocardial injury markers. The results of the current study revealed that the serum levels of cTnI, BNP, CK-MB and Mb in the LPS group were significantly higher than those in the control (P<0.05; Fig. 3). In addition, compared with the LPS group, levels of the aforementioned markers were significantly decreased in the miR-146a agonist group, but increased in the miR-146a inhibitor group (P<0.05; Fig. 3).

Expression of myocardial tissue-associated proteins. The expression of NF-κB, TLR-4, TNF-α and ICAM-1 in rat myocardium tissue of the LPS group and the miR-146a inhibitor group was significantly increased compared with the control group and expression was significantly increased in the

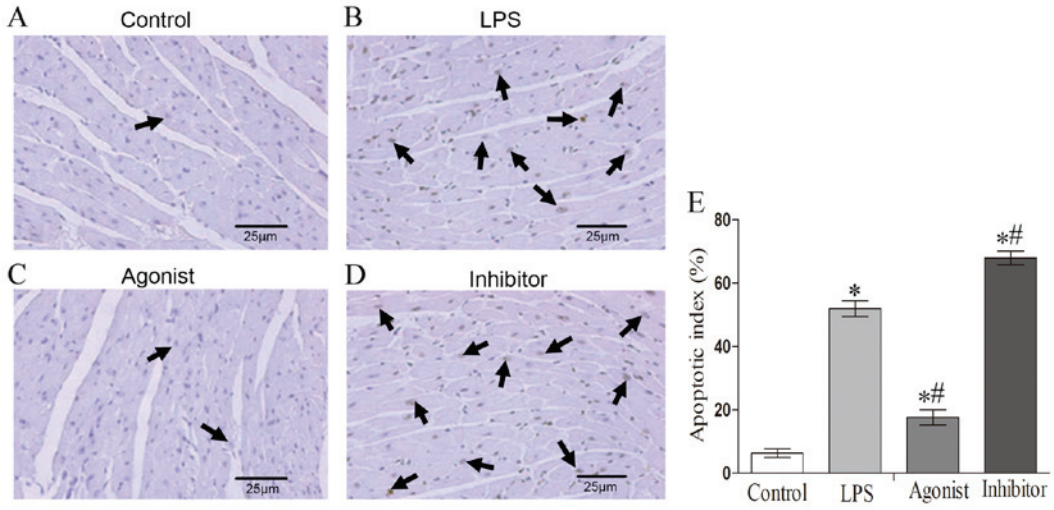

Figure 2. Detection of cardiomyocyte apoptosis in four groups of rats. Cardiomyocyte apoptosis of rat myocardial tissue in the (A) control, (B) model (C) agonist and (D) miR-146a inhibitor group. miR, microRNA; LPS, lipopolysaccaride. (E) Quantitative analysis of apoptotic index. *P<0.05 vs. the control group; #P<0.05 vs. the LPS group.

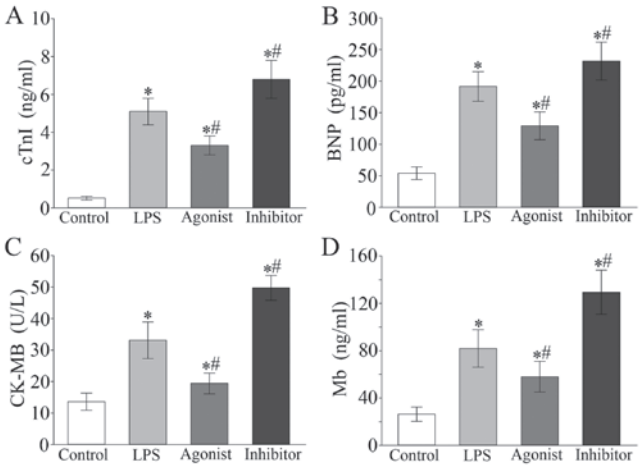

Figure 3. Levels of myocardial injury markers in four groups of rats. Comparison of serum (A) cTnI, (B) BNP, (C) CK-MB and (D) Mb levels in the indicated groups. *P<0.05 vs. the control group; #P<0.05 vs. the LPS group. cTnI, cardiac troponin I; BNP, B-type natriuretic peptide; CK-MB, creatine kinase myocardial bound; Mb, myoglobin; LPS, lipopolysaccaride.

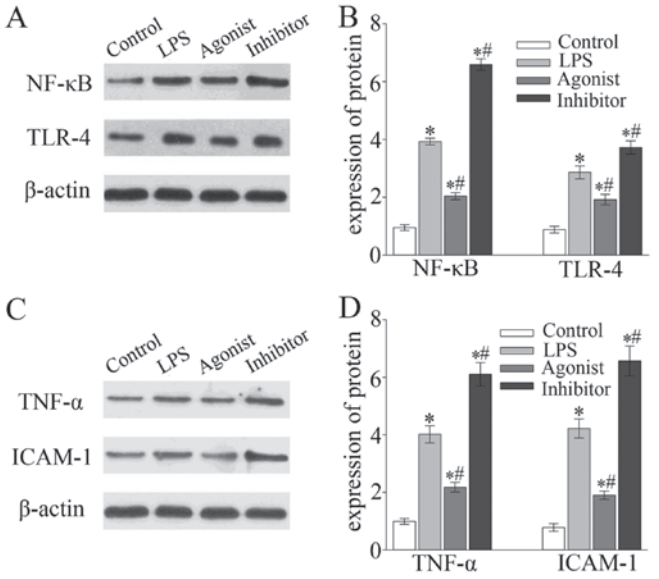

Figure 4. TLR-4/NF-κB-associated protein expression in the myocardial tissue of the four treatment groups. (A) Western blot analysis and (B) subsequent quantification of NF-κB and TLR-4 expression in the myocardial tissue of four groups of rats. (C) Westen blot analysis and (D) subsequent quantification of TNF-α and ICAM-1 expression in the myocardial tissue of rats. *P<0.05 vs. the control group; #P<0.05 vs. the model group. TLR-4, toll-like receptor-4; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule-1; LPS, lipopolysaccaride.

inhibitor group compared with the LPS group (P<0.05; Fig. 4). In the miR-146a agonist group, the expression of NF-κB, TLR-4, TNF-α and ICAM-1 was also significantly upregulated when compared with the control group, but the expression was significantly lower than that of the LPS group (P<0.05; Fig. 4).

Expression of myocardial tissue-associated mRNAs. The mRNA expression of miR-146a, TNF-α, IL-1α and IL-1β in the myocardial tissue of the model group were significantly higher compared with the control group (P<0.05; Fig. 5). In the miR-146a agonist group, miR-146a expression significantly increased, but the expression of TNF-α, IL-1α and IL-1β significantly decreased compared with the LPS group (P<0.05; Fig. 5). The expression of miR-146a in the miR-146a inhibition group was significantly downregulated while TNF-α, IL-1α and IL-1β expression was upregulated when compared with the LPS group (P<0.05; Fig. 5).

Discussion

The excessive stimulation of the inflammatory response is significant in the pathogenesis of SIC. Previous studies have revealed that TLR-4 mediates the activation of the Myd88-dependent NF-κB signaling pathway and releases downstream inflammatory factors including TNF-α, IL-6, IL-8, IL-1β, which serve a role in the subsequent sepsis cascade reaction (20-22). After invasion of the body, the pathogenic microorganism promotes the cascadal release of inflammatory factors via cell membrane and intracellular

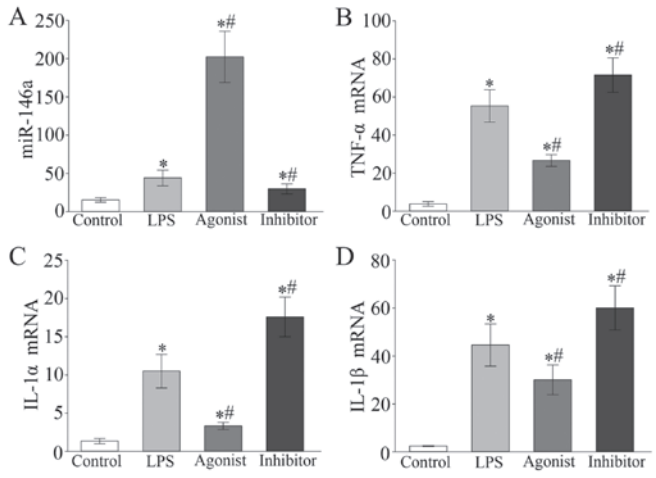

Figure 5. Expression of myocardial tissue-associated mRNA in four groups of rats. (A) miRNA-146a, (B) TNF-α, (C) IL-1α and (D) IL-1β mRNA expression was determined in the myocardial tissue of the four treatment groups. miR, microRNA; TNF-α, tumor necrosis factor-α; IL-1α, interleukin-1α; IL-1β, interleukin-1β; LPS, lipopolysaccaride. *P<0.05 vs. the control group; #P<0.05 vs. the LPS group.

signaling pathways, which are important in the pathogenesis of sepsis-induced myocardial damage (23,24). Therefore, the TLR-4/NF-κB signaling pathway is an important link in sepsis-induced myocardial damage. When sepsis occurs, LPS in the cell wall of Gram-negative bacteria can transduce extracellular signals by activating the intrinsic immune recognition of TLR-4, sequentially activating IRAK1 and TRAF6. NF-κB subsequently translocates rapidly from the cytoplasm to the nucleus, initiating the transcription of target genes and releasing downstream inflammatory factors including TNF-α and IL-6 (25-27). TNF-α and IL-6 inhibit the myocardium by inhibiting Ca^{2+} transport, regulating the nitric oxide pathway, degrading key contractile proteins, affecting mitochondrial function and activating intracellular signal transduction which leads to the myocardial dysfunction observed in sepsis (28,29).

miRNAs are a class of endogenous regulatory non-coding RNAs present in eukaryotes and are ~20-25 nucleotides in length (30). Mature miRNAs are produced from a long primordial transcript that can be cut by a series of nucleases (10). The target mRNA is recognized by base-pair pairing and is subsequently silenced or repressed depending on the degree of mIRNA complementation (31). miRNAs including miRNA-125b, miRNA-150, miRNA-155, miRNA-223, miRNA-132 and miRNA-146a, serve a significant role in the regulation of inflammatory responses by inhibiting the expression of inflammation-associated genes (32-37). A previous study has demonstrated that activated miR-146a is rapidly upregulated in human monocytes and certain targets signaling molecules including IRAK1 and TRAF6, indicating that miRNA-146a may be involved in the negative feedback regulation of the inflammatory signaling pathway (38). Consistent with this, miR-146a promotes toxin tolerance and cross-tolerance in the body by regulating monocyte production of TNF-α (38). miR-146a is the first regulator of the miRNA family that has been revealed to be associated with inflammation, which makes it an early diagnostic marker for sepsis with high specificity and sensitivity (38,39). In recent years, it has been demonstrated that miRNA-146a regulates the TLR-4/NF-κB signaling pathway and downstream inflammatory factors via negative feedback regulation (40).

The results of the current study demonstrate that in the LPS group, the expression of cTnI, BNP, CK-MB, Mb and NF-κB, the protein expression of TLR-4, TNF-α and ICAM-1 and the mRNA expression of miR-146a, TNF-α, IL-1α and IL-1β were significantly higher compared with the control group. Compared with the LPS group, miR-146a levels in the miR-146a agonist group significantly increased, while the expression of cTnI, BNP, CK-MB, Mb and NF-κB-associated proteins and the mRNA expression of TNF-α, IL-1α and IL-1β were downregulated. The results indicated that the further upregulation of miR-146a in heart tissue may produce a negative feedback regulatory response within the TLR-4/NF-κB signaling pathway, thereby inhibiting the inflammatory cascade and reducing the release of inflammatory factors which directly impair tissue damage and therefore improve SIC. The expression of miRNA-146a in the miRNA-146a inhibition group was significantly lower compared with the LPS group, while the expression of cTnI, BNP, CK-MB, Mb and NF-κB-associated proteins were significantly higher than those in the LPS group. These results indicated that in the case of miR-146a inhibition, the NF-κB signaling pathway was activated, the expression of downstream inflammatory factors was increased and myocardial damage was therefore more severe in rats. It was thus demonstrated that miR-146a may regulate the TLR-4/NF-κB signaling pathway and downstream inflammatory factors via negative feedback mechanisms. This is an important signal transduction mechanism and a key regulatory pathway in the pathogenesis of SIC.

In summary, the TLR-4/NF-κB, signaling pathway is an important signal transduction mechanism and a key regulatory pathway in SIC. miR-146a may regulate the TLR-4/NF-κB signaling pathway and downstream inflammatory factors via negative feedback mechanisms, so as to inhibit the inflammatory response and improve sepsis-induced cardiac dysfunction. However, blood and specimens were only collected 24 h after LPS injection in the current study. The role of miR-146a in sepsis-induced cardiac dysfunction over a longer time period is still unknown. Investigation over extended experimental time and further study into the mechanisms of miR-146a in sepsis-induced cardiac dysfunction is therefore required.

In conclusion, miR-146a may regulate the TLR-4/NF-κB signaling pathway via negative feedback mechanisms, thereby improving inflammation and cardiac dysfunction in sepsis-induced cardiomyopathy.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JX and WF designed the study, performed the experiments and wrote the manuscript. JX, LZ and XF established the animal models. XD and ZZ collected the data. JX and LZ analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Animal Ethics Committee of Kunming Medical University Animal Center (Kunming, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Tønnesen E and Larsen K: Severe sepsis and septic shock. Ugeskr Laeger 176: V03130200, 2014 (In Danish).
2. Sharawy N and Lehmann C: New directions for sepsis and septic shock research. J Surg Res 194: 520-527, 2015.
3. Cao Z and Robinson RA: The role of proteomics in understanding biological mechanisms of sepsis. Proteomics Clin Appl 8: 35-52, 2014.
4. Sato R and Nasu M: A review of sepsis-induced cardiomyopathy. J Intensive Care 3: 48, 2015.
5. Sato R, Kuriyama A, Takada T, Nasu M and Luthe SK: Prevalence and risk factors of sepsis-induced cardiomyopathy: A retrospective cohort study. Medicine (Baltimore) 95: e5031, 2016.
6. Hobai IA, Edgecomb J, LaBarge K and Colucci WS: Dysregulation of intracellular calcium transporters in animal models of sepsis-induced cardiomyopathy. Shock 43: 3-15, 2015.
7. Romero-Bermejo FJ, Ruiz-Bailen M, Gil-Cebrian J and Huertos-Ranchal MJ: Sepsis-induced cardiomyopathy. Curr Cardiol Rev 7: 163-183, 2011.
8. Lv X and Wang H: Pathophysiology of sepsis-induced myocardial dysfunction. Mil Med Res 3: 30, 2016.
9. Espín-Pérez A, Krauskopf J, Chadeau-Hyam M, van Veldhoven K, Chung F, Cullinan P, Piepers J, van Herwijnen M, Kubesch N, Carrasco-Turigas G, *et al*: Short-term transcriptome and microRNAs responses to exposure to different air pollutants in two population studies. Environ Pollut 242: 182-190, 2018.
10. Bernardo BC, Ooi JY, Lin RC and McMullen JR: miRNA therapeutics: A new class of drugs with potential therapeutic applications in the heart. Future Med Chem 7: 1771-1792, 2015.
11. Zhou F, Wang W, Xing Y, Wang T, Xu X and Wang J: NF-κB target microRNAs and their target genes in TNFα-stimulated HeLa cells. Biochim Biophys Acta 1839: 344-354, 2014.
12. Wu ZW, Liu YF, Wang S and Li B: miRNA-146a induces vascular smooth muscle cell apoptosis in a rat model of coronary heart disease via NF-κB pathway. Genet Mol Res 14: 18703-18712, 2015.
13. Wang X, Ha T, Liu L, Zou J, Zhang X, Kalbfleisch J, Gao X, Williams D and Li C: Increased expression of microRNA-164a decreases myocardial ischemia/reperfusion injury. Cardiovasc Res 97: 432-442, 2013.
14. Gao M, Wang X, Zhang X, Ha T, Ma H, Liu L, Kalbfleisch JH, Gao X, Kao RL, Williams DL and Li C: Attenuation of cardiac dysfunction in polymicrobial sepsis by microRNA-146a is mediated via targeting of IRAK1 and TRAF6 expression. J Immunol 195: 672-682, 2015.
15. Taganov KD, Boldin MP, Chang KJ and Baltimore D: NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA 103: 12481-12486, 2006.
16. Sun W, Wang Q, Guo Y, Zhao Y, Wang X, Zhang Z, Deng G and Guo M: Selenium suppresses inflammation by inducing microRNA-146a in Staphylococcus aureus-infected mouse mastitis model. Oncotarget 8: 110949-110964, 2017.
17. Wu ZW, Liu YF, Wang S and Li B: Corrigendum miRNA-146a induces vascular smooth muscle cell apoptosis in a rat model of coronary heart disease via NF-κB pathway-Genet. Mol. Res. 14 (4): 18703-18712. Genet Mol Res 15, 2016.
18. Li J, Xie C, Zhuang J, Li H, Yao Y, Shao C and Wang H: Resveratrol attenuates inflammation in the rat heart subjected to ischemia-reperfusion: Role of the TLR4/NF-κB signaling pathway. Mol Med Rep 11: 1120-1126, 2015.
19. Karra R, Knecht AK, Kikuchi K and Poss KD: Myocardial NF-κB activation is essential for zebrafish heart regeneration. Proc Natl Acad Sci USA 112: 13255-13260, 2015.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. Yang SF, Zhuang TF, Si YM, Qi KY and Zhao J: Coriolus versicolor mushroom polysaccharides exert immunoregulatory effects on mouse B cells via membrane Ig and TLR-4 to activate the MAPK and NF-κB signaling pathways. Mol Immunol 64: 144-151, 2015.
22. Kim E, Kim HC, Lee S, Ryu HG, Park YH, Kim JH, Lim YJ and Park HP: Dexmedetomidine confers neuroprotection against transient global cerebral ischemia/reperfusion injury in rats by inhibiting inflammation through inactivation of the TLR-4/NF-κB pathway. Neurosci Lett 649: 20-27, 2017.
23. Bi W, Zhu L, Jing X, Zeng Z, Liang Y, Xu A, Liu J, Xiao S, Yang L, Shi Q, *et al*: Rifampicin improves neuronal apoptosis in LPS-stimulated co-cultured BV2 cells through inhibition of the TLR-4 pathway. Mol Med Rep 10: 1793-1799, 2014.
24. Cimolai MC, Alvarez S, Bode C and Bugger H: Mitochondrial mechanisms in septic cardiomyopathy. Int J Mol Sci 16: 17763-17778, 2015.
25. Zhou Q, Pan X, Wang L, Wang X and Xiong D: The protective role of neuregulin-1: A potential therapy for sepsis-induced cardiomyopathy. Eur J Pharmacol 788: 234-240, 2016.
26. Park H, Huang X, Lu C, Cairo MS and Zhou X: MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. J Biol Chem 290: 2831-2841, 2015.
27. Wei J, Wang J, Zhou Y, Yan S, Li K and Lin H: MicroRNA-146a contributes to SCI recovery via regulating TRAF6 and IRAK1 expression. Biomed Res Int 2016: 4013487, 2016.
28. Jiang W, Kong L, Ni Q, Lu Y, Ding W, Liu G, Pu L, Tang W and Kong L: miR-146a ameliorates liver ischemia/reperfusion injury by suppressing IRAK1 and TRAF6. PLoS One 9: e101530, 2014.
29. Hiram R, Rizcallah E, Sirois C, Sirois M, Morin C, Fortin S and Rousseau E: Resolvin D1 reverses reactivity and Ca2+ sensitivity induced by ET-1, TNF-α, and IL-6 in the human pulmonary artery. Am J Physiol Heart Circ Physiol 307: H1547-H1558, 2014.
30. Hiram R, Rizcallah E, Marouan S, Sirois C, Sirois M, Morin C, Fortin S and Rousseau E: Resolvin E1 normalizes contractility, Ca2+ sensitivity and smooth muscle cell migration rate in TNF-α- and IL-6-pretreated human pulmonary arteries. Am J Physiol Lung Cell Mol Physiol 309: L776-L788, 2015.
31. Dumache R, Rogobete AF, Bedreag OH, Sarandan M, Cradigati AC, Papurica M, Dumbuleu CM, Nartita R and Sandesc D: Use of miRNAs as biomarkers in sepsis. Anal Cell Pathol (Amst) 2015: 186716, 2015.
32. Landskroner-Eiger S, Moneke I and Sessa WC: miRNAs as modulators of angiogenesis. Cold Spring Harb Perspect Med 3: a006643, 2013.
33. Zhou S, Zhang P, Liang P and Huang X: The expression of miR-125b regulates angiogenesis during the recovery of heat-denatured HUVECs. Burns 41: 803-811, 2015.
34. Feng J, Yang Y, Zhang P, Wang F, Ma Y, Qin H and Wang Y: miR-150 functions as a tumour suppressor in human colorectal cancer by targeting c-Myb. J Cell Mol Med 18: 2125-2134, 2014.
35. Slezak-Prochazka I, Kluiver J, de Jong D, Smigielska-Czepiel K, Kortman G, Winkle M, Rutgers B, Koerts J, Visser L, Diepstra A, *et al*: Inhibition of the miR-155 target NIAM phenocopies the growth promoting effect of miR-155 in B-cell lymphoma. Oncotarget 7: 2391-2400, 2016.
36. Meloche J, Le Guen M, Potus F, Vinck J, Ranchoux B, Johnson I, Antigny F, Tremblay E, Breuils-Bonnet S, Perros F, *et al*: miR-223 reverses experimental pulmonary arterial hypertension. Am J Physiol Cell Physiol 309: C363-C372, 2015.

37. Hadar A, Milanesi E, Walczak M, Puzianowska-Kuźnicka M, Kuźnicki J, Squassina A, Niola P, Chillotti C, Attems J, Gozes I and Gurwitz D: SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's Disease. Sci Rep 8: 8465, 2018.
38. Bae SC and Lee YH: MiR-146a levels in rheumatoid arthritis and their correlation with disease activity: A meta-analysis. Int J Rheum Dis 21: 1335-1342, 2018.
39. Hu Q, Song J, Ding B, Cui Y, Liang J and Han S: miR-146a promotes cervical cancer cell viability via targeting IRAK1 and TRAF6. Oncol Rep 39: 3015-3024, 2018.
40. Zhang QB, Qing YF, Yin CC, Zhou L, Liu XS, Mi QS and Zhou JG: Mice with miR-146a deficiency develop severe gouty arthritis via dysregulation of TRAF 6, IRAK 1 and NALP3 inflammasome. Arthritis Res Ther 20: 45, 2018.