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Original Article

The Role of Mitochondrial ATP-Sensitive Potassium Channels in Cardioprotective and Anti-Inflammatory Effects of Troxerutin in Myocardial Reperfusion Injury

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Abstract

A major clinical challenge in ischemic heart disease is the prevention of myocardial injury following ischemia/reperfusion (I/R). Application of natural pharmaceuticals seems to be clinically interesting due to their multiplex activities. Protective effects of troxerutin (TXR) in myocardial I/R injury have been ever demonstrated, nevertheless, the purpose of this study is to explore the role of mitochondrial adenosine triphosphate -sensitive potassium (mitoK_{ATP}) channels and toll-like receptor 4 (TLR4)-nuclear factor kappa B (NF-κB) pathway in cardioprotective effects of TXR against I/R injury in rats. Male Wistar rats (n=72, 250–300 g, 12 weeks old) were randomized into groups with/without I/R and/or TXR and 5-hydroxydecanoate (5-HD), alone or in combination. To induce I/R model, the langendorff-perfused hearts were subjected to left anterior descending coronary artery (LAD) ligation and re-opening. TXR (150mg/kg/day) was administered for 4 weeks before I/R. Moreover, 5-HD (100 μM) was added to the perfusion solution before the ischemia. Finally, myocardial infarct size, LDH release, protein expression levels of TLR4 and NF-κB, and the levels of pro-inflammatory cytokines (TNF-α and IL-1β) were assessed. TXR preconditioning significantly reduced IS and LDH release ($P<0.05$). Furthermore, it decreased the expression of TLR4 and NF-κB and the level of pro-inflammatory cytokines ($P<0.05$ to $P<0.01$). Inhibition of mitoK_{ATP} channels by 5-HD significantly reversed the cardioprotective effects of TXR. This work shed some light on the knowledge about the mechanisms involved in the anti-inflammatory effect of TXR preconditioning in myocardial I/R injury. This effect may be partly mediated through mitoK_{ATP} channels opening and subsequent suppression of the TLR4/NF-κB pathway.

Keywords: Cardioprotection, Inflammation, Ischemic heart disease, Mitochondrial ATP-sensitive potassium channel, Myocardial reperfusion injury, Troxerutin.

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1. Introduction

Ischemic heart diseases (IHD) are the major cause of death worldwide, especially in industrialized societies [1]. The most effective treatment for limiting infarct size and preventing subsequent heart failure after

ischemia is timely interventional or surgical reperfusion, but even then, mortality and morbidity remain significant due to myocardial ischemia/reperfusion (MI/R) injury. Even though early reperfusion of the ischemic region is essential for the survival of cardiomyocytes, reperfusion itself leads to destructive responses in circulating cells and cardiomyocytes, and a series of pathophysiological and biochemical changes [2, 3]. MI/R injury leads to contractile dysfunction, arrhythmias, and increased infarct size by cardiomyocytes apoptosis, necrosis, and other pathophysiological mechanisms [4].

One of the main mechanisms involved in the pathophysiology of MI/R injury is the activation of inflammatory pathways and increased production of cytokines and interleukins [5-8]. The inflammatory response has been shown to begin with ischemia and continue for several hours throughout the reperfusion phase. Activation of toll-like receptor 4 (TLR4) following myocardial reperfusion is involved in reactive oxygen species (ROS) overproduction, cytokine releasing, and neutrophil activation [4, 9]. TLR4 can activate and transfer the nuclear factor-kappa B (NF-κB) to the nucleus, leading to the transcription and release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) and augmentation of the initial inflammatory response. Accumulation of pro-inflammatory cytokines in the ischemic region causes tissue injury, oxygen-free radicals releasing, cardiomyocyte damage, and myocardial dysfunction [9, 10]. Numerous studies have shown that the opening of mitochondrial adenosine triphosphate (ATP)-sensitive potassium (mitoK_{ATP}) channels in the outer membrane of the mitochondrion may play an essential role in preventing MI/R injury [11, 12]. These channels have a protective role in the primary stages of inflammatory responses by maintaining mitochondrial integrity and regulating mitochondrial matrix volume [13]. Hence, modulation of mitoK_{ATP} channels and TLR4/NF-κB signaling-directed inflammatory responses seems to be an interesting approach to achieve cardioprotection in patients with MI/R injury.

Troxerutin (TXR), also known as vitamin P4, is a natural bioflavonoid with antioxidant, anti-inflammatory, and anti-apoptotic activities against MI/R injury under diabetic and normal conditions [14- 16]. Also, the protective effects of TXR in several tissues such as the brain [17], liver [18], and kidney [19] have been proven. In our previous works, we have reported that preconditioning with TXR had anti-arrhythmic and anti-inflammatory effects against I/R injury in diabetic hearts [16]. Also, pretreatment with TXR in combination with ischemic postconditioning could protect the heart against I/R injury by preventing cell-cell interaction and pro-inflammatory cytokines releasing [20]. However, the mechanisms involved in the anti-inflammatory effects of TXR in the setting of MI/R injury need to be investigated in depth. Given the potential anti-inflammatory effect of TXR, the present study aims to explore the role of mitoK_{ATP} channels and TLR4/NF-κB pathway in the cardioprotective and anti-inflammatory effects of TXR against MI/R injury in rats. Insight into this aspect is expected to contribute to a better introduction of TXR as a protective agent in cardiovascular diseases.

2. Materials and Methods

2.1. Animals and chemicals

Male Wistar rats (n=72, 250–300 g, 12 weeks old) were purchased from the animal center of Tabriz University of Medical Sciences. All the rats were housed in the animal room under a 12 hours' light/12 hours' dark schedule at a temperature of 25 ± 2°C and a humidity of 50 ± 10% and had free access to standard food and water. Study protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th Edition, NRC 2011) and approved by the ethical committee of Tabriz University of Medical Sciences (Ethics approval number: 92-3729). TXR was obtained from Sigma (St. Louis, MO, USA), and 5-hydroxydecanoate (5-HD) was purchased from Tocris Bioscience (Avonmouth, UK). All substances for the Krebs-Henseleit (K-H) solution were obtained from Merck Company (Munich, Germany), and all other chemicals and reagents were obtained from commercial sources in the highest quality available.

2.2. Langendorff isolated heart perfusion and induction of regional I/R

Animals were heparinized (500 IU/kg), then anesthetized with an intraperitoneal injection of a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg). After opening the chest in layers, the heart was rapidly removed and then mounted on a pressure-constant Langendorff perfusion apparatus (ML176-V; AD Instruments, New South Wales, Australia). The isolated heart was retrogradely perfused via the aorta with K-H solution that contained (in mmol/L) $MgSO_4$ 1.2; KCl 4.8; NaCl 118.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $CaCl_2$ 1.7; and glucose 11.1 at pH 7.4 under a constant perfusion pressure of 80 mmHg. The perfusion solution was gassed with a mixture of 95% O_2 and 5% CO_2 at 37 ± 0.5°C. Throughout the stabilization, the coronary flow rate was 12–14 ml/min. For maintaining the temperature near 37°C, the isolated heart was surrounded by a homoeothermic glass cover. Before the induction of regional ischemia, the isolated hearts in the Langendorff setting were perfused with K-H solution for 15-20 minutes to stabilize cardiac activity. Then regional ischemia was established by ligation of the left anterior descending (LAD) coronary artery for 30 minutes close to its origin. Reperfusion was induced by LAD re-opening for 45 minutes. The efficiency of coronary ligation and sufficient perfusion were approved by reducing coronary flow to about 30–40% of its baseline values and returning coronary flow, respectively.

2.3. Experimental design

Rats were randomly divided into the following six experimental groups (n = 12 in each group): (1) Control: non-treated healthy group without experiencing I/R; (2) TXR: TXR-receiving group without experiencing I/R; (3) MI/R: non-treated group experiencing I/R; (4) MI/R+TXR: TXR-receiving group experiencing I/R; (5) MI/R+5-HD: 5-HD-receiving group experiencing I/R; and (6) MI/R+TXR+5-HD: TXR plus 5-HD-receiving group experiencing I/R.

In TXR-receiving groups, TXR (150 mg/kg) was orally administered through a gavage tube once a day for 4 weeks before the induction of

MI/R. In 5-HD-receiving groups, the hearts were perfused with a K-H solution containing 100 µM 5-HD, as a mitoK_{ATP} channel blocker, at 20 minutes before the ischemia. At the end of the reperfusion, evaluation of infarct size (IS), lactate dehydrogenase (LDH) release, protein expression, and cytokine levels was performed.

2.4. Evaluation of area at risk (AAR) and IS

To differentiate between ischemic and non-ischemic areas, the LAD was re-occluded following 45 minutes of reperfusion, then 0.25% Evans blue dye (3-4 ml, Sigma-Aldrich, St. Louis, MO, USA) was infused via the aortic cannula into the coronary system. After the hearts were frozen at -20°C for 24 hours, the hearts were sliced into 2 mm sections, then incubated for 15 minutes in 1% 2,3,5-triphenyl tetrazolium chloride phosphate-buffered solution (pH 7.4) at 37°C. Next, the slices were placed in 10% formalin for 24–48 hours. Using Image J software (NIH, Bethesda, USA), the determination of AAR and IS was performed. The AAR was reported as a percentage of the left ventricle (AAR/LV × 100). The IS was reported as a percentage of AAR (IS/AAR × 100).

2.5. Measurement of LDH release

LDH release into the coronary effluent was measured by a colorimetric method using a specific laboratory kit (Pars Azmoon Co., Karaj, Iran) and autoanalyzer (Alcyon 300, Abbott Labs, Santa Clara, CA, USA), in compliance with the manufacturer's methods. A spectrophotometer detected the absorbance of the solution for LDH at 492 nm. The results were reported in IU/L.

2.6. Preparation of tissue homogenates

At the end of the reperfusion, the heart samples isolated from the ischemic zone of the left ventricle were immediately frozen in liquid nitrogen and stored at –80°C. Samples were prepared by a modified protocol for tissue homogenization. The ventricular tissue was weighed, then cut into pieces in 5 ml of ice-cold lysis buffer (1 mM EDTA, 1 mM KCL, 1mM Na_3VO_4, 1 mM KH_2PO_4, 50 mM Tris-HCl pH 7.4, 1 mM NaF, 1% triton X100, and protease inhibitor cocktail) and homogenized using a Polytron PT-10/ST homogenizer. After centrifuging the homogenate (10,000 RCF) for 10 minutes at 4°C, the supernatants were removed from the homogenates and rapidly frozen at –80°C. To determine protein concentration in samples, the Bradford method was used.

2.7. Western blotting

The protein expression levels of TLR4 and NF-κB in isolated hearts of all groups were measured by western blotting. Fresh-frozen samples were dissected and homogenized in radio-immunoprecipitation assay (RIPA) lysis buffer (Sigma-Aldrich, St. Louis, MO, USA). The resulting solutions were centrifuged at 13000 × g for 20 minutes, and the supernatants were collected. Using a UV 3000 ultraviolet spectrophotometer (NanoDrop, Wilmington, DE), the concentration of total protein was determined. Then, equal amounts of proteins (50 µg) were loaded into the electrophoresis chamber in 10–15% SDS-polyacrylamide gel. After that, separated soluble proteins were

electro-transferred to a polyvinylidene-difluoride membrane (Sigma-Aldrich, St. Louis, MO, USA), then incubated with 5% non-fat dry milk solution in Tris-buffered saline-Tween 20 (TBST, pH 7.4) in room temperature for 2 hours to block non-specific bindings. The membranes were incubated with primary antibodies at 1:1000 against TLR4 and NF-κB (Cell Signaling Technology, USA) diluted in blocking buffer overnight at 4°C on a shaker. To control for equal loading, membranes were also incubated for β-actin antibody (1:1000, Cell Signaling Technology, USA), diluted in TBST overnight at 4°C. Then blots were incubated with goat anti-rabbit horseradish peroxidase-conjugated IgG for 1 hour at room temperature. For detecting the immunoreactivity, protein bands were incubated with enhanced chemiluminescence (ECL) substrate (Millipore, MA, USA) and then were exposed to X-ray hyper film inside a hyper cassette in a dark room for 5 minutes, and the chemiluminescence of the antibody binding was visualized using a visualizing machine. To quantify the intensity of protein bands in the blots, densitometry analysis was used, then normalized with the intensity of the β-actin band as a loading control. The measured values were reported in arbitrary units (AU).

2.8. Determination of cardiac inflammatory cytokines

For measuring the levels of inflammatory cytokines (TNF-α and IL-1β), rat-specific enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, San Diego, USA) were used according to the manufacturer's methods. The results were reported as pg/mg.

2.9. Statistical analysis

The analysis was carried out using SPSS software 25 (SPSS Inc., Chicago, IL, USA). To determine the statistical significance between groups, a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used. All values were expressed as means ± standard errors of means (SEM). A p-value below 0.05 ($P<0.05$) was considered a statistically significant difference.

3. Results

3.1. Myocardial AAR and IS

As shown in **figure 1**, there were no significant differences in the AAR between all of the hearts subjected to 30 minutes of ischemia and 45 minutes of reperfusion. The IS was significantly increased in the MI/R group as compared to the Control group ($P < 0.001$). Preadministration of TXR significantly reduced IS as compared to MI/R hearts ($P < 0.05$). However, adding 5-HD to the perfusion solution significantly abolished the IS-lowering effect of TXR. There was no significant change in IS between MI/R and MI/R+5-HD groups.

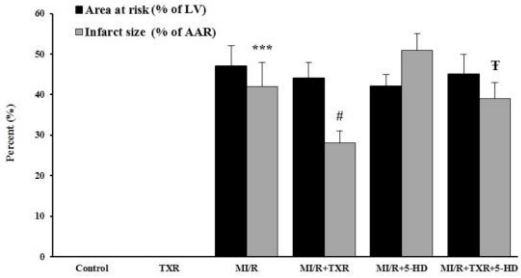

Figure 1. Area at risk (AAR) and infarct size (IS) percentages in different groups. The data were expressed as mean ± SEM (n = 6 for each group). (***$P < 0.001$ vs. Control group, #$P < 0.05$ vs. MI/R group, Ŧ$P < 0.05$ vs. MI/R+TXR group). TXR: troxerutin; MI/R: myocardial ischemia/reperfusion; MI/R+TXR: myocardial ischemia/reperfusion plus troxerutin; MI/R+5-HD: myocardial ischemia/reperfusion plus 5-hydroxydecanoate; MI/R+TXR+5-HD: myocardial ischemia/reperfusion plus troxerutin plus 5-hydroxydecanoate.

3.2. LDH release

Figuer 2 shows the levels of LDH release into the coronary effluent of treated and untreated I/R hearts. The levels of LDH release were significantly increased in the MI/R group as compared to the Control group ($P < 0.001$). Preconditioning with TXR significantly reduced the levels of LDH release into the coronary effluent during reperfusion in comparison with MI/R hearts ($P < 0.05$). Adding 5-HD to the perfusion solution could prevent the positive effects of TXR. No significant difference was found between MI/R and MI/R+5-HD groups.

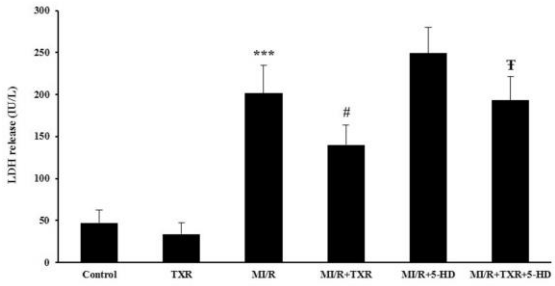

Figure 2. Lactate dehydrogenase (LDH) release into the coronary effluent in different groups. The data were expressed as mean ± SEM (n = 6 for each group). (***P < 0.001 vs. Control group, #P < 0.05 vs. MI/R group, ŦP < 0.05 vs. MI/R+TXR group). TXR: troxerutin; MI/R: myocardial ischemia/reperfusion; MI/R+TXR: myocardial ischemia/reperfusion plus troxerutin; MI/R+5-HD: myocardial ischemia/reperfusion plus 5-hydroxydecanoate; MI/R+TXR+5-HD: myocardial ischemia/reperfusion plus troxerutin plus 5-hydroxydecanoate.

3.3. TLR4 and NF-κB proteins expression

As shown in **figure 3A**, upregulation of TLR4 protein was observed following induction of MI/R ($P < 0.01$). TXR preconditioning caused a significant reduction in the expression of TLR4 protein as compared to MI/R hearts ($P < 0.05$). This positive effect of TXR was partially eliminated after blocking the mitoK$_{ATP}$ channels by 5-HD administration. There was no significant difference between MI/R and MI/R+5-HD groups.

Figure 3B shows that induction of MI/R led to an increased expression level of NF-κB protein in comparison with the Control group ($P < 0.001$). However, the expression level of NF-κB protein was significantly decreased in rats receiving TXR as compared to the MI/R group ($P < 0.01$). However, TXR in the presence of 5-HD could not significantly decrease the expression of NF-κB as compared to the MI/R+TXR group. No significant difference was found between MI/R and MI/R+5-HD groups.

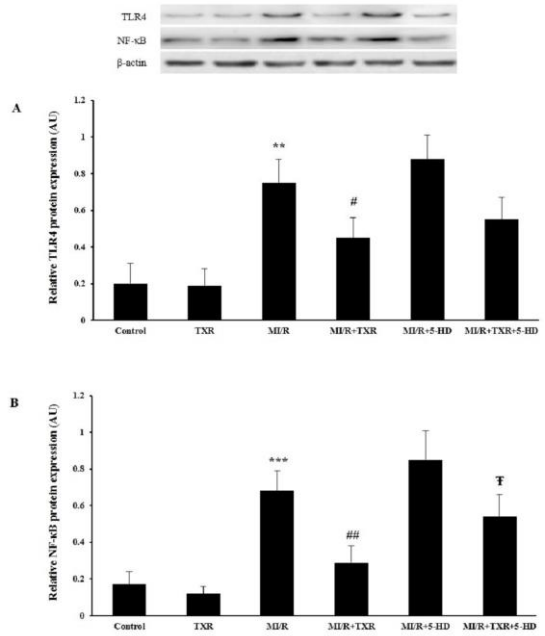

Figure 3. Western blot analysis of toll-like receptor 4 (TLR4) and nuclear factor kappa B (NF-κB) correlated to the β-actin band in different groups. The data were expressed as mean ± SEM (n = 6 for each group). (**P < 0.01 and ***P < 0.001 vs. Control group, #P < 0.05 and ##P < 0.01 vs. MI/R group, ŦP < 0.05 vs. MI/R+TXR group). TXR: troxerutin; MI/R: myocardial ischemia/reperfusion; MI/R+TXR: myocardial ischemia/reperfusion plus troxerutin; MI/R+5-HD: myocardial ischemia/reperfusion plus 5-hydroxydecanoate; MI/R+TXR+5-HD: myocardial ischemia/reperfusion plus troxerutin plus 5-hydroxydecanoate.

3.4. The levels of cardiac TNF-α

As shown in **figure 4**, significant elevation in the levels of TNF-α was detected in the MI/R group in comparison with the Control group ($P < 0.001$). Application of TXR

preconditioning significantly reduced the levels of TNF-α as compared to MI/R hearts ($P < 0.01$). Administration of 5-HD reversed the effect of TXR on the alteration of TNF-α levels. TNF-α levels did not differ between MI/R and MI/R+5-HD groups.

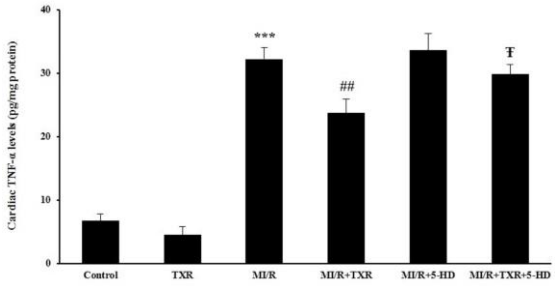

Figure 4. The levels of tumor necrosis factor-alpha (TNF-α) in different groups. The data were expressed as mean ± SEM (n = 6 for each group). (***P < 0.001 vs. Control group, ## P < 0.01 vs. MI/R group, ŦP < 0.05 vs. MI/R+TXR group). TXR: troxerutin; MI/R: myocardial ischemia/reperfusion; MI/R+TXR: myocardial ischemia/reperfusion plus troxerutin; MI/R+5-HD: myocardial ischemia/reperfusion plus 5-hydroxydecanoate; MI/R+TXR+5-HD: myocardial ischemia/reperfusion plus troxerutin plus 5-hydroxydecanoate.

3.5. The levels of cardiac IL-1β

Induction of MI/R significantly increased the levels of IL-1β in comparison with the Control group ($P < 0.001$). Preconditioning with TXR significantly reduced the levels of IL-1β as compared to MI/R hearts ($P < 0.05$). In addition, the levels of IL-1β were significantly decreased in the TXR-receiving group without experiencing I/R as compared to the Control group ($P < 0.05$). Blocking the mitoK$_{ATP}$ channels by 5-HD significantly abolished the IL-1β-lowering influence of TXR. The levels of IL-1β did not differ between MI/R and MI/R+5-HD groups (**Figure 5**).

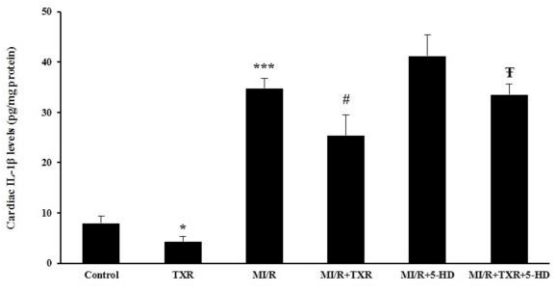

Figure 5. The levels of interleukin-1beta (IL-1β) in different groups. The data were expressed as mean ± SEM (n = 6 for each group). (*P < 0.05 and ***P < 0.001 vs. Control group, #P < 0.05 vs. MI/R group, ŦP < 0.05 vs. MI/R+TXR group). TXR: troxerutin; MI/R: myocardial ischemia/reperfusion; MI/R+TXR: myocardial ischemia/reperfusion plus troxerutin; MI/R+5-HD: myocardial ischemia/reperfusion plus 5-hydroxydecanoate; MI/R+TXR+5-HD: myocardial ischemia/reperfusion plus troxerutin plus 5-hydroxydecanoate.

4. Discussion

The present study revealed the therapeutic potential of TXR in mitigating MI/R damage in the isolated rat heart. Preconditioning with TXR over 4 weeks in rats diminished myocardial IS and LDH release following MI/R injury. Besides, this study documented that TXR preconditioning had potent cardioprotective and anti-inflammatory activity by reducing the activity of the TLR4/NF-κB signaling pathway. The beneficial effect of TXR was reversed by using 5-HD, as a mitoK$_{ATP}$ channel blocker, indicating that TXR may exert its cardioprotective and anti-inflammatory effect under MI/R injury by the opening of mitoK$_{ATP}$ channels. The results obtained from the present study can lead to a better understanding of the cardioprotective activity of TXR as a natural bioflavonoid in MI/R injury.

Several experimental studies have demonstrated the cardioprotective effects of TXR by evaluating important cellular signalings, but

the mechanisms of anti-inflammatory effects of TXR in MI/R damage need to be studied in depth. The specific pathophysiological mechanisms underlying MI/R injury are complex and need further investigation, however, several studies demonstrated that inflammatory response participates in MI/R injury. It has been proven that an important protein that is involved in the inflammatory response of MI/R damage is TLR4 [9]. In particular, TLR4 triggers the release of cytokines following MI/R injury [21-23] via the MyD88 pathway, and NF-κB can be activated through this dependent pathway. NF-κB pathway participates in the stress reaction and tissue damage [9, 24]. After translocation of NF-κB to the nucleus within the first few minutes following reperfusion, NF-κB promotes cell dysfunction and death through induction of pro-inflammatory and pro-apoptotic genes expression [4]. Therefore, inhibition of the TLR4/NF-κB signaling pathway can be considered as an important therapeutic target to reduce MI/R injury. Consistent with previous studies, our data showed that I/R led to increased expression levels of TLR4 and NF-κB proteins, and this accelerated inflammatory response caused a decrease in cardiac resistance to reperfusion injury. Interestingly, preadministration of TXR for 4 weeks could alleviate TLR4 and NF-κB expression, and ultimately protect the heart against I/R damage. It can be said that TXR had potent anti-inflammatory properties by reducing the activity of the TLR4/NF-κB pathway.

TNF-α and IL-1β play a key role in MI/R injury by causing cardiac dysfunction and cardiomyocyte necrosis and apoptosis [9]. Activation of myocardial TNF-α and TNF receptors has a complex role in MI/R damage and protection from it. Excessive expression of TNF-α and stimulation of cardiomyocyte TNF receptor type 1 can lead to cardiac cell death, fibrosis, hypertrophy, and contractile dysfunction. Whereas, lower expression of TNF-α and stimulation of cardiomyocyte TNF receptor type 2 can induce protective impacts [25]. Our study revealed that I/R caused a significant increase in the myocardial levels of TNF-α and IL-1β, which was associated with increased myocardial injury. However, pretreatment with TXR reduced TNF-α and IL-1β levels in the heart, indicating that the positive effect of TXR against MI/R damage may be attributed to the reduction of inflammatory cytokines such as TNF-α and IL-1β via decreasing the activity of the TLR4/NF-κB signaling pathway.

Studies have demonstrated that the mitoK_{ATP} channel, which is considered as an end effector of cardioprotective pathways, has cardioprotective activity in MI/R injury by regulating mitochondrial matrix volume and decreasing calcium uptake by mitochondria [26]. It has been suggested that the opening of the mitoK_{ATP} channel decreases pro-apoptotic mediators and improves mitochondrial function [13], while administration of 5-HD, as a mitoK_{ATP} channel blocker, reverses the positive effects of preconditioning and postconditioning modalities on myocardial IS [27]. In the present study, it was revealed that the anti-inflammatory effects of TXR were reversed when the hearts were perfused with a K-H solution containing 5-HD. It can be said that the positive effects of TXR may be partially mediated through the mitoK_{ATP} channels opening, and there is probably an association between the activation of mitoK_{ATP} channels and the inhibitory effect of TXR on

inflammatory response under MI/R injury. In addition, it can be indirectly demonstrated that the activation of mitoK_{ATP} channels by TXR can protect the functional integrity of mitochondria, prevent mitochondrial swelling, inhibit mitochondrial permeability transition pore (mPTP) opening, and reduce the production and release of inflammatory mediators from the mitochondria in MI/R condition [2, 11]. Conforming to our study, many previous works demonstrated that TXR had protective effects against cardiovascular diseases and oxidative and inflammatory damage in numerous organs [20, 28-32]. TXR has been shown to inhibit the endoplasmic reticulum stress pathway and reduce intracellular inflammatory proteins and enzyme activities in specific tissues [32-35]. Moreover, in our previous works, we have shown that TXR preconditioning could protect the diabetic I/R hearts by preventing myocardial apoptosis through glycogen synthase kinase-3β (GSK-3β) phosphorylation [36]. In addition, we proved that TXR preconditioning had protective effects in doxorubicin-induced cardiotoxicity in rats via anti-oxidative activities and restoring mitochondrial function [37].

5. Conclusion

The results of the present work contribute to some valuable conclusions. Preconditioning with TXR could provide cardioprotective effects under MI/R damage. Cardioprotective effects of TXR were partly mediated through the modulation of inflammatory response, including reduction of TNF-α and IL-1β levels via decreasing the activity of TLR4/NF-κB signaling pathway. The opening of mitoK_{ATP} channels plays a crucial role in the cardioprotective and anti-inflammatory effects of TXR in MI/R injury conditions. However, further attempts are required to examine the effects of TXR on the other important pathways involved in mitoK_{ATP} channels-dependent cardioprotection under I/R injury.

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None.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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