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Research Article

Cardioprotective Effects of Glatiramer Against Ischemia-Reperfusion Injury in Coronary Artery Ligation Model in Rats Through Activation of AKT-GSK-3β-TNF-α-Nrf2 Signalling Pathway

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Abstract

Background and Objective: Glatiramer is extensively used in the management of multiple sclerosis. However, recent studies have shown its widespread usefulness in preventing injury to other tissues including cardiomyocytes from oxygen-glucose deprivation injury. The present study was aimed to explore the cardioprotective potential of glatiramer in attenuating IR-injury in coronary artery ligation models in rats. **Materials and Methods:** Male Wistar albino rats were subjected to left coronary artery ligation for 30 min followed by reperfusion for 120 min. Glatiramer (0.5, 1.0 and 2.0 mg kg^{-1}) was administered 30 min before subjecting to IR injury. The levels of cTnT and CK-MB were quantified to assess the degree of myocardial injury, while the levels of TNF-α, p-AKT, p-GSK-3β/GSK-3β ratio and nuclear: cytoplasmic ratios of Nrf2 were estimated in the heart homogenates. **Results:** Glatiramer abolished IR-induced increase in the release in cTnT and CK-MB along with the reduction in the TNF-α level in a dose-dependent manner. It also restored IR-induced decrease in p-AKT levels, p-GSK-3β/GSK-3β ratio and nuclear: cytoplasmic ratio of Nrf2 in the heart. **Conclusion:** Glatiramer may attenuate myocardial injury in the coronary artery ligation model in rats, which may be possibly attributed to the activation of the AKT-GSK-3β signalling pathway along with the decrease in inflammation and increase in antioxidant activities in rat hearts.

Key words: Glatiramer, ischemia, heart, inflammation, reperfusion, cardioprotection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ischemia refers to a state of decrease in blood flow, which is different from hypoxia in which there is a decrease in oxygen supply. Nevertheless, these two terms ischemia and hypoxia are used interchangeably in clinical setup, where ischemia and hypoxia are found to coexist[1]. Persistent myocardial ischemia in the form of myocardial infarction needs immediate treatment and this is done by recanalizing the occluded blood vessel, either through thrombolytic agents or using percutaneous angioplasty[2]. However, the institution of reperfusion also contributes to enhancing ischemia-induced myocardial injury and collectively, the term ischemia-reperfusion-induced myocardial injury is used to describe the injury as a result of ischemia and reperfusion[3]. However, there is no specific treatment to attenuate ischemia-reperfusion injury in a clinical setup and there is a need for novel interventions to attenuate ischemia-reperfusion-induced myocardial injury.

Glatiramer is an immunomodulator and it has been approved for relapsing-remitting multiple sclerosis[4]. Its beneficial effects have been attributed to multiple mechanisms including a decrease in the release of inflammatory cytokines[5]. Apart from multiple sclerosis, it is effective in many other disease states including ischemia-reperfusion-induced cerebral injury[6], cognitive dysfunction[7], renal injury[8], spinal cord injury[9] and epileptic seizures[10] etc. A recent study has also described the beneficial effects of glatiramer in oxygen-glucose deprivation-induced injury to cardiomyocytes[11]. However, its role in an *in vivo* model of cardiac ischemia-reperfusion injury has not been explored.

Left coronary artery occlusion in rats has been widely employed to produce ischemia in rats and it represents an *in vivo* model of ischemia-reperfusion injury, a true representative of ischemia taking place in humans[7]. Accordingly, the present study was designed to investigate the cardioprotective effects of different doses of glatiramer in an *in vivo* model of ischemia-reperfusion injury in rats. Considering the key role of AKT[12], GSK-3β[13], TNF-α[14] and Nrf2, a transcriptional factor controlling the antioxidant activities[15] in ischemia-reperfusion injury, the present study also explored the possible role of AKT, GSK-3β, TNF-α and Nrf2 in glatiramer mediated beneficial effects in ischemia-reperfusion injury in rats.

MATERIALS AND METHODS

Study area: All experimental studies were conducted in the Department of Cardiology, Shanxi Cardiovascular Hospital, Taiyuan, Shanxi Province, 030024, China between March-June, 2021.

Animals and drugs: Male Wistar albino rats (300-350 g) were used for this study and these were kept in the animal house in standardized laboratory conditions. The animal experiments were approved by the Research Ethical Approval Committee of Shanxi Cardiovascular Hospital and the approval number was SXV2. 0/2021.01.05. The doses of glatiramer were selected[8].

Induction of myocardial ischemia-reperfusion injury: The animals were anaesthetized and a left-sided thoracotomy was performed to open the pericardium and the heart was exteriorized with a cardiac holder consisting of a plastic loop. The left anterior descending coronary artery (LAD) was localized and a 5.0 silk suture was used to ligate the LAD for 10 min to induce ischemia[16]. Thereafter, the ligation was removed and blood flow was restored to induce reperfusion injury. After 120 min of reperfusion, rats were sacrificed to remove blood samples and hearts for further processing.

Quantitative assessment of myocardial injury: The extent of myocardial injury was assessed by quantifying the circulating levels of cardiac troponin (cTnI) and heart specific-isoform of creatine kinase (CK-MB). The levels of cTnT and CK-MB were measured using commercially available kits (MyBioSource, San Diego, CA, USA).

Estimation of biochemical parameters in the heart: The hearts were removed and homogenized in PBS buffer (pH: 7.4). Thereafter, the homogenized samples were centrifuged to separate supernatant. In the supernatants, the estimation of different parameters of TNF-α (Abcam, Cambridge, USA), p-AKT levels (MyBioSource, San Diego, CA, USA), p-GSK-3β/GSK-3β ratio (MyBioSource, San Diego, CA, USA), nuclear: cytoplasmic ratio of Nrf2 (MyBioSource, San Diego, CA, USA) was done using commercially available ELISA kits.

Experimental design: There were five groups and each group comprised of 10 animals.

Non-ischemic: No surgery was performed and no drug was given to rats. The animals were kept for 150 min and rats were sacrificed to collect blood (for quantification of heart injury parameters) and hearts (for biochemical analysis to unfold the mechanisms).

IR injury: Surgery was performed and LAD was ligated for 30 min to induce ischemic injury and it will be followed by reperfusion for 120 min to induce reperfusion injury. Afterwards, the rats were sacrificed to collect blood and hearts for biochemical analysis.

Glatiramer (0.5 mg kg^{-1}) in IR injury: Glatiramer (0.5 mg kg^{-1}) was administered in rats 30 min before performing coronary artery ligation and thereafter, the same procedure was followed as described in group II.

Glatiramer (1.0 mg kg^{-1}) in IR injury: Glatiramer (1.0 mg kg^{-1}) was administered in rats 30 min before performing coronary artery ligation and thereafter, the same procedure was followed as described in group II.

Glatiramer (2.0 mg kg^{-1}) in IR injury: Glatiramer (2.0 mg kg^{-1}) was administered in rats 30 min before performing coronary artery ligation and thereafter, the same procedure was followed as described in group II.

Statistical analysis: The data were represented in the form of Mean±SD. The data were analysed using One way ANOVA followed by Tukey's *post hoc* test. The value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of glatiramer on ischemia-reperfusion-induced cardiac injury: There was a significant increase in myocardial injury in response to left coronary artery ligation, which was assessed by measuring the levels of cardiac injury-specific biomarkers i.e. cTnT in Fig. 1 and CK-MB in Fig. 2. The levels of cTnT were increased from 97 pg mL^{-1} in non-ischemic to 2482 pg mL^{-1} in ischemia-subjected rats; while the levels of CK-MB increased from 475 U L^{-1} in non-ischemic to 1769 U L^{-1} in ischemia-subjected rats. However, treatment with glatiramer (0.5, 1.0 and 2.0 mg kg^{-1}) significantly attenuated the ischemia-reperfusion-induced increase in the release of cTnT and CK-MB in a dose-dependent manner. The levels of cTnT were decreased to 1746, 900 and 319 pg mL^{-1} and the levels of CK-MB were decreased to 1285, 870 and 672.5 U L^{-1} in Glatiramer-treated animals with doses of 0.5, 1.0 and 2.0 mg kg^{-1}. It signifies the importance of glatiramer in conferring cardioprotective actions in ischemia-reperfusion-subjected rats.

Effect of glatiramer on ischemia-reperfusion-induced other biochemical parameters: There was a significant increase in inflammation in hearts in response to ischemia-reperfusion as it was assessed by an increase in the TNF-α levels

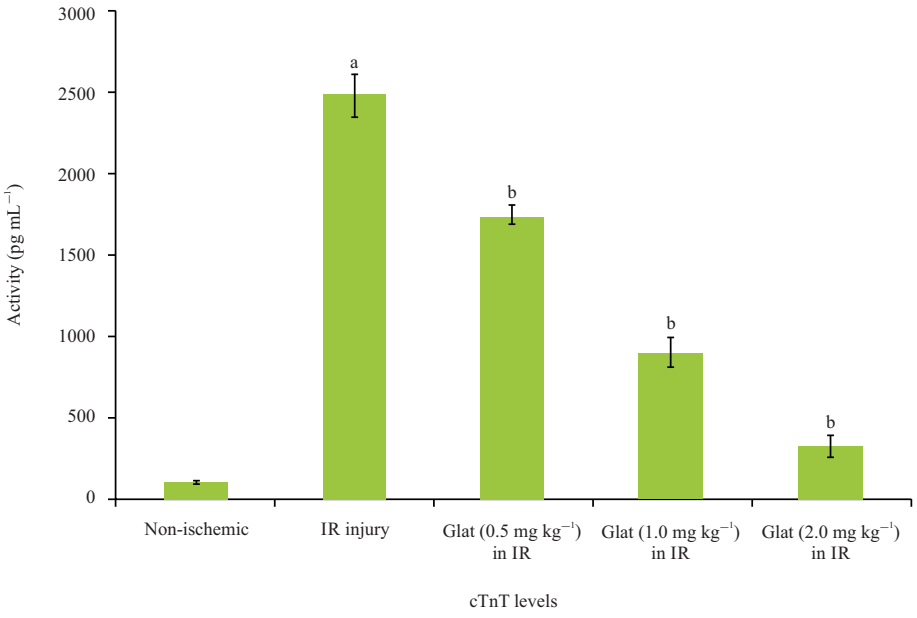

Fig. 1: Effect of different doses of glatiramer on the release of cTnT in the blood in coronary artery ligation model in rats

Value was in Mean±SD, [a]$p<0.05$ vs. non-ischemic, [b]$p<0.05$ vs. IR injury

(95.5 pg mL^{-1} in non-ischemic vs 458 pg mL^{-1} in ischemia) in the heart homogenates in Fig. 3. Moreover, there was a significant decrease in the p-AKT levels (100% in non-ischemic to 46.5% in Ischemia) in Fig. 4 and a decrease in the p-GSK-3β/GSK-3β ratio (1.16 in non-ischemic to 0.343 in ischemia) in Fig. 5 in the heart homogenates of ischemia-reperfusion-subjected rats. The decrease in p-AKT and decrease in p-GSK-3β/GSK-3β ratio suggests the inhibition of enzymatic activities of AKT and activation of GSK-3β. Furthermore, the nuclear: cytoplasmic ratio of Nrf2 (1.45 in non-ischemic to 0.35 in ischemia) in Fig. 6 was significantly reduced in

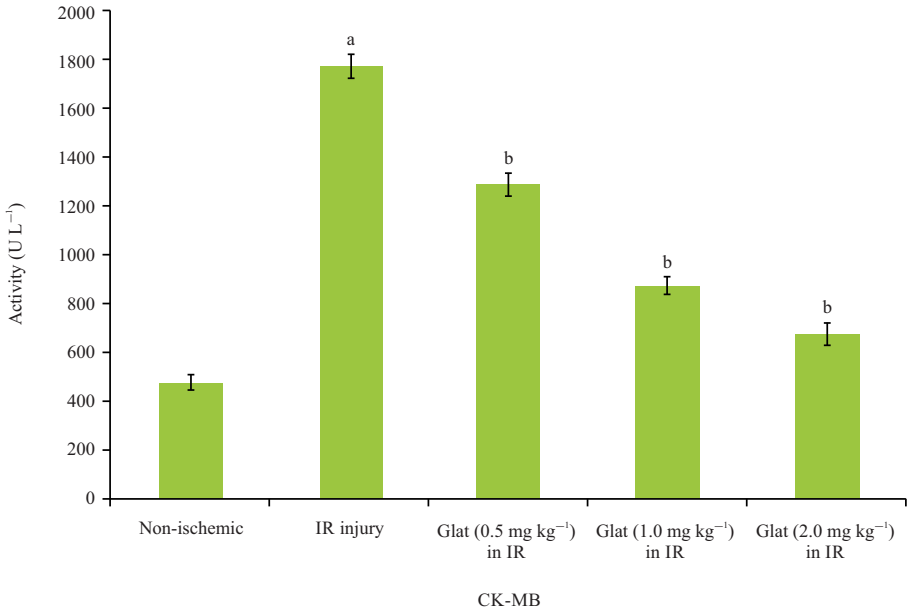

Fig. 2: Effect of different doses of glatiramer on the release of CK-MB in the blood in coronary artery ligation model in rats
Value was in Mean±SD, [a]p<0.05 vs. non-ischemic, [b]p<0.05 vs. IR injury

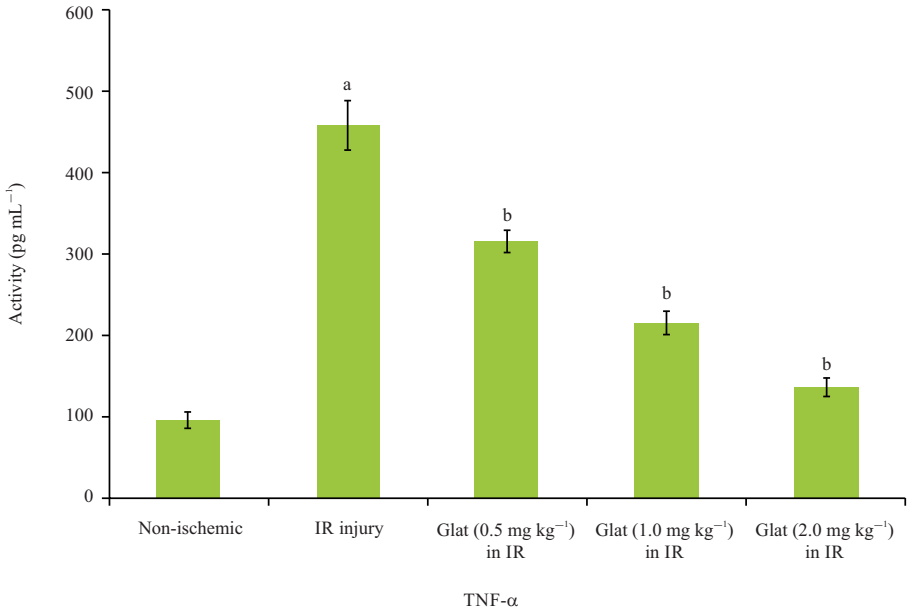

Fig. 3: Effect of different doses of glatiramer on the TNF-a levels in the heart homogenates coronary artery ligation model in rats
Value was in Mean±SD, [a]p<0.05 vs. non-ischemic, [b]p<0.05 vs. IR injury

ischemia-reperfusion-subjected rat hearts. However, treatment with glatiramer (0.5, 1.0 and 2.0 mg kg^{-1}) significantly attenuated ischemia-reperfusion-induced deleterious changes in biochemical parameters in a dose-dependent manner. Glatiramer (0.5, 1.0 and 2.0 mg kg^{-1}) reduced the levels of TNF-α (315.2, 215, 136.3 pg mL^{-1}), increased the levels of p-AKT (57.4, 72.9, 86%), increased the ratio of p-GSK-3β/GSK-3β (0.532, 0.743, 0.871) and nuclear: cytoplasmic Nrf2 (0.55, 0.76, 1.17) in a dose-dependent manner.

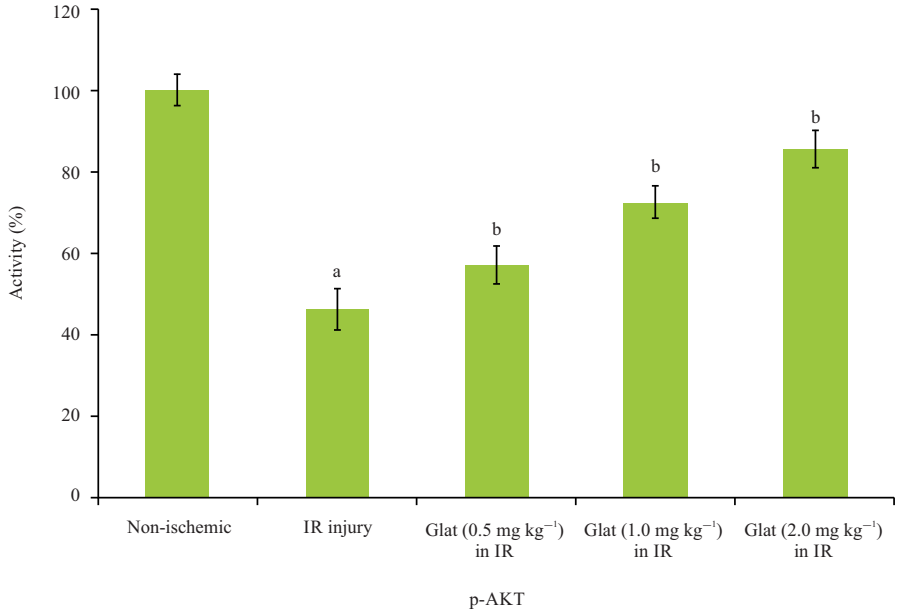

Fig. 4: Effect of different doses of glatiramer on the p-AKT expression in heart homogenates in coronary artery ligation model in rats

Value was in Mean±SD, [a]p<0.05 vs. non-ischemic, [b]p<0.05 vs. IR injury

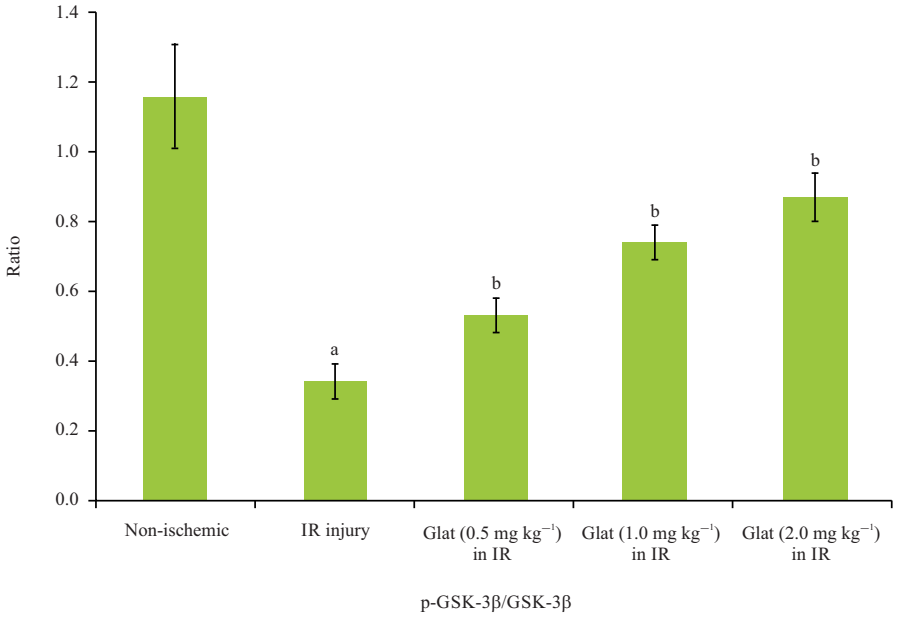

Fig. 5: Effect of different doses of glatiramer on the p-GSK-3ß/GSK-3ß ratio in heart homogenates in coronary artery ligation model in rats

Value was in Mean±SD, [a]p<0.05 vs. non-ischemic, [b]p<0.05 vs. IR injury

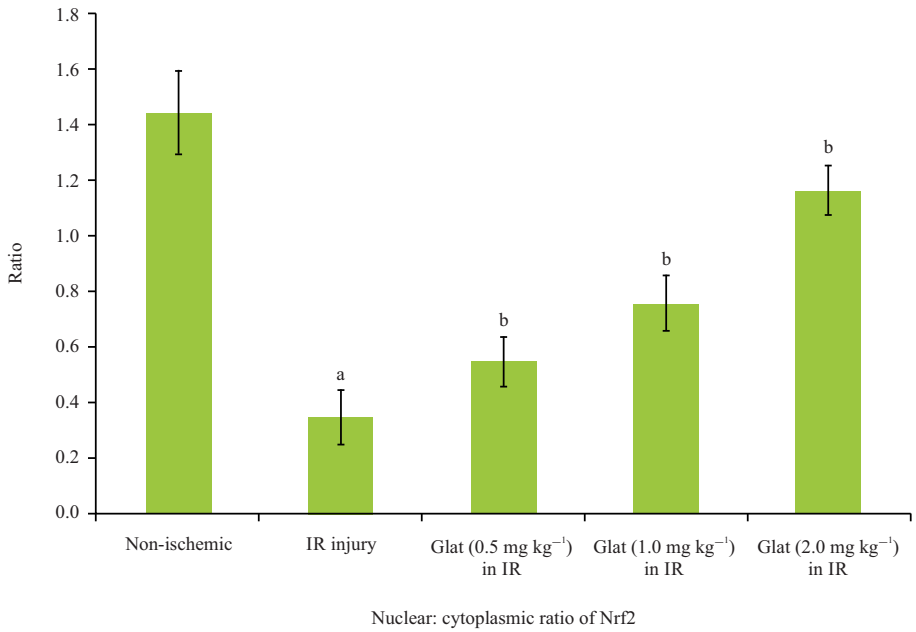

Fig. 6: Effect of different doses of glatiramer on the nuclear: cytoplasmic ratio of Nrf2 ratio in heart homogenates in coronary artery ligation model in rats

Value was in Mean±SD, [a]p<0.05 vs. non-ischemic, [b]p<0.05 vs. IR injury

DISCUSSION

In the present investigation, there was a significant increase in the myocardial injury in response to occlusion of left descending coronary artery ligation. The increase in myocardial injury was assessed by noting the increase in the release of heart injury-specific biomarkers including cTnT and CK-MB in the blood after ischemia and reperfusion injury. Coronary artery ligation is a commonly employed model to assess myocardial injury in laboratory models[17,18] and it closely mimics the ischemic conditions in humans[8]. cTnT and CK-MB are very specific biomarkers to assess myocardial injury[19] and thus, their assessment in this study specifically depicts myocardial injury. There have been studies documenting that ischemia-reperfusion injury in the form of coronary artery ligation produce significant cardiac injury in rats[20,21]. In these studies, the increase in cardiac injury markers were found to be significantly increased following coronary artery ligation, which was also observed in the current study in which there was a significant increase in the levels of cardiac injury biomarkers in coronary artery ligation model.

In this present study, administration of glatiramer in three different doses produced cardioprotective effects and significantly attenuated the release of cTnT and CK-MB from the heart into the bloodstream in response to ischemia and reperfusion injury. It depicts the cardioprotective effects of glatiramer in a dose-dependent manner in the ischemia-reperfusion injury model in rats. Glatiramer is an immunomodulator and has been used in the management of replacing-remitting multiple sclerosis[22]. Apart from it, it has been shown to produce beneficial effects in ischemia-reperfusion induced cerebral injury in non-diabetic[23] as well as diabetic rats[6] along with improvement in hypo perfusion-induced cognitive dysfunction[7]. A very recent study has shown its potential in attenuating oxygen-glucose deprivation-induced injury to cardiomyocytes[11]. However, to the best of our knowledge, it is the first study showing the beneficial effects of glatiramer in attenuating ischemia-reperfusion-induced myocardial injury.

In this study, the glatiramer also attenuated the ischemia-reperfusion-induced increase in TNF-α levels and increased the ratio of nuclear: cytoplasmic Nrf2. There have been studies showing the important role of TNF-α in producing ischemia-reperfusion-induced cardiac injury[24] and a significant rise in the levels of TNF-α has been documented in previous studies involving coronary artery ligation in rats[25]. Nrf2 is a transcriptional factor and its increase in nuclear: cytoplasmic ratio suggests an increase antioxidant activity[26]. There have been previous studies showing that the ratio of Nrf2 is significantly reduced in hearts after coronary artery ligation-induced ischemia-reperfusion injury[27]. Furthermore, an increase in nuclear: cytoplasmic ratio of Nrf2 has been shown to confer cardioprotection against ischemic injury[28]. Previous studies have shown that glatiramer may attenuate the

expression of TNF-α and exhibit strong anti-inflammatory effects[29]. However, it is the first study showing the effects of glatiramer on increasing the expression of Nrf2. Based on these, it is hypothesized that a glatiramer-mediated decrease in inflammation and increase in antioxidant activity may contribute to attenuating ischemia-reperfusion-induced myocardial injury.

In this investigation, glatiramer also normalized ischemia-reperfusion-induced decrease in p-AKT levels and p-GSK-3β/GSK-3β ratio in rat hearts. AKT and GSK-3β constitute an important intracellular signalling pathway[30,31]. The activation of AKT (depicted by an increase in p-AKT) leads to phosphorylation of GSK-3β and phosphorylation of GSK-3β (p-GSK-3β) leads to a decrease in enzymatic activity of GSK-3β[32,33]. Therefore, it may be proposed that treatment of glatiramer led to an increase in the activation of AKT, which subsequently increased the phosphorylation of GSK-3β to decrease its activity. The previous studies have shown that coronary artery ligation leads to a decrease in p-AKT levels[34] and p-GSK-3β/GSK-3β ratio in hearts[35]. Moreover, there have been various studies showing that activation of AKT and inhibition of GSK-3β attenuates ischemia-reperfusion-induced cardiac injury[36,37]. Moreover, there has been a previous study showing that glatiramer may activate AKT-GSK-3β linked pathway[38]. Therefore, it may be proposed that glatiramer may produce beneficial effects in ischemia-reperfusion injury by activating the AKT-GSK-3β signalling pathway.

CONCLUSION

Glatiramer may attenuate myocardial injury in the coronary artery ligation model in rats, which may be possibly attributed to the activation of the AKT-GSK-3β signalling pathway along with the decrease in inflammation and increase in antioxidant activities in rat hearts.

SIGNIFICANCE STATEMENT

This study discovered that glatiramer can be beneficial for treating the ischemia-reperfusion-induced cardiac injury. Till now the therapeutic effects of glatiramer in ischemia-reperfusion-induced heart injury were not discovered. Moreover, the other researchers were not able to find the molecular mechanism involved in glatiramer-mediated cardioprotection, which involves activation of the AKT-GSK-3β signalling pathway. This study will help the researchers to uncover the cardioprotective uses of glatiramer in ischemic hearts.

REFERENCES

1. Ambrose, J.A., 2006. Myocardial ischemia and infarction. J. Am. Coll. Cardiol., 47: D13-D17.
2. Bates, D.W., E. Miller, S.J. Bernstein, P.J. Hauptman and L.L. Leape, 1997. Coronary angiography and angioplasty after acute myocardial infarction. Ann. Internal Med., 126: 539-550.
3. Frank, A., M. Bonney, S. Bonney, L. Weitzel, M. Koeppen and T. Eckle, 2012. Myocardial ischemia reperfusion injury. Semin. Cardiothorac. Vasc. Anesthesia, 16: 123-132.
4. Mantia, L.L., L.M. Munari and R. Lovati, 2010. Glatiramer acetate for multiple sclerosis. Cochrane Database Syst. Rev., Vol. 2014. 10.1002/14651858.cd004678.pub2.
5. Arnon, R. and R. Aharoni, 2019. Glatiramer acetate: From bench to bed and back. Isr Med Assoc J., 21: 151-157.
6. Mangin, G., M. Poittevin, C. Charriaut-Marlangue, C. Giannesini, T. Merkoulova-Rainon and N. Kubis, 2019. Glatiramer acetate reduces infarct volume in diabetic mice with cerebral ischemia and prevents long-term memory loss. Brain Behav. Immun., 80: 315-327.
7. Chen, L., Y. Yao, C. Wei, Y. Sun and X. Ma *et al*., 2015. T cell immunity to glatiramer acetate ameliorates cognitive deficits induced by chronic cerebral hypoperfusion by modulating the microenvironment. Sci. Rep., Vol. 5. 10.1038/srep14308.
8. Nezamoleslami, S., M. Sheibani, A.R. Dehpour, P. Mobasheran and H. Shafaroodi, 2020. Glatiramer acetate attenuates renal ischemia reperfusion injury in rat model. Exp. Mol. Pathol., Vol. 112. 10.1016/j.yexmp.2019.104329.
9. Askarifirouzjaei, H., L. Khajoueinejad, A.S. Farrokhi, M.T. Tahoori, M. Fazeli, T. Tiraihi and A.A. Pourfathollah, 2019. Implications of immunotherapy with high-dose glatiramer acetate in acute phase of spinal cord injury in rats. Immunopharmacol. Immunotoxicol., 41: 150-162.
10. You, Y., Y. Zhao, H. Bai, Z. Liu, F. Meng, H. Zhang and R. Xu, 2013. Glatiramer acetate, an anti-demyelination drug, reduced rats' epileptic seizures induced by pentylenetetrazol via protection of myelin sheath. Eur. J. Pharm. Sci., 49: 366-370.
11. Du, J., W. Lv, S. Yang, J. Liu, J. Zhen and J. Leng, 2020. Glatiramer acetate protects against oxygen-glucose deprivation/reperfusion-induced injury by inhibiting Egr-1 in H9c2 cells. Mol. Immunol., 120: 61-66.
12. Liu, Q., Z. Li, Y. Liu, Q. Xiao and X. Peng *et al*., 2018. Hydromorphine postconditioning protects isolated rat heart against ischemia-reperfusion injury via activating P13K/Akt/eNOS signaling. Cardiovasc. Ther., Vol. 36. 10.1111/1755-5922.12481.
13. Wei, D., H. Xu, X. Gai and Y. Jiang, 2019. Astragaloside IV alleviates myocardial ischemia-reperfusion injury in rats through regulating PI3K/AKT/GSK-3β signaling pathways. Acta Cirurgica Bras., Vol. 34. 10.1590/s0102-865020190070000008.

14. Huang, X.W., M.D. Pan, P.H. Du and L.X. Wang, 2018. Arginase-2 protects myocardial ischemia-reperfusion injury via NF-κB/TNF-α pathway. Eur. Rev. Med. Pharmacol. Sci., 22: 6529-6537.
15. Shen, Y., X. Liu, J. Shi and X. Wu, 2019. Involvement of Nrf2 in myocardial ischemia and reperfusion injury. Int. J. Biol. Macromol., 125: 496-502.
16. Samsamshariat, S.A., Z.A. Samsamshariat and M.R. Movahed, 2005. A novel method for safe and accurate left anterior descending coronary artery ligation for research in rats. Cardiovasc. Revasculariz. Med., 6: 121-123.
17. Moon, C., M. Krawczyk, D. Ahn, I. Ahmet, D. Paik, E.G. Lakatta and M.I. Talan, 2003. Erythropoietin reduces myocardial infarction and left ventricular functional decline after coronary artery ligation in rats. Proc. Nat. Acad. Sci. USA, 100: 11612-11617.
18. Yin, Y., Q. Zhang, Q. Zhao, G. Ding and C. Wei *et al*., 2019. Tongxinluo attenuates myocardiac fibrosis after acute myocardial infarction in rats via inhibition of endothelial-to-mesenchymal transition. BioMed Res. Int., Vol. 2019. 10.1155/2019/6595437.
19. Emadi, N., M.H. Nemati, M. Ghorbani and E. Allahyari, 2019. The effect of high-dose vitamin C on biochemical markers of myocardial injury in coronary artery bypass surgery. Braz. J. Cardiovasc. Surg., 34: 517-524.
20. Zhang, X., L.F. Huang, L. Hua, H.K. Feng and B. Shen, 2019. Resveratrol protects myocardial apoptosis induced by ischemia-reperfusion in rats with acute myocardial infarction via blocking P13K/Akt/e-NOS pathway. Eur. Rev. Med. Pharmacol. Sci., 23: 1789-1796.
21. Dong, L.Y., X.X. Qiu, Y. Zhuang and S. Xue, 2019. Y-27632, a Rho-kinase inhibitor, attenuates myocardial ischemia-reperfusion injury in rats. Int. J. Mol. Med., 43: 1911-1919.
22. Mantia, L.L., C.D. Pietrantonj, M. Rovaris, G. Rigon and S. Frau *et al*., 2016. Interferons-beta versus glatiramer acetate for relapsing-remitting multiple sclerosis. Cochrane Database Syst. Rev., Vol. 11. 10.1002/14651858.cd009333.pub3.
23. Cruz, Y., J. Lorea, H. Mestre, J.H. Kim-Lee and J. Herrera *et al*., 2015. Copolymer-1 promotes neurogenesis and improves functional recovery after acute ischemic stroke in rats. PLOS ONE, Vol. 10. 10.1371/journal.pone.0121854.
24. Zhou, C.N., W. Yao, Y.N. Gong, Y. Li, C.H. Wang and Y.F. Huo, 2019. 22-oxacalcitriol protects myocardial ischemia-reperfusion injury by suppressing NF-κB/TNF-α pathway. Eur. Rev. Med. Pharmacol. Sci., 23: 5495-5502.
25. Wang, D.S., L.Y. Yan, D.Z. Yang, Y. Lyu, L.H. Fang, S.B. Wang and G.H. Du, 2020. Formononetin ameliorates myocardial ischemia/reperfusion injury in rats by suppressing the ROS-TXNIP-NLRP3 pathway. Biochem. Biophys. Res. Commun., 525: 759-766.
26. Zhang, Z., J. Qu, C. Zheng, P. Zhang and W. Zhou *et al*., 2018. Nrf2 antioxidant pathway suppresses numb-mediated epithelial–mesenchymal transition during pulmonary fibrosis. Cell Death Dis., Vol. 9. 10.1038/s41419-017-0198-x.
27. Rani, N. and D.S. Arya, 2020. Chrysin rescues rat myocardium from ischemia-reperfusion injury via PPAR-γ/Nrf2 activation. Eur. J. Pharmacol., Vol. 883. 10.1016/j.ejphar.2020.173389.
28. Xu, G., X. Zhao, J. Fu and X. Wang, 2019. Resveratrol increase myocardial Nrf2 expression in type 2 diabetic rats and alleviate myocardial ischemia/reperfusion injury (MIRI). Ann. Palliative Med., 8: 565-575.
29. Li, Q.Q., D.R. Burt and C.T. Bever, 2001. Glatiramer acetate inhibition of tumor necrosis factor-α-induced RANTES expression and release from U-251 MG human astrocytic cells. J. Neurochem., 77: 1208-1217.
30. Yang, W., Y. Liu, Q.Q. Xu, Y.F. Xian and Z.X. Lin, 2020. Sulforaphene ameliorates neuroinflammation and hyperphosphorylated tau protein via regulating the PI3K/Akt/GSK-3ß pathway in experimental models of alzheimer's disease. Oxid. Med. Cell. Longevity, Vol. 2010. 10.1155/2020/4754195.
31. Xin, Y., Y. Bai, X. Jiang, S. Zhou and Y. Wang *et al*., 2018. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 via stimulating the Akt/GSK-3β/Fyn pathway. Redox Biol., 15: 405-417.
32. Beurel, E., S.F. Grieco and R.S. Jope, 2015. Glycogen synthase kinase-3 (GSK3): Regulation, actions and diseases. Pharmacol. Ther., 148: 114-131.
33. Fang, X., S.X. Yu, Y. Lu, R.C. Bast, J.R. Woodgett and G.B. Mills, 2000. Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. Proc. Nat. Acad. Sci. USA, 97: 11960-11965.
34. Wang, D., X. Zhang, D. Li, W. Hao and F. Meng *et al*., 2017. Kaempferide protects against myocardial ischemia/reperfusion injury through activation of the PI3K/Akt/GSK-3β pathway. Mediators Inflammation, Vol. 2017. 10.1155/2017/5278218.
35. Zeng, Z., Q. Wang, X. Yang, Y. Ren and S. Jiao *et al*., 2019. Qishen granule attenuates cardiac fibrosis by regulating TGF-β/Smad3 and GSK-3β pathway. Phytomedicine, Vol. 62. 10.1016/j.phymed.2019.152949.
36. Arslan, F., R.C. Lai, M.B. Smeets, L. Akeroyd and A. Choo *et al*., 2013. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res., 10: 301-312.
37. Murphy, E. and C. Steenbergen, 2005. Inhibition of GSK-3β as a target for cardioprotection: The importance of timing, location, duration and degree of inhibition. Expert Opin. Ther. Targets, 9: 447-456.
38. Carpintero, R., K.J. Brandt, L. Gruaz, N. Molnarfi, P.H. Lalive and D. Burger, 2010. Glatiramer acetate triggers PI3Kδ/AKT and MEK/ERK pathways to induce IL-1 receptor antagonist in human monocytes. Proc. Nat. Acad. Sci. USA, 107: 17692-17697.