Tropical Journal of Pharmaceutical Research November 2021; 20 (11): 2273-2278
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i11.6

Original Research Article

Dexmedetomidine mitigates myocardial ischemia-reperfusion injury via regulation of HMGB1-TLR4-NF-κB signaling axis

Ting Wen[1], Jia Liu[2], Shibiao Chen[1], Benchao Hou[1], Gan Li[1], Chaxiu Yu[1]*
[1]Department of Anesthesiology, The First Affiliated Hospital of Nanchang University, [2]Department of Laboratory, Jiangxi Provincial Mental Hospital, Nanchang 330006, Jiangxi Province, China

For correspondence: **Email: pj295h@163.com*

Sent for review: 1 August 2021 *Revised accepted: 22 October 2021*

Abstract

Purpose: *To study the effect of dexmedetomidine (Dex) on myocardial ischemia-reperfusion injury (MIRI), and the associated mechanism of action.*
Methods: *Sixty Sprague-Dawley (SD) rats were assigned to sham, ischemia-reperfusion (I/R), Dex, and MD groups (methyllycaconitine prior to injection with Dex), with 15 rats in each group. Pathological changes in myocardial tissues were determined in all groups. Protein expression levels of HMGB1, TLR4, NF-κB and myeloid differentiation protein 88 (MyD88) in serum and myocardial tissues were assayed and compared.*
Results: *Protein levels of HMGB1, TLR4, MyD88 and NF-κB were significantly higher in heart muscle I/R rats than those in sham group, but lower in heart muscle of rats in Dex group than in heart muscle of I/R rats ($p < 0.05$). However, they were significantly up-regulated in MD group, relative to Dex group ($p < 0.05$).*
Conclusion: *Dex exerts a protective effect against ischemia/reperfusion-induced myocardial damage via HMGB1-TLR4-NF-κB signal axis via CAP, and thus, is a potential agent for the management of myocardial disease.*

Keywords: *Ischemia/reperfusion injury, Dexmedetomidine, activation of high mobility group box 1, HMGB1, Toll-like receptor 4, Myocardial ischemia reperfusion injury*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Myocardial ischemia reperfusion injury (MIRI) is a major cause of severe postoperative complications and death in patients undergoing coronary artery reconstruction [1]. Currently, there are many reports on MIRI, but the mechanism of MIRI has not been fully elucidated, and thus, is a potential agent for the management of myocardial disease. Inflammatory response after MIRI is an important factor that exacerbates the disease and secondary injury, during which a large number of inflammatory factors and inflammatory mediators are released [2].

Currently, it is recognized that cholinergic anti-inflammatory pathway (CAP) is the main route

used by the vagus nerve to regulate inflammatory responses, and it is an important link to inflammatory response [3]. In recent years, CAP has been gradually applied in the protection of organs such as heart, liver and kidney from ischemia-reperfusion injury, through a mechanism involving blockage of the inflammatory pathway by activating alpha7 nicotinic acetylcholine receptor (α7nAchR) [4,5].

Clinical investigations have demonstrated that MIRI induces inflammatory cascade through the activation of HMGB1 protein, which causes myocardial tissue damage. In addition, HMGB1 binds to Toll-like receptor 4 (TLR4) and activates NF-κB mediated by myeloid differentiation factor 88 (MyD88) and Toll interleukin receptor-related regulatory molecules. This in turn, induces the nuclear transfer of NF-κB and IRF-3, as well as regulation of the expressions of related cytokines, eventually impairing myocardial systolic function, leading to myocardial tissue damage [6,7]. Clinical investigations have revealed that Dex protects organs by blocking lipopolysaccharide (LPS)-induced inflammatory response [8,9]. However, there are limited studies on the inflammatory response of Dex to MRI in rats, as well as its mechanism of action. Therefore, the purpose of the present investigation was to study the influence of Dex on I/R myocardial lesion in rats, and the pathways involved.

EXPERIMENTAL

Animals

Sixty SD rats aged 10 weeks were obtained from Nanjing Junke Biological Engineering Co. Ltd. [Batch number: SCXK (Su) 2020-0006]. The rats were fed in single cages and kept at temperature range of 20 to 24 ºC in the animal room under 9:00 am to 21:00 pm photoperiod. Drinking water and feed were provided freely. This research was approved by the Animal Ethical Committee of Jiangxi Provincial Mental Hospital (approval no. 20190873), and carried out according to “Principles of Laboratory Animal Care” (NIH publication no. 85-23, revised 1985) [10].

Main reagents and equipment

The reagents and instruments used, and their suppliers (in brackets) were: Dex (Shanghai Guangrui Biotechnology Co. Ltd); chloral hydrate (Beijing Eta Biotechnology Co. Ltd); methyllycaconitine, ELISA kits for IL-6 and TNF-α (AmyJet Scientific Co. Ltd); TLR4 antibody (Wuhan Yipu Biotechnology Co. Ltd.); HMGB1 antibody (Wuhan Feien Biotechnology Co. Ltd.); NF-κB antibody (Shanghai Lianmai Biological Engineering Co. Ltd.); MyD88 antibody (Shenzhen Haodi Huatuo Biotechnology Co. Ltd); BCA protein quantitative kit (Shenyang Wanjie Biotechnology Co. Ltd); PVDF membrane (Zhejiang Yuxiang Biotechnology Co. Ltd), and ECL ultra-sensitive luminescent solution (Beijing Eta Biological Technology Co. Ltd).

The equipment used were small animal ventilator (Yuanyang Zhenhua Teaching Instrument Co. Ltd); centrifuge (Nanjing Beideng Medical Co. Ltd); ultra-low temperature refrigerator (Hangzhou Nuoding Scientific Equipment Co. Ltd); intravenous in-dwelling needles (Henan Zeyuan Medical Equipment Sales Co. Ltd); ophthalmic surgical instruments (Beijing Baoyuan Xingye Technology Co. Ltd); multifunctional shaker (Shenzhen Saijin Biological Technology Co. Ltd); inverted microscope (Shanghai Tusen Vision Technology Co. Ltd), and chemiluminescence imager (Suzhou Aibituo Biotechnology Co. Ltd).

Study design

Sixty SD rats were randomly divided into sham operation, I/R, Dex and MD groups, with 15 animals per group. Sham operation rats were injected with normal saline in the right jugular vein for 25 min, and the rats were threaded without ligation. In the other three groups of rats, induction of myocardial ischemia reperfusion was done via ligation of the anterior descending branch of the left coronary artery for 30 min and restoring the perfusion for 120 min. Before establishing the model, rats in the I/R group were injected with normal saline in the right jugular vein for 25 min; those in Dex group received Dex injection, while the MD rats were injected intraperitoneally with 10 μM methyllycaconitine prior to injection with Dex.

At 120 min after reperfusion, the left anterior descending coronary artery was ligated again, and the rats were sacrificed via decapitation. The heart tissues were excised after confirmation of successful establishment of ischemia. After separation of atria from the right ventricles, pathological changes and myocardial infarction in rats in each group were determined histologically using H & E and TTC staining, and the extent of myocardial infarction was evaluated.

Right cervical venous blood (2 mL) was collected from rats in each group, and centrifuged to obtain serum. A portion of myocardial tissue from each rat was used to prepare myocardial tissue homogenate. The concentrations of IL-6 and

TNF-α in serum and myocardial tissues were determined with ELISA.

Protein was extracted from heart muscle, and the protein expression levels of HMGB1, TLR4, NF-κB and MyD88 were measured with western blotting.

Statistical analysis

Myocardial infarction area, and HMGB1, TLR4, NF-κB and MyD88 protein expression levels in each group are presented as mean ± standard deviation. For statistical analyses, pairwise comparison was performed using SNK-q test, while multi-group comparison was done with ANOVA using SPSS software package. Values of $p < 0.05$ indicated that differences are statistically significant.

RESULTS

Pathological changes in rat myocardial tissue

There was orderly arrangement of myocardial fibers in sham rats, and normal cell morphology; the cell membranes were intact, and the nuclei were normal. In I/R group, the myocardial cells were disordered, with swelling, necrosis, massive neutrophil infiltration and severe myocardial tissue damage. In contrast, in the Dex group, myocardial fibers were arranged neatly, neutrophil infiltration was reduced, myocardial cell necrosis was relatively decreased, and myocardial tissue injury was significantly mitigated. In the MD group, the myocardial fibers of rats were broken, swollen and necrotic, and the cellular arrangement was disordered, while the cells were neutrophil-infiltrated. These results are shown in Figures 1A -1D.

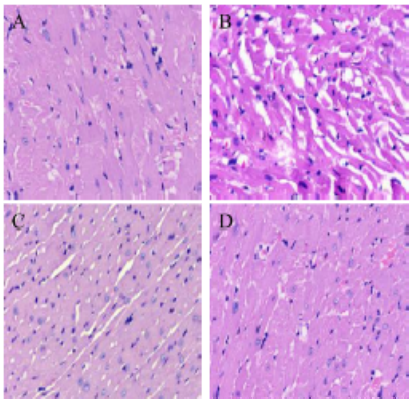

Figure 1: Photomicrographs showing pathological changes in heart muscles. A & B: Sham & I/R rats, respectively; C: Dex group; D: MD group (H & E staining, ×200)

Myocardial infarction area

The area of myocardial infarction was significantly higher in the I/R group than in the sham operation group, but was markedly lower in the Dex group than in I/R rats. Myocardial infarction area in the MD group was markedly higher than that in the Dex group ($p < 0.05$). These data are presented in Table 1.

Table 1: Area of myocardial infarction in each group of rats

Group	Infarct size (%)
Sham	0.00
I/R	56.11±6.69[a]
Dex	26.99±1.21[b]
MD	43.15±3.61[c]
F	161.650
P-value	< 0.001

Data are shown as mean ± SD (n = 15). [a, b & c]$P < 0.05$, vs sham, I/R and MD rats, respectively

Levels of inflammatory factors in heart muscle and serum

The levels of interleukin-6 and tumor necrosis factor-α in serum and myocardial tissue were significantly higher in I/R rats than in sham operation rats, but they were markedly reduced in Dex-exposed rats group, when compared to I/R rats ($p < 0.05$). Their concentrations in heart muscle and serum in MD rat group were markedly raised, relative to those in Dex-treated rats. These results are shown in Table 2.

Rat myocardial tissue levels of proteins linked to HMGB1-TLR4-NF-κB signal axis

The protein levels of TLR4, HMGB1, MyD88 and NF-κB in myocardial tissues of rats were markedly up-regulated in I/R rats, relative to sham operation rats, but they were markedly lower in Dex-exposed rats than in I/R rats. These protein expressions were markedly higher in myocardial tissues of rats in MD rats than those in Dex-treated rats ($p < 0.05$). These results are presented in Figure 2.

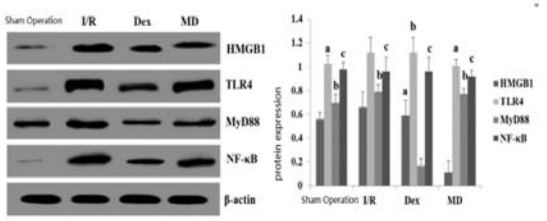

Figure 2: Levels of proteins linked to HMGB1-TLR4-NF-κB signal axis

Table 2: Levels of inflammatory factors in myocardial tissue and serum

	Myocardial tissue		Serum	
Group	***IL-6 (pg/mL)***	***TNF-α (pg/mL)***	***IL-6 (pg/mL)***	***TNF-α (pg/mL)***
Sham	135.44±15.66	179.13±24.19	95.11±17.59	156.31±15.32
I/R	335.43±15.25[a]	620.25±21.69[a]	337.39±41.10[a]	637.08±24.56[a]
Dex	211.34±11.94[b]	279.91±24.25[b]	153.72±10.39[b]	233.55±9.41[b]
MD	244.85±15.86[c]	467.19±31.25[c]	231.19±20.31[c]	387.25±31.09[c]
F	472.840	881.700	261.340	1424.470
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Data are shown as mean ± SD. [a,b & c]*P* < 0.05, vs sham operated, I/R and MD rats, respectively

DISCUSSION

Dexmedetomidine (Dex) is a highly selective α2 adrenergic receptor agonist that plays an anti-inflammatory and myocardial protective role [11]. In recent years, several investigations have been carried out on the organ protective influence of Dex, especially its cardio-protective effect, which has been shown to significantly mitigate inflammatory response in MIRI-related links [12]. It has been reported that Dex effectively reduced the serum levels of endotoxin-induced inflammatory factors in severe septicemia, and also significantly reduced mortality of rats from shock [13].

Studies have also shown that Dex inhibited inflammatory response by blocking sympathetic nerve and relatively exciting vagus nerve, and also activated CAP to a certain extent [14]. However, the mechanism through which Dex mitigated ischemia reperfusion injury appears controversial. In a study in which an MIRI rat model was pretreated with Dex, it was shown that Dex significantly reduced the myocardial infarction area [15]. It might be that Dex reduced coronary blood flow, leading to lack of blood supply to myocardial tissue before ischemia occurs, thereby triggering ischemic pre-adaptation in myocardial tissue, and protection from I/R lesion.

A study using various models has shown that the myocardial infarction area of Dex-preconditioned group was significantly lower than those of the other groups [16]. However, it was suggested that the mechanism of action of Dex was not mediated by ischemia preconditioning, but by activation of phosphatidylinositol-3 kinase (PI3K)/serine threonine kinase (Akt) during preconditioning and ischemia reperfusion.

In the present study, the injury caused by various types of ischemic heart disease and coronary recanalization in clinical practice were simulated through the preparation of a classical MIRI rat model. It was found that that the myocardial infarction area had marked increase, the heart muscles were damaged, and the release of inflammatory factors was markedly increased in the MIRI rats. However, after Dex treatment, the myocardiopathy area was significantly reduced, myocardiopathy was markedly reduced, and the release of inflammation-related factors was significantly suppressed. One of the important components of CAP is α7nAChR. It participates in anti-inflammatory responses of many tissues and organs. In recent years, α7nAChR has been found to be expressed in cardiomyocytes [17]. Therefore, in this study, it was hypothesized that the vagus nerve may play a role in reducing cardiac inflammatory response through α7nAChR mediated CAP.

Methyllycaconitine is a selective α7nAChR antagonist which binds to α7nAChR, leading to inhibition of acetylcholine action. In this study, it was found that the protective effect of Dex was significantly blocked by methyllycaconitine in MIRI rats, suggesting that Dex pretreatment may exert a protective role in MIRI rats, most likely through regulation of CAP.

Currently, it is recognized that TLR4/MyD88/NF-κB pathway is the main route involved in TLR4-associated inflammatory response, and plays an crucial role in inflammatory response in MIRI. It has been reported that HMGB1 is an extremely important endogenous substance in inflammatory response-related links: it binds to TLR4, leading to series of inflammatory responses [18-19]. In this study, it was found that the protein concentrations of NF-κB, TLR4, MyD88 and NF-κB in myocardial tissue of MIRI rats were markedly raised, but the levels of these proteins were markedly decreased by Dex. However, the effect of Dex was blocked by methyl lycaconitine, suggesting that Dex may regulate this signal axis through CAP.

CONCLUSION

Dexmedetomidine (Dex) regulates HMGB1-TLR4-NF-κB signal axis in rats via CAP. Therefore, it a protective potential in the management of ischemia/reperfusion-induced myocardial injury.

DECLARATIONS

Acknowledgement

This research was supported by Science and Technology Plan of Department of Education in 2013 (no. GJJ13134) and Science and Technology Plan of the National Health Commission in 2020 (no. 20203098).

Conflict of interest

No conflict of interest is associated with this work.

Authors' contribution

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chaxiu Yu designed the study, supervised the data collection, and analyzed the data. Ting Wen interpreted the data and prepared the manuscript for publication. Jia Liu, Shibiao Chen, Benchao Hou and Gan Li supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

REFERENCES

1. *Yang SF, Huang PH. Mir-25 is a potential treatment target for myocardial ischemic-reperfusion injury. J Chin Med Assoc 2020; 83(5): 419-420.*
2. *Li Z, Zhang Y, Ding N, Zhao Y, Ye Z, Shen L, Yi H, Zhu Y. Inhibition of lncRNA XIST Improves Myocardial I/R Injury by Targeting miR-133a through Inhibition of Autophagy and Regulation of SOCS2. Mol Ther Nucleic Acids 2019; 18: 764-773.*
3. *Yamada M, Ichinose M. The cholinergic anti-inflammatory pathway: an innovative treatment strategy for respiratory diseases and their comorbidities. Curr Opin Pharmacol 2018; 40: 18-25.*
4. *Yi L, Lyn YJ, Peng C, Zhu RL, Bai SS, Liu L, Wang PX, Zhou H, Dong Y. Sinomenine inhibits fibroblast-like synoviocyte proliferation by regulating α7nAChR expression via ERK/Egr-1 pathway. Int Immunopharmacol 2018; 56: 65-70.*
5. *Kong W, Kang K, Gao Y, Liu H, Meng X, Cao Y, Yang S, Liu W, Zhang J, Yu K, et al. GTS-21 Protected Against LPS-Induced Sepsis Myocardial Injury in Mice Through α7nAChR. Inflammation 2018; 41(3): 1073-1083.*
6. *Wang Q, Zhang S, Liu T, Wang H, Liu K, Wang Q, Zeng W. Sarm1/Myd88-5 Regulates Neuronal Intrinsic Immune Response to Traumatic Axonal Injuries. Cell Rep 2018; 23(3): 716-724.*
7. *Zhang F, Zhu Y, Fan G, Hu S. MicroRNA-2682-3p inhibits osteosarcoma cell proliferation by targeting CCND2, MMP8 and Myd88. Oncol Lett 2018; 16(3): 3359-3364.*
8. *Haque MA, Jantan I, Harikrishnan H. Zerumbone suppresses the activation of inflammatory mediators in LPS-stimulated U937 macrophages through MyD88-dependent NF-κB/MAPK/PI3K-Akt signaling pathways. Int Immunopharmacol 2018; 55: 312-322.*
9. *Li H, Zhang X, Chen M, Chen J, Gao T, Yao S. Dexmedetomidine inhibits inflammation in microglia cells under stimulation of LPS and ATP by c-Fos/NLRP3/caspase-1 cascades. EXCLI J 2018; 17: 302-311.*
10. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
11. *Rahimzadeh P, Faiz SHR, Imani F, Derakhshan P, Amniati S. Comparative addition of dexmedetomidine and fentanyl to intrathecal bupivacaine in orthopedic procedure in lower limbs. BMC Anesthesiol 2018; 18(1): 55-62.*
12. *Nie Y, Tu W, Shen X, Yu W, Yu Y, Song X, Wang S, Luo A, Cao M, Wu X, et al. Dexmedetomidine Added to Sufentanil Patient-Controlled Intravenous Analgesia Relieves the Postoperative Pain after Cesarean Delivery: A Prospective Randomized Controlled Multicenter Study. Sci Rep 2018; 8(1): 9940-9952.*
13. *Xiong M, Chen B, Hu Z, Gupta S, Li Z, Liu J, He J, Patel S, Eloy JD, Xu B. Dose Comparison of Dexmedetomidine Sedation following Spinal Anesthesia: Parturient versus Nonpregnant Women-A Randomized Trial. Anesthesiol Res Pract 2020; 2020: 1059807.*
14. *Taniguchi T, Kurita A, Kobayashi K, Yamamoto K, Inaba H. Dose-and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats. J Anesth 2008; 22(3): 221-228.*
15. *Okada H, Kurita T, Mochizuki T, Morita K, Sato S. The cardioprotective effect of dexmedetomidine on global ischaemia in isolated rat hearts. Resuscitation 2007; 74(3): 538-545.*
16. *Mimuro S, Katoh T, Suzuki A, Yu S, Adachi YU, Uraoka M, Sano H, Sato S. Deterioration of myocardial injury due to dexmedetomidine administration after myocardial ischaemia. Resuscitation 2010; 81(12): 1714-1717.*
17. *Ulleryd MA, Mjornstedt F, Panagaki D, Yang LJ, Engevall K, Gutierrez S, Wang Y, Gan LM, Nilsson H, Michaelsson E, et al. Stimulation of alpha 7 nicotinic*

acetylcholine receptor (α7nAChR) inhibits atherosclerosis via immunomodulatory effects on myeloid cells. Atherosclerosis 2019; 287: 122-133.

18. *Gu H, Wang C, Li J, Yang Y, Sun W, Jiang C, Li Y, Ni M, Liu WT, Cheng Z, et al. High mobility group box-1-toll-like receptor 4-phosphatidylinositol 3-kinase/protein kinase B-mediated generation of matrix metalloproteinase-9 in the dorsal root ganglion promotes chemotherapy-induced peripheral neuropathy. Int J Cancer 2020; 146(10): 2810-2821.*
19. *Yaoxia L, Tao W, Min Z, Ping C, Yerong Y. Down-regulation of myocardial infarction associated transcript 1 improves myocardial ischemia-reperfusion injury in aged diabetic rats by inhibition of activation of NF-κB signaling pathway. Chem Biol Interact 2019; 300: 111-122.*