Pharmacological Reports 71 (2019) 682–687

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Cardioprotective effects of cerebrolysin on the lesion severity and inflammatory factors in a rat model of isoproterenol-induced myocardial injury

Abolfazl Ardjmand[a,b], Mohammad Esmaeil Shahaboddin[c], Tahere Mazoochi[d], Azhdar Heydari[a,b], Gholamreza Ghavipanjeh[a,b,*]

[a] Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran
[b] Department of Physiology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[c] Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[d] Department of Pathology, Kashan University of Medical Sciences, Kashan, Iran

ARTICLE INFO

Article history:
Received 9 September 2018
Received in revised form 24 February 2019
Accepted 11 March 2019
Available online 16 March 2019

Keywords:
Isoproterenol
Cerebrolysin
Inflammatory factor
Myocardial injury
Rat

ABSTRACT

Background: Myocardial injury (MI) is an important heart condition and a major cause of morbidity and mortality worldwide. The current study was designed to investigate the cardioprotective effects of cerebrolysin (CLY) on the lesion severity and inflammatory factors in male rats using isoproterenol (ISO)-induced MI model.

Methods: MI in rats was induced by injecting ISO (100 mg/kg) subcutaneously (*sc*) on the first 2 days. Then, CLY (5 ml/kg) was injected intraperitoneally (*ip*) post-treatment for 7 days. On the 3rd day, creatine phosphokinase (CK-MB) and cardiac troponin I (cTnI) levels in serum and, on the 10th day, the TNF-α and IL6 levels in serum and heart tissue were measured by enzyme-linked immunosorbent assay (ELISA). Finally, the heart of each rat was dissected out and stained for histopathological examination.

Results: On the 3rd day, the serum CK-MB and cTnI levels in the ISO and CLY + ISO groups were significantly increased compared with that in the control and CLY + Sal groups. One week after the induction of MI, ISO administration showed a significant increase in the serum level of TNF-α in the ISO group compared with that in the control and CLY + Sal groups. Also, our findings showed only a moderate reduction in inflammatory cell infiltration and extent of edema following CLY treatment in the CLY + ISO group. Also, CLY induced vascular proliferation in the heart tissue.

Conclusions: We conclude that the severity of pathological changes induced by ISO in MI (e.g. inflammation and edema) can be limited by CLY treatment.

Introduction

Myocardial injury (MI), an important heart condition, is one of the major causes of morbidity and mortality worldwide [1,2]. According to the World Health Organization report, 12 million deaths due to MI occur throughout the world each year. Despite the progress made over the last few decades in the treatment of MI with the introduction of pharmacological and mechanical interventions, MI remains a major cause of death [3,4]. It has been established that the limitation of the extent of the injury is an important factor in reducing the consequences of MI [3,4].

MI occurs when there is an imbalance between the coronary blood supply and myocardial demand [1]. Serum levels of creatine phosphokinase (CK-MB) and cardiac troponins (cTnI) are considered as sensitive and specific diagnostic markers of MI [1]. Increased levels of cTnI and inflammation-related proteins are frequently associated with MI [5].

Oxidative stress is caused by an imbalance in the body's redox condition, in which the increased free radicals in the body result in tissue damage [6]. Studies have shown that there is increased production of reactive oxygen species (ROS) in myocardial ischemia and MI [7]. Research evidence suggests that ROS plays a key role in the development of MI [8]. Some studies have shown that oxidative stress can activate the production of proinflammatory cytokines, e.g., tumor necrosis factor alpha (TNF-α), interleukin-1β (IL1β), and interleukin 6 (IL6) [9]. In fact, accumulation and

* Corresponding author.
E-mail address: Ghavipanjeh-gh@kaums.ac.ir (G. Ghavipanjeh).

http://dx.doi.org/10.1016/j.pharep.2019.03.003
1734-1140/

activation of monocytes and macrophages in the area of necrosis cause release of the cytokines [9].

Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic receptor agonist [1]. It has been known that ISO in supramaximal doses has deleterious effects on the heart [1,10]. ISO causes complex biochemical and structural changes leading to cell damage and necrosis *via* the induction of oxidative stress, myocardial damage, and depletion of the energy reserve of cardiac muscle cells [10,11]. The pathophysiological changes following ISO administration are comparable to those occurring in human myocardial pathologies [12].

Cerebrolysin (CLY), a peptide derivative, acts as a growth and neurotrophic factor in the brain [13,14]. It is a mixture of different neurotrophic factors, e.g., brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, nerve growth factor, ciliary neurotrophic factor, and other peptide fragments [15]. It has been suggested that CLY exerts its effect by the inhibition of apoptosis and production of ROS and, also, lipid peroxidation products, which can contribute to the inhibition of neuronal death and neuroinflammation [13,14]. Moreover, it has been reported that CLY can promote neuronal survival and stimulate neurogenesis [13,14].

Therefore, the important question that remains unanswered is whether CLY can improve the myocardial function, promote myocyte survival, and increase cardiac blood flow. Hence, the present study was designed to investigate the cardioprotective effects of CLY on the lesion severity and inflammatory factors in a rat model of ISO-induced MI.

Materials and methods

Animals

Thirty-two adult male Wistar rats (150–200 g), supplied by Physiology Research Center, Kashan University of Medical Sciences (KAUMS) (Kashan, Iran), were used for the study. The animals were kept in standard cages under a 12 h light–dark cycle at 22 °C–24 °C with 50% humidity for one week before the experiment. The rats had free access to food and water. All the experimental procedures used in this study were performed in accordance with the ethical guidelines of KAUMS, and the study was approved by the Ethics Committee of KAUMS and registered under the code IR.KAUMS.MEDNT.REC.1396.8.

Drugs

ISO was purchased from Merck (Germany). CLY was obtained from EVER Neuro Pharma GmbH (Austria) (5 ml vial, 1 ml contains 215.2 mg of CLY).

Induction of myocardial injury

ISO was dissolved in physiological saline and injected subcutaneously (*sc*) to rats (100 mg/kg/day) at an interval of 24 h for 2 days (days 1 and 2) to induce experimental MI [10,16,17].

Experimental protocols

After acclimatization, the rats were divided into 4 groups of 8 rats each. Group 1 (control) was given saline (*sc*) for 2 days (days 1 and 2), and then, saline intraperitoneally (*ip*) for 7 days (days 3–9); Group 2 (ISO) was given ISO (100 mg/kg/day, *sc*) for 2 consecutive days (days 1 and 2), and then, saline (*ip*) for 7 days (days 3–9); Group 3 (CLY + ISO) was given ISO (100 mg/kg/day, *sc*) for 2 consecutive days (days 1 and 2), and then, CLY (5 ml/kg, *ip*) [14] for 7 days (days 3–9); Group 4 (CLY + Sal) was given saline (*sc*) for 2 days (days 1 and 2), and then, CLY (5 ml/kg, *ip*) for 7 days (days 3–9).

Serum biochemical assays

Blood samples were taken *via* tail vein on the 3rd day (24 h after the second injection of ISO to measure CK-MB, cTnI, and confirm the induction of MI) and also on the 10th day (24 h after the last injection of CLY) *via* cardiac puncture before sacrificing. Blood samples were centrifuged, and the prepared serum was used for the biochemical analysis. Also, on the 10th day, the heart of each rat was excised and washed with phosphate buffer saline [10,14]. The apex of the heart samples was fixed in 10% buffered formaldehyde for histopathological evaluation, and the remaining tissue was stored at −70 °C for further analyses.

Biomarker analyses

I Cardiac biomarkers

The serum levels of CK-MB and cTnI were measured as markers for cardiac damage. The serum levels of CK-MB and cTnI were determined spectrophotometrically at 450 nm using commercially available kits (Zellbio, Germany) and enzyme-linked immunosorbent assay (ELISA) (Mybiosourc, USA) [14], respectively.

II Inflammatory markers

On the 10th day and before sacrificing, the blood samples were collected by cardiac puncture. After leaving the samples for 30 min at room temperature and allowing them to clot, the samples were centrifuged, and the serum was collected. The TNF-α and IL6 levels in serum and heart tissue were measured by ELISA kit (Eastbiopharm, China) according to the manufacturer's instructions.

Histopathological examination

After the completion of the behavioral experiments and sacrificing the animals, the heart of each rat was immediately dissected out and washed with phosphate buffer saline [10,14]. The cardiac apex of the samples was excised and fixed in buffered formaldehyde solution (10%). The apex of the samples was embedded in paraffin. Then, the sectioned tissues were stained with hematoxylin and eosin to evaluate the necrosis histologically. The prepared slides were examined under light microscopy, and the histopathological photomicrographs (thickness 4 μm) were prepared [7,10,12]. The histological findings were reported in the order of severity and extent of myocardial necrosis for each specimen. Two blinded pathologists separately examined the photomicrographs. Based on a minimum of 10 fields for each specimen, the slides were evaluated for myocardial necrosis, inflammatory cell infiltration, and edema and scored accordingly on a scale of severe (+++), moderate (++), mild (+), and nothing (−) [8,18,19].

Statistical analyses

All data were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A $p < 0.05$ was considered as statistically significant.

Results

Cardiac biomarkers

Fig. 1 shows the CK-MB level in serum on the 3rd day (24 h after the second injection of ISO) in all groups. The one-way ANOVA showed a significant difference between the groups ($p < 0.05$).

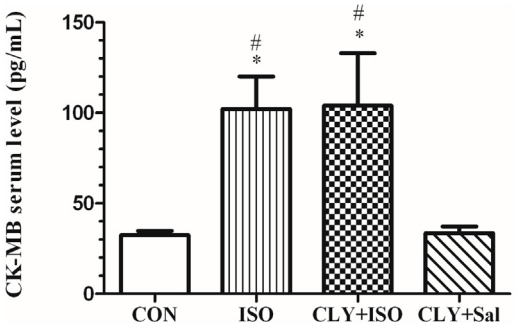

Fig. 1. Serum level of CK-MB (ng/ml) on the 3rd (24 h after the second injection of ISO) in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM. * indicates significant difference compared to Control group ($p < 0.05$). # indicates significant difference compared to (CLY+Sal) group ($p < 0.05$).

Also, Tukey's *post hoc* test showed a significant difference between the ISO, control and CLY + Sal groups ($p < 0.05$); CLY + ISO, control and CLY + Sal groups ($p < 0.05$).

Fig. 2 shows the cTnI level in serum on the 3rd day (24 h after the second injection of ISO) in all groups. The ANOVA showed a significant difference between the groups ($p < 0.01$). Also, Tukey's post hoc test showed a significant difference between the ISO and control groups ($p < 0.01$); ISO and CLY + Sal groups ($p < 0.01$); CLY + ISO, control and CLY + Sal groups ($p < 0.05$).

Inflammatory biomarkers

Serum level of TNF-α

Fig. 3 shows the TNF-α level in serum on the 10th day (24 h after the last injection of CLY) in all groups. The ANOVA showed a significant difference between the groups ($p < 0.01$). Also, Tukey's post hoc test showed a significant difference between the ISO and control groups ($p < 0.05$) and ISO and CLY + Sal groups ($p < 0.01$).

Serum level of IL6

Fig. 4 shows the IL6 level in serum on the 10th day in all groups (24 h after the last injection of CLY). The serum level of IL6 did not show a significant difference between the groups.

TNF-α level in heart tissue

Fig. 5 shows the TNF-α level in heart tissue on the 10th day in all groups (24 h after the last injection of CLY). The tissue level of TNF-α did not show a significant difference between the groups.

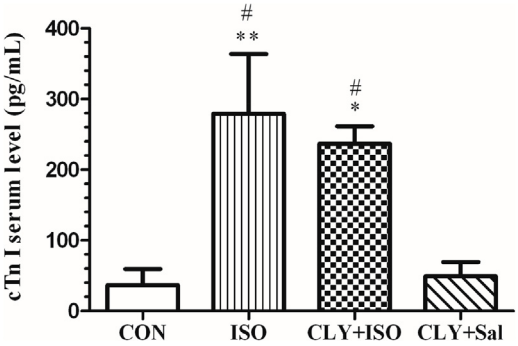

Fig. 2. Serum level of cTnI (pg/ml) on the 3rd (24 h after the second injection of ISO) in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM. * indicates significant difference compared to Control group ($p < 0.05$). # indicates significant difference compared to (CLY+Sal) group ($p < 0.05$).

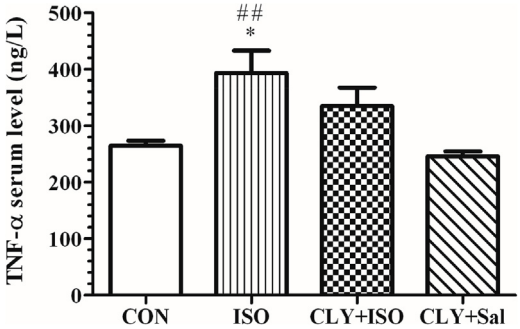

Fig. 3. Serum level of TNF-α (ng/L), one week after the induction of MI in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM. * indicates significant difference compared to Control group ($p < 0.05$). ## indicates significant difference compared to (CLY + Sal) group ($p < 0.01$).

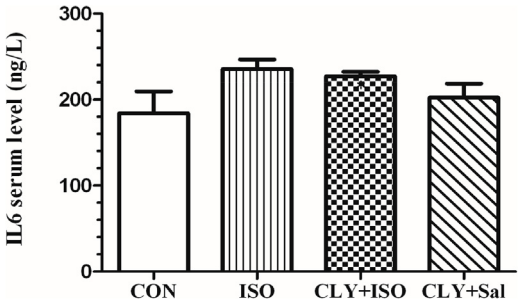

Fig. 4. Serum level of IL6 (ng/L), one week after the induction of MI in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM.

IL6 level in heart tissue

Fig. 6 shows the IL6 level in heart tissue on the 10th day in all groups (24 h after the last injection of CLY). The tissue level of IL6 did not show a significant difference between the groups.

Histopathological study

Histopathological examination of the tissue sections in the control and CLY + Sal groups showed no evidence of microscopic changes (Fig. 7, Table 1). In the ISO group, the heart tissue showed focal confluent necrosis of muscle fibers with inflammatory cell infiltration, edema, and fibroblastic proliferation, confirming ISO-induced MI. The CLY + ISO group showed moderate infiltration of

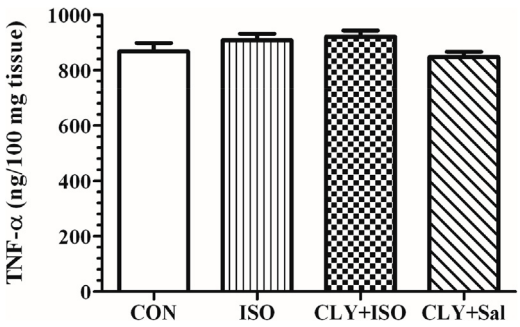

Fig. 5. TNF-α tissue level (ng/100 mg tissue) one week after the induction of MI in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM.

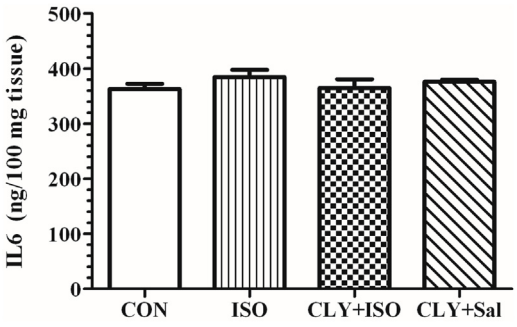

Fig. 6. IL6 (ng/ml) tissue level (ng/100 mg tissue) one week after the induction of MI in four groups (number of 8 in each group). CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline. Data are expressed as mean ± SEM.

inflammatory cells and edema (Fig. 7, Table 1). The CLY + ISO group also showed vascular proliferation (Fig. 7).

Discussion

This study was designed to investigate the effect of CLY on ISO-induced MI, MI markers, and inflammatory factors (TNF-α and IL6) in male rats.

Our results showed that on the 3rd day, the serum CK-MB and cTnI levels in the ISO and CLY + ISO groups were significantly increased compared with that in the control and CLY + Sal groups. However, the CLY + Sal group showed no difference compared with the control group. For one week after MI (10th day), the ISO administration resulted in a significant increase in serum level of TNF-α in ISO group compared with that in the control and CLY + Sal groups. Also, our findings showed only a moderate reduction in inflammatory cell infiltration and extent of edema following CLY treatment in the CLY + ISO group. Also, CLY induced vascular proliferation in the heart tissue.

Catecholamines play an important role in the regulation of myocardial contractility and metabolism [18]. Diverse mechanisms for ISO-induced MI have been reported by researchers. For example, ISO acting on β- adrenoceptors results in positive inotropic and chronotropic effects. Thus, ISO produces partial ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension [2]. Other probable mechanisms may include the increased cyclic adenosine monophosphate level, increased intracellular Ca^{2+} overload, depletion of high-energy phosphate stores, and oxidative stress [2]. Increased generation of cytotoxic free radicals due to the autoxidation of metabolic products of ISO is one of the well-known mechanisms of ISO-induced myocardial necrosis. Following ISO administration, the heart weight increases significantly, with the body weight relatively unchanged [2,11].

Table 1
Effect of CLY on histopathological changes in the ISO-induced MI in rats. ISO, Isoproterenol: CON, Control; CLY, Cerebrolysin; Sal, saline. CON: received 2 days injections of saline (*sc*) and 7 days injections of saline (*ip*); ISO: received 2 days injections of isoproterenol (100 mg/kg/*sc*) and 7 days injections of saline (*ip*); CLY + ISO: received 2 days injections of isoproterenol (100 mg/kg/*sc*) and 7 days injections of CLY (5 ml/kg/*ip*). CLY + Sal: received 2 days injections of saline (*sc*) and 7 days injections of CLY (5 ml/kg/*ip*).

Group	Myonecrosis	Inflammation	Edema
CON	–	–	–
ISO	+++	+++	+++
CLY + ISO	+++	++	++
CLY + Sal	–	–	–

Previous studies have shown that the use of other animal models for the induction of experimental MI (e.g. banding, β-adrenergic agonist microinfusion, and ligation) have some disadvantages, such as infection, other post-injury complications, and high morbidity and mortality [20]. However, the ISO-induced MI model, as reported by some researchers, is a reproducible [21], rapid, simple, and non-invasive method, mimicking the acute type of clinical MI [20]. Reportedly, in ISO-induced MI model, the injury occurs in the subendocardium of the left ventricle with acute extensive myofibrillary degeneration [22].

As previously reported, the serum levels of CK-MB and cTnI are very sensitive and specific diagnostic markers for MI [1]. Our results on the 3rd day confirmed the findings of the previous studies that ISO-induced significant increases in serum CK-MB and cTnI levels which are considered as the reason for the severe damage of heart tissue [23,24].

Various factors, such as inflammation and oxidative stress, are involved in the pathogenesis of MI. It is reported that the plasma

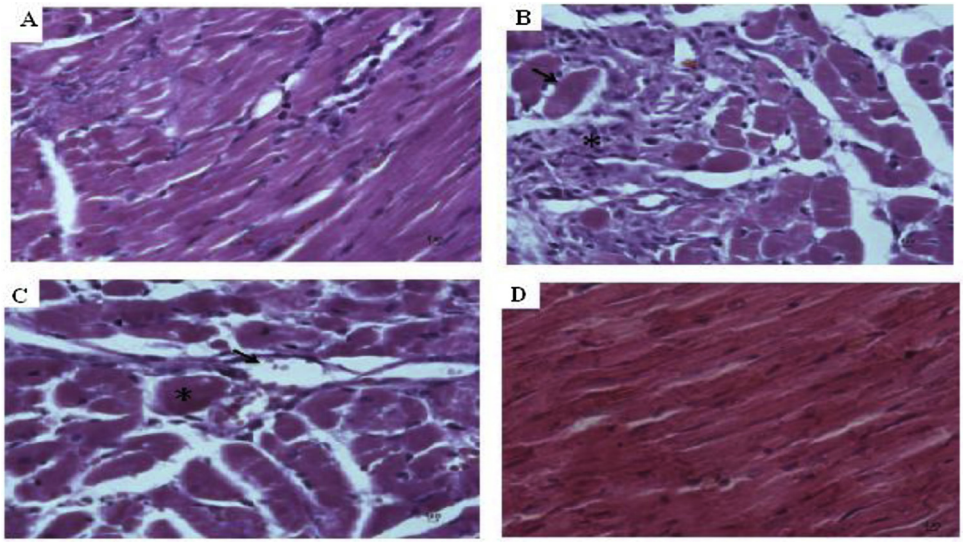

Fig. 7. Effect of Cerebrolysin on the rat heart histopathology in the ISO-induced MI in rats. (**A**) Control group shows normal histological pattern (H&E; original magnification ×400); (**B**) ISO group shows cell necrosis (arrow) and infiltration of inflammatory cells (asterisks) (H&E; original magnification ×400); (**C**) CLY + ISO group shows vascular proliferation (arrow) and cell necrosis (asterisks) with less edema (H&E; original magnification ×400); (**D**) (CLY + Sal) group shows normal histological pattern (H&E; original magnification ×400); CON, control; ISO, Isoproterenol; (CLY + ISO), Cerebrolysin + Isoproterenol; (CLY + Sal), Cerebrolysin + Saline.

levels of TNF-α and IL6 increase immediately after an acute MI [9]. However, our results showed that one week after the induction of MI, there was a significant increase only in the TNF-α serum level in the ISO group compared with the level in the control group. Nonetheless, IL6 serum level did not show a significant increase compared with that in the control group. According to previous studies, while 2–3 days after the induction of MI the concentration of IL6 in serum increases, its concentration reduces gradually in the following days [25,26]. The possible explanation for the discrepancy seen is that in our study, the IL6 concentration was high on the first day after the induction of MI, but because the subsequent measurement was done one week later, we could not detect the elevated IL6 concentration. Therefore, we suggest that the serum level of IL6 be checked daily for one week after the induction of MI. In addition, we observed an elevated TNF-α level, one week after the induction of MI, due to its higher stability [27].

Earlier studies have suggested several mechanisms for CLY activity, such as the inhibition of apoptosis, production of free radicals, and lipid peroxidation [28]. All these pathways contribute to the inhibition of neuronal death and inflammation in neurodegenerative diseases [28–30]. As an example, in a study using the focal ischemic stroke model in rats, it has been reported that CLY administered 24 or 48 h after the stroke onset resulted in a decrease in the extent of the infarct [31].

Our study result also showed that the serum level of TNF-α in the CLY + ISO group was reduced, but not significantly, in comparison with that in the ISO group. It is suggested that if the dose or duration of the CLY treatment is increased, the serum level of TNF-α will significantly be reduced in the CLY + ISO group compared with that in the ISO group.

Also, the results showed a slight, but non-significant, increase in tissue TNF-α level one week after MI between the ISO and CLY + ISO groups in comparison with the control group. Similarly, Deten et al. reported that there was only a slight, but non-significant, increase in IL6 expression in myocardium 12 weeks after MI [26]. On the other hand, Prabhu et al. showed a prominent increase in IL6 expression in myocardium 12 weeks after MI [32]. Also, the results of Ono et al. showed an increase in IL6 expression 8 and 20 weeks after MI [33]. However, we did not observe a significant difference in heart tissue levels of IL6 between the 4 groups for one week after MI. One possible reason for the observed effect is that, in our study, the IL6 and TNF-α may have been expressed at the genomic level but did not result in a product. Otherwise, in the case of a gene product, the alternative reason might be that the concentration of the product was far less than the sensitivity of the ELISA kits.

In contrast, since ISO produces partial ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension [2], one may suggest that due to the fewer accumulation of leukocytes and macrophages in cardiac ischemia resulting from reduced blood flow, the TNF-α and IL6 levels in the heart tissue would be low.

To the best of our knowledge, there is only one study that shows the ameliorative effect of CLY on heart ischemia. Boshra et al. reported that pretreatment with CLY could protect myocardium against ischemia in rats. In that study, the observed effect was attributed to the antioxidant and anti-apoptotic properties of CLY [14]. This ameliorative effect of CLY on heart tissue suggests its possible use in the experimental treatment of heart disorders, such as myocardial infarction [14]. Nonetheless, our findings showed that CLY post-treatment did not have a significant effect on MI and only moderately reduced the inflammation and tissue edema.

In addition, in our study, the histopathological examination of the ISO-injected heart tissue showed an abnormal histological structure of the myocardial bundle with inflammatory cell infiltration, edema, and fragmentation of muscle fibers, which indicates the involvement of oxidative stress and inflammatory processes in ISO-induced MI. However, CLY treatment in ISO-injected rats resulted in a moderate histopathological change, confirmed by a reduction in inflammatory cell infiltration and edema in the CLY + ISO group. Furthermore, in our study, CLY induced vascular proliferation in the heart tissue. It is likely that the vascular proliferation results in a better blood supply to the heart, thereby, reducing the inflammation and extent of edema.

In summary, based on the findings of the present study, we conclude that administration of CLY, after the induction of experimental MI in rats, produces only a non-significant reduction in inflammation and extent of tissue edema which may be due to the induction of vascular proliferation in the heart tissue.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgments

This study has been funded and supported by the Deputy of Research, Kashan University of Medical Sciences (KAUMS, Grant No: 96055). This study was part of a larger research study entitled “The cerebrolysin effects on animal model of myocardial injury”.

References

[1] Mnafgui K, Hajji R, Derbali F, Khlif I, Kraiem F, Ellefi H, et al. Protective effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. Cardiovasc Toxicol 2016;16(2):147–55.
[2] Pipaliya H, Vaghasiya J. Cardio protective effect of vitamin A against isoproterenol-induced myocardial infarction. J Nutr Sci Vitamino 2012;58 (6):402–7.
[3] Burns RJ, Gibbons RJ, Yi Q, Roberts RS, Miller TD, Schaer GL, et al. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. J Am Coll Cardiol 2002;39 (1):30–6.
[4] Karlsson LO, Bergh N, Li L, Bissessar E, Bobrova I, Gross GJ, et al. Dose-dependent cardioprotection of enkephalin analogue Eribis peptide 94 and cardiac expression of opioid receptors in a porcine model of ischaemia and reperfusion. Eur J Pharmacol 2012;674(2-3):378–83.
[5] Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction. Eur Heart J 2018;40(3):237–69.
[6] Dayem AA, Choi HY, Kim JH, Cho SG. Role of oxidative stress in stem, cancer, and cancer stem cells. Cancers (Basel) 2010;2(2):859–84.
[7] Zhou R, Xu Q, Zheng P, Yan L, Zheng J, Dai G. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur J Pharmacol 2008;586(1–3):244–50.
[8] Goyal SN, Sharma C, Mahajan UB, Patil CR, Agrawal YO, Kumari S, et al. Protective effects of cardamom in isoproterenol-induced myocardial infarction in rats. Int J Mol Sci 2015;16(11):27457–69.
[9] Puhakka M, Magga J, Hietakorpi S, Penttilä I, Uusimaa P, Risteli J. Interleukin-6 and tumor necrosis factor alpha in relation to myocardial infarct size and collagen formation. J Card Fail 2003;9(4):325–32.
[10] Kannan MM, Quine SD. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. Metabolism 2013;62 (1):52–61.
[11] Saleh AI, Abdel Maksoud SM, El-Maraghy SA, Gad MZ. Protective effect of L-arginine in experimentally induced myocardial ischemia: comparison with aspirin. J Cardiovasc Pharmacol Ther 2011;16(1):53–62.
[12] Karthikeyan K, Bai BS, Devaraj SN. Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int J Cardiol 2007;115(3):326–33.
[13] Amiri-Nikpour MR, Nazarbaghi S, Ahmadi-Salmasi B, Mokari T, Tahamtan U, et al. Cerebrolysin effects on neurological outcomes and cerebral blood flow in acute ischemic stroke. Neuropsychiatr Dis Treat 2014;10:2299–306.
[14] Boshra V, Atwa A. Effect of cerebrolysin on oxidative stress-induced apoptosis in an experimental rat model of myocardial ischemia. Physiol Int 2016;103 (3):310–20.
[15] Menon Pk, Muresanu DF, Sharma A, Mossler H, Sharma HS. Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. CNS Neurol Disord Drug Targets 2012;11(1):40–9.
[16] Kannan MM, Quine SD. Ellagic acid ameliorates isoproterenol induced oxidative stress: evidence from electrocardiological, biochemical and histological study. Eur J Pharmacol 2011;659(1):45–52.
[17] Punithavathi V, Prince PSM. Pretreatment with a combination of quercetin and α-tocopherol ameliorates adenosine triphosphatases and lysosomal enzymes in myocardial infarcted rats. Life Sci 2010;86(5–6):178–84.

[18] Haleagrahara N, Varkkey J, Chakravarthi S. Cardioprotective effects of glycyrrhizic acid against isoproterenol-induced myocardial ischemia in rats. Int J Mol Sci 2011;12(10):7100–13.
[19] Buwa CC, Mahajan UB, Patil CR, Goyal SN. Apigenin attenuates β-receptor-stimulated myocardial injury via safeguarding cardiac functions and escalation of antioxidant defence system. Cardiovasc Toxicol 2016;16(3):286–97.
[20] Brooks WW, Conrad CH. Isoproterenol-induced myocardial injury and diastolic dysfunction in mice: structural and functional correlates. Comp Med 2009;59:339–43.
[21] Shukla SK, Sharma SB. β-Adrenoreceptor agonist isoproterenol alters oxidative status, inflammatory signaling, injury markers and apoptotic cell death in myocardium of rats. Indian J Clin Biochem 2015;30:27–34.
[22] Acikel M, Buyukokuroglu ME, Erdogan F, Aksoy H, Bozkurt E, Senocak H. Protective effects of dantrolene against myocardial injury induced by isoproterenol in rats: biochemical and histological findings. Int J Cardiol 2005;98:389–94.
[23] Evran B, Karpuzoğlu H, Develi S, Kalaz EB, Soluk-Tekkeşin M, Olgaç V, et al. Effects of carnosine on prooxidant–antioxidant status in heart tissue, plasma and erythrocytes of rats with isoproterenol-induced myocardial infarction. Pharmacol Rep 2014;66(1):81–6.
[24] Radhiga T, Rajamanickam C, Sundaresan A, Ezhumalai M, Pugalendi KV. Effect of ursolic acid treatment on apoptosis and DNA damage in isoproterenol-induced myocardial infarction. Biochimie 2012;94(5):1135–42.
[25] Tashiro H, Shimokawa H, Yamamoto K, Nagano M, Momohara M, Muramatu K, et al. Monocyte-related cytokines in acute myocardial infarction. Am Heart J 1995;130(3 Pt1):446–52.
[26] Deten A, Volz HC, Briest W, Zimmer HG. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. Cardiovasc Res 2002;55(2):329–40.
[27] Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000;101(18):2149–53.
[28] Formichi P, Radi E, Battisti C, Di Maio G, Muresanu D, et al. Cerebrolysin administration reduces oxidative stress-induced apoptosis in limphocytes from healthy individuals. J Cell Mol Med 2012;16(11):2840–3.
[29] Álvarez XA, Lombardi V, Corzo L, Pérez P, Pichel V, Laredo M, et al. Oral cerebrolysin enhances brain alpha activity and improves cognitive performance in elderly control subjects. J Neural Transm Suppl 2000;59:315–28.
[30] Álvarez XA, Lombardi V, Fernández-Novoa L, García M, Sampedro C, Cagiao A, et al. Cerebrolysin reduces microglial activation in vivo and in vitro: a potential mechanism of neuroprotection. J Neural Transm Suppl 2000;59:281–92.
[31] Ren J, Sietsma D, Qiu S, Moessler H, Finklestein SP. Cerebrolysin enhances functional recovery following focal cerebral infarction in rats. Restor Neurol Neurosci 2007;25(1):25–31.
[32] Prabhu SD, Chandrasekar B, Murray DR, Freeman GL. β-Adrenergic blockade in developing heart failure: effects on myocardial inflammatory cytokines, nitric oxide, and remodeling. Circulation 2000;101(17):2103–9.
[33] Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S. Cytokine gene expression after myocardial infarction in rat hearts: possible implication in left ventricular remodeling. Circulation 1998;98(2):149–56.