Dexmedetomidine-mediated regulation of miR-17-3p in H9C2 cells after hypoxia/reoxygenation injury

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Abstract. Patients with heart disease often suffer from ischemia, which can be treated by reperfusion. However, this treatment can lead to the development of ischemia/reperfusion (I/R) injury, an inflammatory condition that can cause further heart damage. Dexmedetomidine (Dex), an α_2-adrenoceptor agonist, and the microRNA (miR)-17-3p, have both been suggested to alleviate I/R injury and cardiac tissue inflammation. The aim of the present study was to investigate whether Dex and miR-17-3p could act together to prevent I/R injury. H9C2 cells, a myoblast cell line used as a model of rat cardiomyocytes, were cultured in a hypoxic environment for 3 h, and then reoxygenated for 3 h. This hypoxia/reoxygenation (H/R) was used to model I/R. Cell Counting kit-8 was used to determine cell viability and an annexin V-FITC/propidium iodide apoptosis kit used to analyze cell apoptosis. A dual luciferase reporter assay was used to determine the interaction between miR-17-3p and toll-like receptor 4 (TLR4). Western blotting and reverse transcription-quantitative PCR were used to determine protein levels and mRNA expression of TLR4 and galectin-3. A concentration of 0.1-10 μmol/l Dex attenuated H/R injury, which was accompanied by increased miR-17-3p levels. Additionally, the inhibition of miR-17-3p exacerbated H/R injury and reduced the effect of Dex on H/R injury. H/R led to an increased galectin-3 level compared with that in control cells, and Dex or miR-17-3p inhibitor did not markedly affect the level of galectin-3, indicating that Dex alleviated the effects of I/R injury through other pathways. Inhibition of miR-17-3p in Dex-induced H9C2 cells during H/R increased the expression of inflammatory mediators including tumor necrosis factor-α, interleukin (IL)-6, IL-1β and phosphorylated NFκB subunit p65, while Dex reduced the H/R-induced expression of these inflammatory mediators. Inhibition of TLR4 also attenuated H/R injury. In summary, the findings of the present study indicated that Dex reduced H/R injury in H9C2 cell via the modulation of inflammatory signaling pathways, and these inflammatory factors could be regulated by miR-17-3p.

Introduction

Coronary heart diseases, including myocardial infarction and ischemic heart disease, are a leading cause of mortality and morbidity worldwide, causing at least 370,000 deaths in the USA each year (1). Patients with coronary heart disease, which is characterized by impaired blood supply to the heart, present with myocardial ischemic injury in the clinic. Coronary heart perfusion or reperfusion is a commonly used and effective therapy in protection of the heart against ischemic injury. However, perfusion can cause additional damage to the myocardium, termed ischemia/reperfusion (I/R) injury. Though reperfusion attenuates myocardial ischemia, it can increase the incidence of arrhythmia and myocardial stunning, and increase infarct size (2-4). It would therefore be beneficial to develop a drug to prevent I/R injury.

I/R injury can be modeled *in vitro* using a hypoxia/reoxygenation (H/R) technique. In this model cells are placed in an hypoxic environment and then returned to a normoxic environment. This can be a useful tool to investigate cardioprotective strategies against myocardial injury (5-7).

Dexmedetomidine (Dex), (+)-4-(S)-[1-(2,3-dimethylphenyl) ethyl]-1H-imidazole, is a selective and potent α2-adrenoceptor agonist, that is prescribed as an anti-anxiety medication, a sedative and an analgesic (8,9). As an α2-adrenoceptor agonist, Dex has potential applications as a prophylactic in neuroprotection, which has attracted researchers to study the role of Dex after I/R injury to the brain and other organs (10). Studies in animal models have reported that Dex inhibits hepatic and cerebral I/R by suppressing the inflammatory response (11,12).

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MicroRNAs (miRNAs/miRs) are RNAs with a length of 18-24 bp that can inhibit protein translation by binding to the 3' untranslated region (UTR) of target mRNAs (13). The miR-17-92 cluster, which is one of the most studied miRNA clusters, has six members including miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a and miR-92a (14). The miR-17-92 cluster has been suggested to promote cardiomyocyte proliferation in post-natal and adult hearts (14). Numerous studies have also indicated that miR-17-92 cluster expression is related to the progression of cancer and physiological disorders, such as genetic bone, lung and septal defects (15-18). Additional research suggests that miR-17-3p promoted keratinocyte cells proliferation and metastasis via activating Notch1/NF-κB signal pathways in cutaneous wound healing (19).

In the present study it was hypothesized that regulation of miR-17-3p, a component of the miR-17-92 cluster, may be the method through which Dex reduces I/R and inflammation caused by I/R. The aim of the present study was to investigate whether Dex reduced I/R injury to the myocardium using an H/R model in H9C2 cells and to study the relationship between Dex and miR-17-3p.

Materials and methods

H9C2 cell culture and H/R model. H9C2 cells are myoblasts derived from the rat myocardium, used in the present study as a model of cardiomyocytes, and were acquired from American Type Culture Collection. Gibco, a brand of Thermo Fisher Scientific, Inc., supplied all cell culture reagents. Under normoxic conditions H9C2 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 1% 10,000 U/ml penicillin and 10,000 μg/ml streptomycin. Under hypoxic conditions the cells were cultured in PBS in a hypoxic chamber (50x50x60 cm) filled with 5% CO_2 and 95% N_2 at 37˚C for different times (1, 2, 3 and 4 h). The hypoxic chamber was placed in an aseptic incubator chamber at 37˚C. Gas filling was performed according to the method of Li *et al* (20). After exposure to hypoxia, the cells were reoxygenated with normal culture medium in 5% CO_2 and 95% air at 37˚C for 3 h.

Transfection and treatment. A concentration of 50 nmol/l of miR-17-3p mimic, miR-17-3p inhibitor and a miR-negative control (NC) were obtained from Biomics Biotechnologies (Nantong) Co., Ltd. and were dissolved in FBS-free DMEM medium, containing Lipofectamine™ (Invitrogen; Thermo Fisher Scientific, Inc.), following the manufacturer's protocol. Sequences were 3'-GAUGUUCACGGAAGUGACGUCA-5' (mimics), 3'-CUACAAGUGCCUUCACUGCAGU-5' (inhibitor) and 5'-UUUGUACUACACAAAAGUACUG-3' (NC). A Lipofectamine-medium solution was added to the cells (2x10^5 cells/well) in 24-well plate, and cells were cultured for 2-3 h. The medium was then replaced with normal culture medium and the cells were cultured for a further 24 h. TAK-242 (TAK), an inhibitor of TLR-4 was purchased from MedChemExpress. Dex was purchased from Selleck Chemicals.

Cells were treated with different concentrations of Dex (0, 0.1, 1, 5 and 10 μmol/l) to assess the impact of Dex on cell viability and miR-17-3p levels in H9C2 cells. Cells treated with different concentrations of Dex (0, 0.1, 1, 5 and 10 μmol/l) were applied for detecting function of Dex on cell viability, apoptosis, miR-17-3p, TLR4 and galectin-3 levels in H9C2 cells with H/R (3 h hypoxia/3 h reoxygenation). H9C2 cells treated with mimics, inhibitor, NC or TAK (10 μg/ml) were named the mimics, inhibitor, NC or TAK groups respectively. Untreated H9C2 cells were used as a control. Cells in the inhibitor, NC or TAK groups going through 3 h hypoxia/3 h reoxygenation were named the H/R + inhibitor, H/R + NC or H/R + TAK groups. Cells in the inhibitor, NC or TAK groups, treated with 5 μmol/l Dex, going through 3 h hypoxia/3 h reoxygenation were named the Dex + H/R + Inhibitor, Dex + H/R + NC, or Dex + H/R + TAK groups. Cells that had not received any treatment were used as controls.

Cell viability. Cell Counting kit-8 (CCK-8; Sigma-Aldrich; Merck KGaA) was used to determine cell viability, according to the manufacturer's protocol. The cells (5x10^3 cells/well) were seeded in 96-well plates for 24 h. After the cells had been treated with the appropriate reagents or H/R, the CCK-8 reagent was diluted with FBS-free DMEM at a ratio of 1:9. A total of 200 μl of CCK-8 solution was applied to each well. The plate was put into an incubator (Thermo Fisher Scientific, Inc.) for 1 h. The color change was detected by a microplate reader (Thermo Fisher Scientific, Inc.) at a wavelength of 450 nm.

Apoptosis. An Annexin V-FITC/propidium iodide apoptosis kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used for cell apoptosis analysis. Cells (5.0x10^5/ml) were seeded on a 75 mm plate for 24 h. After cells were treated with the reagents or H/R, medium was collected in a centrifuge tube and the cells were digested with trypsin (Gibco; Thermo Fisher Scientific, Inc.). Medium was mixed with the cell suspension, and the suspension was centrifuged using a Cence centrifuge (Changsha Xiangyi Centrifuge Instrument Co., Ltd.) at 1,200 x g for 5 min at ˚C. The cell pellet was then resuspended in PBS. Apoptosis kit reagents were added to the cells and the fluorescence was detected using a BD FACSCalibur flow cytometer (BD Biosciences) and BD CellQuest™ Pro Software version 5.1 (BD Biosciences). Procedures were conducted following the manufacturer's instructions.

Bioinformatic analysis and dual luciferase assay. The Targetscan web site (http://www.targetscan.org/vert_72/) was used to predict the binding site between miR-17-3p and TLR4. The TLR4 3' UTR or mutant (MUT) TLR4 3' UTR [Sangon Biotech (Shanghai) Co., Ltd] was cloned into a psiCHECK-2 vector (Promega Corporation). Cells were transfected with miR-17-3p mimic, miR-NC or cloned psi-CHECK-2 vector, using Lipofectamine™ (Invitrogen; Thermo Fisher Scientific, Inc.), following the manufacturer's protocol. After 24 h, the fluorescence was tested with a microplate reader (Thermo Fisher Scientific, Inc.) using a Pierce™ Gaussia-Firefly luciferase dual assay kit (Thermo Fisher Scientific, Inc.), according to manufacturer's protocol.

Western blotting. After the cells were treated with the reagents or H/R, the protein was extracted using RIPA lysis buffer (Thermo Fisher Scientific, Inc.) following the

Table I. Primer sequences.

Gene	Primer sequence	
	Forward	Reverse
miR-17-3p	5'-TGCGTTGACGTCACTCCCG-3'	5'-GTGCAGGGTCCGAGGT-3'
Galectin-3	5'-CCGGGATCCATGGCAGACGGCTTC TCACTTAA-3'	5'-CCGCCCATGGCTATCATTAGATCATG GCGTGGGAAGCG-3'
TNF-α	5'-TGAGCACAGAAAGCATGATC-3'	5'-CATCTGCTGGTACCACCAGTT-3'
IL-6	5'-GTGACAACCACGGCCTTCCCTA-3'	5'-GGTAGCTATGGTACTCCA-3'
IL-1β	5'-GACCTGTTCTTTGAGGCTGAC-3'	5'-TCCATCTTCTTCTTTGGGTATTGTT-3'
TLR4	5'-ACCTGTCCCTGAACCCTATG-3'	5'-CTTCTAAACCAGCCAGACC-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-ACGCTTCACGAATTTGCGT-3'
β-actin	5'-GTGGACATCCGCAAAGAC-3'	5'-GAAAGGGTGTAACGCAACT-3'

TNF-α, tumor necrosis factor-α; IL, interleukin; TLR, toll-like receptor.

manufacturer's instructions. The concentration of protein was determined by BCA protein assay kit (Thermo Fisher Scientific, Inc.) using BSA as a standard. 20 μg protein and protein ladder (Thermo-Fisher Scientific, Inc.) was separated by 12% SDS-PAGE and transferred onto PVDF membranes (Sigma-Aldrich; Merck KGaA). The membranes were blocked with 5% BSA (Beijing Solarbio Science & Technology Co., Ltd.) in TBS containing Tween-20% (TBST) at room temperature for 2 h. The primary antibodies, TLR4 (cat. no. ab22048; Abcam), galectin-3 (cat. no. ab2785; Abcam), phosphorylated (p)-p65 (cat. no. 13346; Cell Signaling Technology, Inc.), p65 (cat. no. 6956, Cell Signaling Technology, Inc.) and β-actin (1:5,000, cat. no. 3700; Cell Signaling Technology, Inc.) were incubated with the protein membranes overnight at 4°C. The primary antibodies were diluted with TBST 1:1,000. The protein membranes were then incubated for 2 h with secondary antibody (horseradish peroxidase-conjugated; cat. no. 7076, Cell Signaling Technology, Inc.) at 37°C. Enhanced chemi-luminescence (ECL) detection reagents (Amersham) were used and detected with an Image-Pro Plus version 6.0 (Media Cybernetics, Inc.) system.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the H9C2 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was reverse transcribed using TaqMan MicroRNA reverse transcription kit (Fermentas; Thermo Fisher Scientific, Inc.) at 42°C for 50 min, according to the manufacturer's instructions. SYBR-Green PCR Master mix (Roche Diagnostics) and the TaqMan miRNA PCR kit (Applied Biosystems, Thermo Fisher Scientific, Inc.) were used to perform qPCR assays, using the Opticon RT-PCR detection system (ABI 7500; Thermo Fisher Scientific, Inc.), The amplification primers were designed by Sigma-Aldrich; Merck KGaA. The primers in Table I were used to perform RT-qPCR under the conditions of 95°C for 10 min for pre-denaturation; 30 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 60 sec. U6 and β-actin were used for normalization. The $2^{-\Delta\Delta Cq}$ method was used to determine the relative mRNA level (21).

Statistical analysis. Data were analyzed using Graph Pad Prism version 6 (Graph Pad Software, Inc.). Data are presented as the mean ± SD. One-way ANOVA with Tukey's post-hoc test was used for multiple comparisons. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of hypoxia on H9C2 cell miR-17-3p levels. miR-17-3p mRNA expression increased significantly following 1-4 h of hypoxia when compared with that of control cells without hypoxia. Though miR-17-3p levels appeared to be increasing over the first 3 h of hypoxia duration, miR-17-3p expression was lower after 4 h than at other time-points, though it remained significantly higher than in control cells (Fig. 1A) This result suggested that prolonged H9C2 hypoxia could reduce miR-17-3p expression. After cells were removed from a hypoxic environment they were exposed to reoxygenation for 3 h and miR-17-3p mRNA expression returned to levels comparable with control cells (Fig. 1B).

Effects of Dex on H9C2 cell viability, cell apoptosis and miR-17-3p levels in H/R. Treatment with 0.1-10 μmol/l Dex had no obvious effect on the viability of H9C2 cells (Fig. 1C). A dose of 0.1-10 μmol/l Dex increased the expression of miR-17-3p mRNA compared with that of the control group (0 μmol/l Dex) in a normoxic culture environment (Fig. 1D). H/R decreased cell viability, but Dex (1, 5 and 10 μmol/l) treatment improved H/R exposed-cell viability significantly compared to H/R only control cells (Fig. 1E). Dex (1, 5 and 10 μmol/l) also promoted miR-17-3p mRNA expression significantly in H/R conditions (Fig. 1F). The H/R model promoted H9C2 cell apoptosis, however, Dex (5 and 10 μmol/l) decreased the rate of H9C2 cell apoptosis significantly after H/R (Fig. 1G and H). The results presented in Fig. 1E-H led to the hypothesis that the protective function of Dex on H9C2 cells in H/R was due to regulation of miR-17-3p levels.

Effects of Dex on H9C2 cell expression of TLR4 and galectin-3 in H/R. H/R increased TLR4 and galectin-3 expression at the mRNA
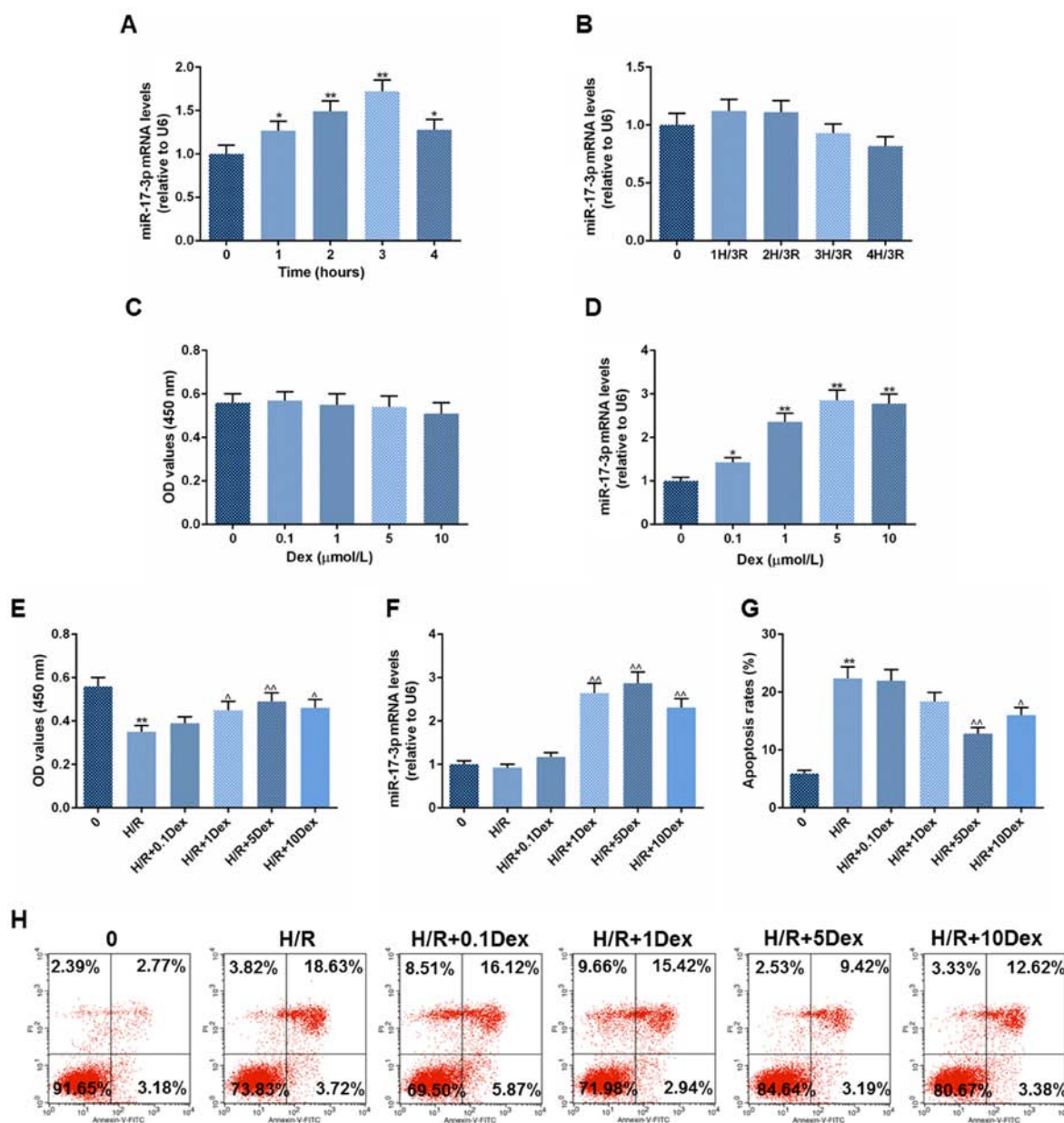
Figure 1. Effects of Dex on H9C2 cell viability and miR-17-3p levels after H/R. H9C2 cells were incubated under hypoxic conditions for 1-4 h and miR-17-3p mRNA levels relative to U6 were determined (A) immediately or (B) after a further 3 h of reoxygenation. H9C2 cells were additionally treated with a range of concentrations of Dex for 24 h under normoxic conditions before (C) cell viability and (D) miR-17-3p expression relative to U6 was determined. An additional group of H9C2 cells was treated with a range of concentrations of Dex for 24 h under normoxic conditions before they were placed in hypoxic conditions for 3 h and then reoxygenated for 3 h. (E) Cell viability, (F) miR-17-3p mRNA level relative to U6, and (G) cell apoptosis (early and late stage) level using (H) flow cytometry were then determined. Data are presented as the mean ± SD. *P<0.05 and **P<0.01 vs. control group, ^P<0.05 and ^^P<0.01 vs. H/R group. Dex, dexmedetomidine; H/R, hypoxia/reoxygenation; miR, microRNA, PI, propidium iodide. 0, the control, is cells with no hypoxia and no reperfusion. 0.1, 1, 5 and 10 Dex are cells treated with 0.1 μmol/l Dex.

and protein level. Treatment with Dex (1, 5, or 10 μmol/l) reduced the expression of TLR4 and galectin-3 during H/R (Fig. 2).

Effects of miR-17-3p inhibitor or mimic on H9C2 cell viability and the expression of TLR4 and galectin-3. miR-17-3p mimic increased miR-17-3p expression (Fig. 3A) and cell viability (Fig. 3B) and lowered TLR4 protein and mRNA expression (Fig. 3C-E), whereas, miR-17-3p inhibitor inhibited miR-17-3p expression and cell viability but enhanced TLR4 expression (Fig. 3). However, both miR-17-3p mimic and miR-17-3p inhibitor did not affect galectin-3 expression. It was hypothesized

that there was an association between miR-17-3p and TLR4 expression.

Effects of Dex and miR-17-3p inhibitor alone or in combination on H9C2 cell viability, apoptosis and miR-17-3p expression in H/R. miR-17-3p inhibitor reduced cell viability in the H/R + inhibitor group compared with the H/R + NC group, and reduced the level of cell viability rescue induced by Dex (5 μmol/l) under H/R conditions in the Dex + H/R + inhibitor group compared with the Dex + H/R + NC group (Fig. 4A). miR-17-3p inhibitor inhibited miR-17-3p mRNA
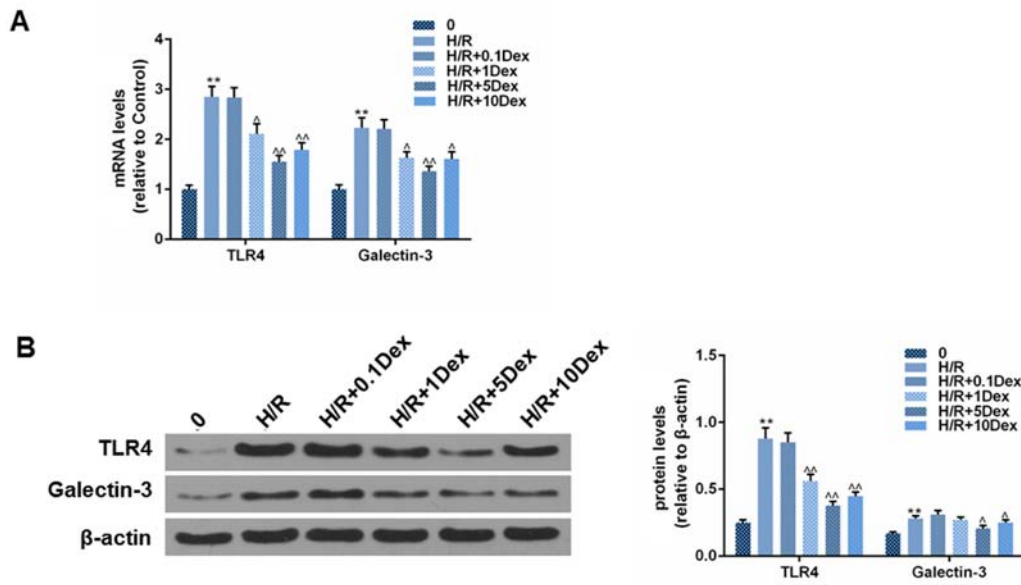
Figure 2. Effects of Dex on TLR4 and galectin-3 expression in H9C2 cells during H/R. H9C2 cells were treated with a range of concentrations of Dex for 24 h, and then the cells were incubated in hypoxic conditions for 3 h before 3 h of reoxygenation. (A) mRNA levels of TLR4 and galectin-3. (B) Protein levels of TLR4 and galectin-3. Data are presented as the mean ± SD. **$P<0.01$ vs. control group, ^$P<0.05$ and ^^$P<0.01$ vs. H/R group. Dex, dexmedetomidine; H/R, hypoxia/reoxygenation; TLR4, toll-like receptor 4.
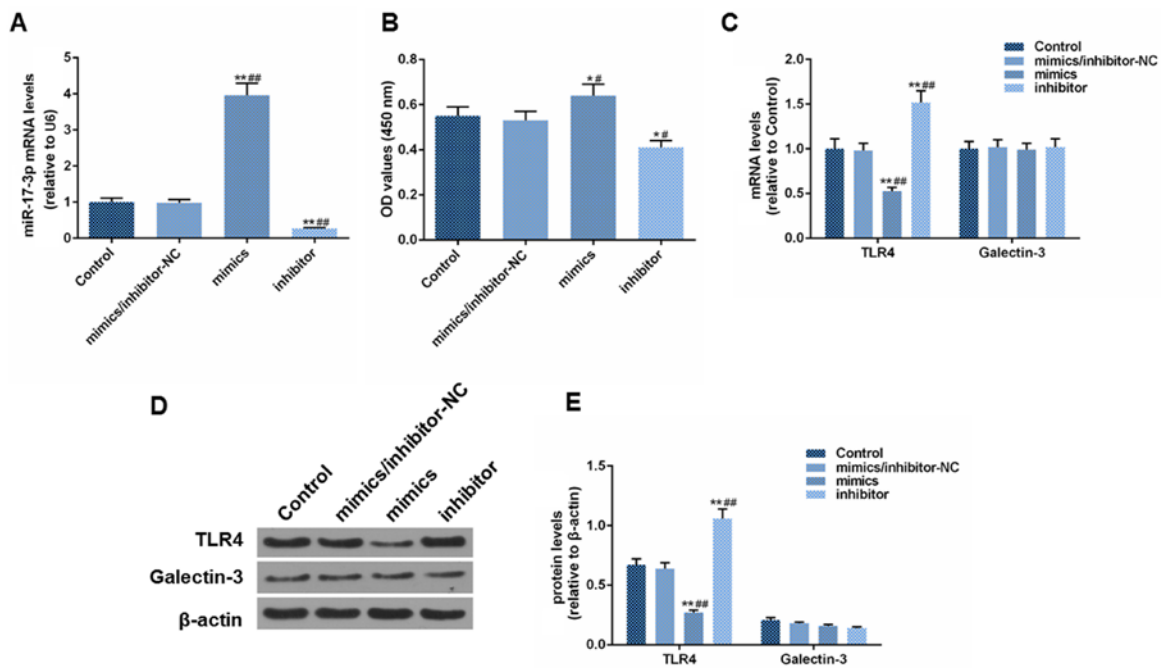

Figure 3. Effects of miR-17-3p mimic and miR-17-3p inhibitor on H9C2 cell viability and expression of TLR4 and galectin-3. H9C2 cells were transfected with miR-17-3p mimic and miR-17-3p inhibitor and the cells were cultured for 24 h. (A) miR-17-3p mRNA level. (B) Cell viability. (C) mRNA levels of TLR4 and galectin-3. (D) Western blotting of TLR-4 and galectin 3 was used to (E) quantify the protein levels. Data are presented as the mean ± SD. *$P<0.05$ and **$P<0.01$ vs. control group, #$P<0.05$ and ##$P<0.01$ vs. NC group. miR, microRNA; NC, negative control; TLR4, toll-like receptor 4.

expression, promoted cell apoptosis in H/R and reduced the protective effect of Dex in H/R (Fig. 4B-D).

Effects of Dex and miR-17-3p inhibitor alone or in combination on H9C2 cell mRNA levels of TNF-α, IL-6, IL-1β, TLR4, galectin-3, p-p65 and p65 in H/R. Treatment with miR-17-3p

inhibitor and exposure to H/R led to increased mRNA levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, TLR4 and increased levels of NF-κB phosphorylated (p)-p65/p65 protein in H9C2 cells (Fig. 4E-G). In addition, miR-17-3p inhibitor treatment increased mRNA levels of TNF-α, IL-6, IL-1β, TLR4 and increased levels of p-p65/p65 protein during H/R
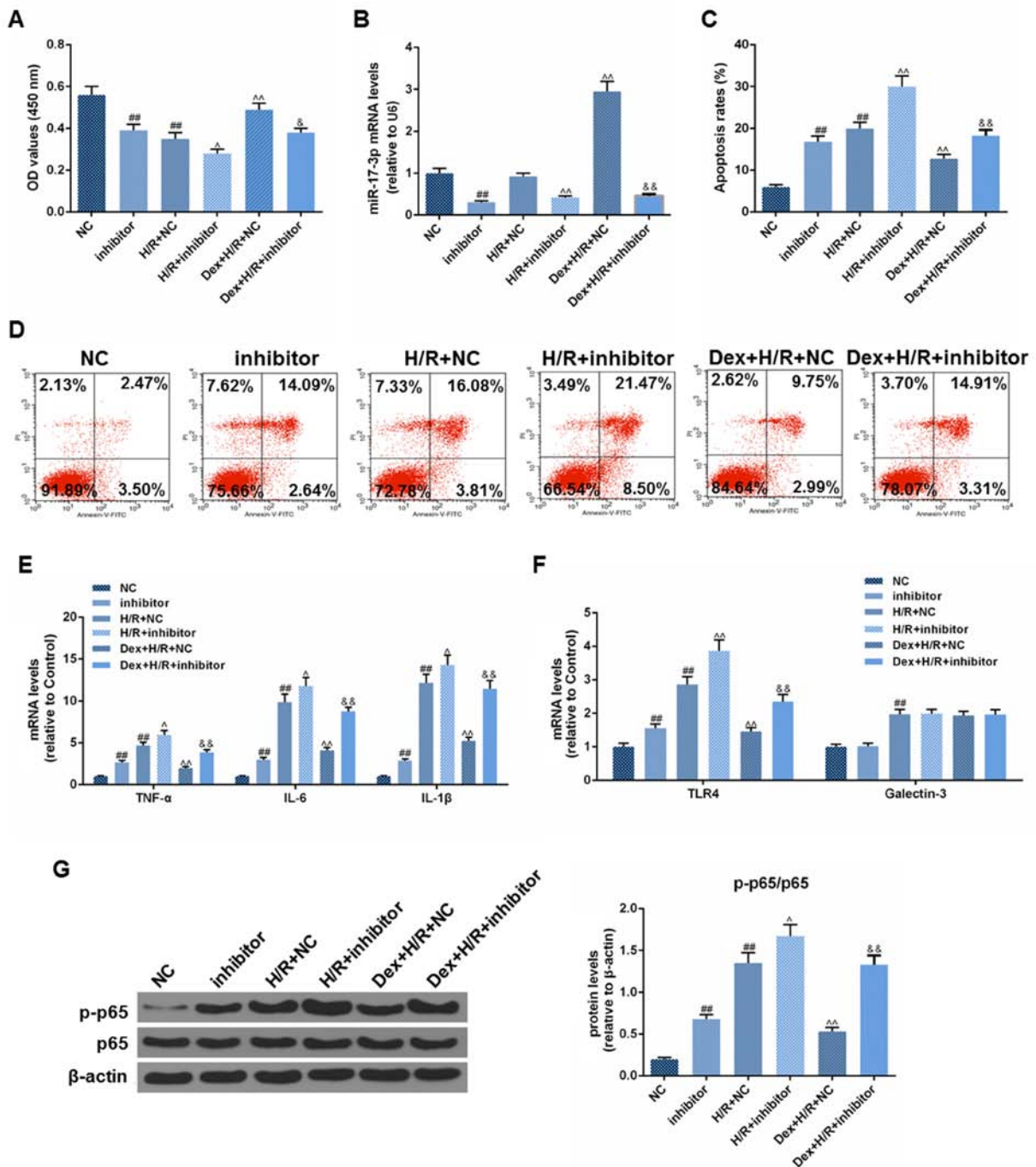
Figure 4. Effects of Dex and miR-17-3p inhibitor alone or in combination on H9C2 cell viability, apoptosis and the levels of miR-17-3p, TNF-α, IL-6, IL-1β, p65, p-p65, galectin-3 and TLR4 during H/R. Dex and miR-17-3p inhibitor alone or in combination were used to treat H9C2 cells for 24 h. Cells were then placed in a hypoxic chamber for 3 h and reoxygenated for 3 h. Measurements were taken of (A) cell viability and (B) mRNA levels of miR-17-3p. (C) Cell apoptosis was determined and (D) representative plots are presented. mRNA levels of (E) TNF-α, IL-6, IL-1β and (F) TLR4 and galectin-3 were determined. (G) The protein levels of p-p65 and p-65 were determined using western blotting. Data are presented as the mean ± SD. [##]P<0.01 vs. NC group, [^]P<0.05 and [^^]P<0.01 vs. H/R + NC group, [&]P<0.05 and [&&]P<0.01 vs. Dex + H/R + NC group. Dex, dexmedetomidine; IL, interleukin; miR, microRNA; NC, negative control; PI, propidium iodide; p, phosphorylated; TNF-α, tumor necrosis factor-α.

and in H9C2 cells treated with Dex during H/R. However, Dex attenuated these H/R-induced changes in TNF-α, IL-6, IL-1β, TLR4 and p-p65/p65 levels (Fig. 4E-G). miR-17-3p inhibitor had no obvious effect on galectin-3 expression (Fig. 4F).

Effects of TAK-242 and Dex alone or in combination on H9C2 cell viability and TLR4 expression. The Targetscan web site

(TargetScan: http://www.targetscan.org/vert_72/) was used to predict the binding site between miR-17-3p and TLR4 (Fig. 5A). miR-17-3p reduced luciferase activity in double fluorescein carrier with TLR4 (Fig. 5B). TAK-242, a TLR4 inhibitor, improved cell viability in H9C2 cells during H/R or in H9C2 cells treated with miR-17-3p inhibitor during H/R (Fig. 5C-F). However, TAK-242 had no significant effect on Dex efficacy in H9C2.
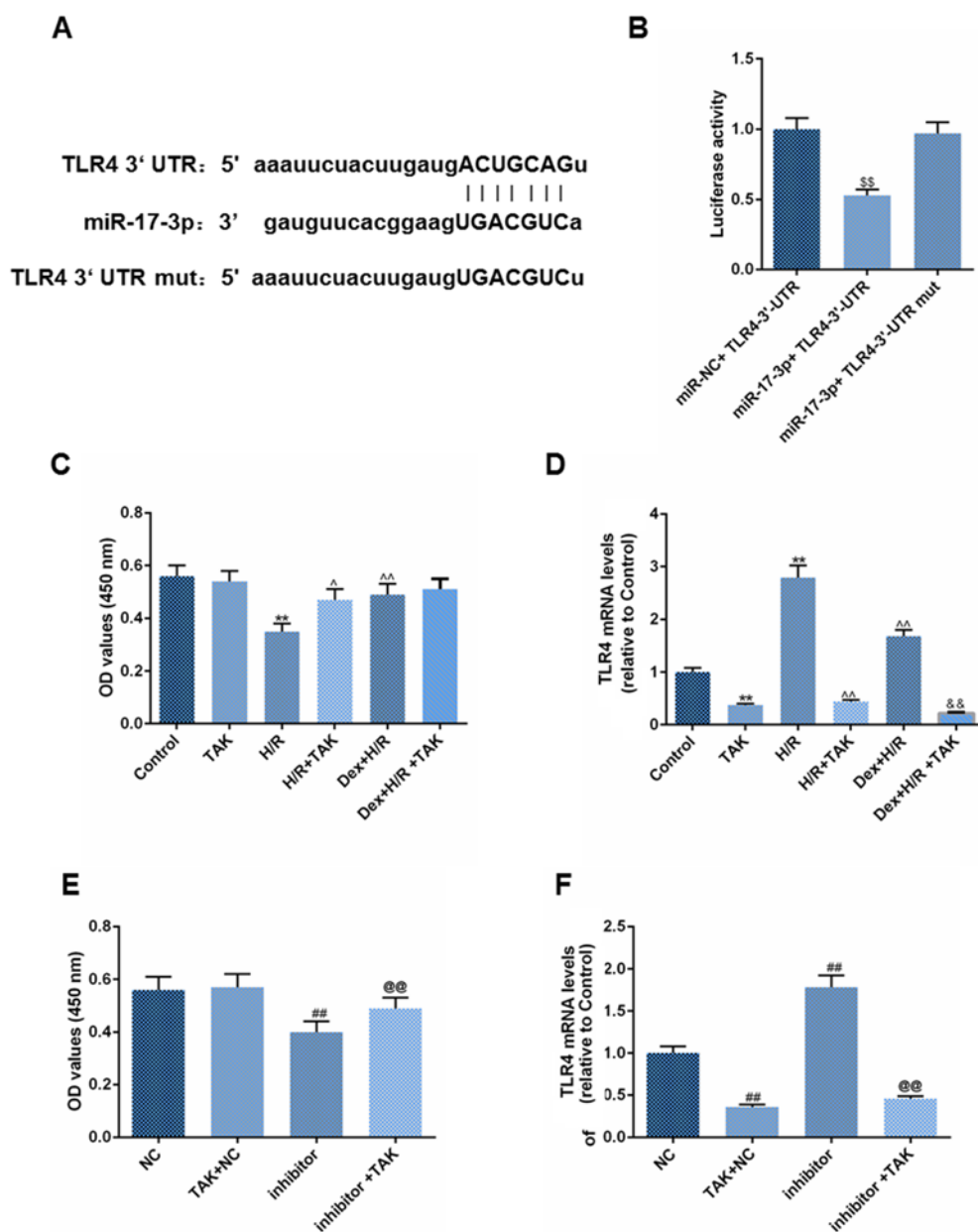
Figure 5. Effects of Dex and TAK-242 alone or in combination on H9C2 cell viability during H/R. (A) The binding site between miR-17-3p and TLR4 was predicted. (B) Cells were transfected with miR-17-3p, miR-NC and TLR4 3'-UTR and cultured for 24 h. Dual luciferase assay was performed to analyze changes in fluorescence. Cells were treated with 1 μmol/l TAK-242 cells for 2 h, and then with Dex for 24 h. Cells were placed in a hypoxic chamber for 3 h and reoxygenated for 3 h. (C) Cell viability and (D) TLR4 mRNA level was then assessed. Cells were treated with1 μmol/l TAK-242 for 2 h, and then transfected with miR-17-3p inhibitor and cultured for 24 h. (E) Cell viability and (F) TLR4 mRNA level was assessed. Data are presented as the mean ± SD. **P<0.01 vs. control group, ##P<0.01 vs. NC group, ^P<0.05 and ^^P<0.01 vs. H/R group, &&P<0.01 vs. Dex + H/R group, $$P<0.01 vs. miR-NC + TLR4 3'UTR group, @@P<0.01 vs. inhibitor group. Dex, dexmedetomidine; miR, microRNA; mut, mutant; NC, negative control; TLR4, toll-like receptor 4; UTR, untranslated region.

Discussion

The results of the present study indicated that Dex (1-10 μmol/l) did not cause severe damage to H9C2 cells, but reduced H/R-induced injury and increased miR-17-3p expression. In order to investigate the relationship between miR-17-3p and Dex, miR-17-3p was used in combination with Dex to prevent H/R-induced H9C2 injury. The results suggested that the protective effect of Dex was reduced when miR-17-3p expression was inhibited. The effect of Dex and miR-17-3p on inflammatory cytokines, TLR4 and galectin-3 expression was also explored.

TLRs are a family of pattern recognition receptors that play an important role in protection against infection (22). Studies suggest that TLRs have an important role in tissue homeostasis through regulation of inflammation and tissue repair (22). TLR4 signaling has been suggested to be important in regenerative biology, as it has been shown to have a pronounced effect on healing in models of injury and inflammatory disease (23,24). TLR4 mRNA levels were increased by H/R. TAK-242, which is an exogenous synthetic antagonist for TLR4, is a small molecule, also known as resatorvid, that binds to TLR4 and inhibits its transduction (25,26). TAK-242 was used to study whether Dex prevents H/R injury through action on TLR4. The results of the

present study revealed that inhibition of TLR4 could attenuate H/R injury, but that it had no obvious effect on the protective effect of Dex. This was unexpected as Chen *et al* (27) reported that Dex relieved retinal I/R injury by acting on TLR4.

Galectin-3, a 32-35 kDa member of galectin family of β-galactoside-binding lectins, plays multiple roles in cell growth, differentiation and aggregation (28). Galectin-3 expression is low in normal rat, murine and human heart; however, galectin-3 expression is rapidly increased in heart failure and progression (29). H/R caused a high expression of galectin-3. However, neither Dex nor miR-17-3p inhibitor caused a marked change in galectin-3 expression after H/R. Hence, Dex might alleviating I/R injury through other pathways.

The present study also evaluated the levels of various inflammatory cytokines. TNF-α is an important inflammatory cytokine, with pleiotropic functions including regulation of apoptosis and survival (30,31). Human IL-6 is made up of 212 amino acids including a 28-amino-acid signal peptide (32) and is rapidly produced in response to infection and tissue injury. Dysregulated synthesis of IL-6 has a pathological effect on inflammation (32). IL-1β, a member of the IL-1 family, has pro-inflammatory activity and promotes tissue injury (33,34). NF-κB was initially discovered in B cells, and is made up of a family of subunits consisting of p65/RelA, p50, p52, RelB and c-Rel (35). p65 activation promotes cardiac fibrosis, inflammation, and apoptosis in a mouse model of heart failure (36). The results of the present study suggest that H/R and miR-17-3p inhibitor increased the inflammatory response; however, Dex decreased this H/R-induced inflammatory response. Inhibition of miR-17-3p in Dex-treated H9C2 during H/R promoted inflammation, suggesting that miR-17-3p plays an important role in the protection induced by Dex.

In the present study the relationship between miR-17-3p and TLR4 was explored, and the results suggested that miR-17-3p binds with TLR4, and the inhibition of miR-17-3p increased TLR4 expression. Whether TLR4 participated in the regulation of inflammatory cytokines including IL-6, IL-1β, TNF-α and p65, in combination with Dex in hypoxia, needs to be further studied.

In conclusion, the findings of the present study suggested that Dex reduced H/R-induced injury in H9C2 cells via inhibition of inflammatory signaling, and that the inflammatory factors were regulated by miR-17-3p.

Availability of data and materials

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

Authors' contributions

TY and YuC made substantial contributions to conception and design of the study. ZY, SX, YaC and WC were responsible for data acquisition, data analysis and interpretation. TY drafted the article and critically revised it for important intellectual content. LW and WL performed the majority of the experiments. All authors approved the final version of the manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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