



Post-infarct treatment with [Pyr1]-apelin-13 reduces myocardial damage through reduction of oxidative injury and nitric oxide enhancement in the rat model of myocardial infarction

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ABSTRACT

Apelin is a newly discovered peptide that has been recently shown to have cardioprotective effects in the animal model of myocardial infarction (MI) and ischemia/reperfusion (I/R) injuries. The aim of the present study was to investigate the long term cardioprotective effect of [Pyr1]-apelin-13 in the rat model of MI. Male Wistar rats ($n=22$) were randomly divided into three groups: (1) sham operated group (2) control MI group and (3) MI treated with apelin (MI-AP group). MI animals were subjected to 30 min of left anterior descending coronary artery (LAD) ligation and 14 days of reperfusion. 24 h after LAD ligation, apelin (10 nmol/kg/day) was administered i.p. for 5 days. Blood sampling was performed at days 1, 3, 5 and 7 after MI for determination of serum changes of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), malondialdehyde (MDA) and nitric oxide (NO). Myocardial infarct size (IS) and hemodynamic function were also measured at the end of the study at day 14. We found out that post infarct treatment with apelin decreases infarct size, serum levels of LDH, CK-MB and MDA and increases heart rate and serum level of NO in the consecutive days, but there were no significant differences in blood pressure in the MI-AP group in comparison with MI. In conclusion, apelin has long term cardioprotective effects against myocardial infarction through attenuation of cardiac tissue injury and lipid peroxidation and enhancement of NO production.

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1. Introduction

Ischemic heart disease causes millions of death worldwide and is still the leading cause of morbidity and mortality in the developed countries [14,27]. Following myocardial infarction (MI), repair of myocardium takes place at the site of cardiomyocyte loss that conserves structural integrity and is essential to the cardiac repair [46]. In the clinical setting, severe myocardial damage caused by ischemia/reperfusion (I/R) injury is a common problem in patients with ischemic heart disease. The ischemia insult along with reperfusion injury is referred to as I/R injury [41,46]. There are limited successes in the efforts that have been investigated to reduce I/R injuries. Two strategies, for cardioprotection against I/R injuries are mechanical and pharmacological pre- and post-conditioning. Post-conditioning has beneficial effects in patients with MI and is a safe and simple strategy in lowering reperfusion injury in the clinical setting [41]. It is well accepted that reactive oxygen species (ROS) and other reactive species are generated in the

ischemic myocardium and peri-infarct area, and directly cause cell death by damaging the cell membrane lipids, proteins, carbohydrates and DNA, thereby leading to qualitative and quantitative alterations of the myocardium [12,29]. Moreover, inflammatory cytokines signaling pathways induced by ROS can regulate cell survival and death [12]. It has also been reported that nitric oxide (NO) has cardioprotective effects against myocardial remodeling and I/R injuries following MI [49]. NO participates in the control of myocardial contractility and heart rate, limits remodeling following MI and has a cardioprotective role in the ischemic pre and post conditioning. Many studies suggest that endogenous and exogenous NO is one of the most prominent defenses systems against myocardial I/R injuries, which could limit myocardial damages [34]. Thus, oxygen free radicals and NO play important roles in the pathophysiology of cardiovascular diseases [49]. The extent of oxidative stress and the severity of myocardial damages after that might depend on the imbalance between ROS overproduction and antioxidant defense system in the heart [39].

Some investigators suggested that the myocardium adapts to I/R injuries via synthesis of various growth factors and cytokines. Having knowledge of these endogenous mechanisms might open new window for limitation of I/R injuries [5,18,51]. Various

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intrinsic peptides have cardioprotective effects in the experimental studies when added during early and/or late phases of reperfusion following lethal ischemia. These endogenous factors found in the body include ghrelin, adiponectin, adrenomedullin, leptin, insulin, neuropeptide Y, erythropoietin and glucagon-like peptide-1 [2,3,5,20,30,35,47]. The adipocytokines are peptide hormones that are synthesized by adipocytes, have roles in metabolic control and disease [40]. Apelin is a new adipocytokine, initially isolated from bovine stomach tissue extracts by Tatemoto in 1998 [44] that has been recently focused on in many studies. The apelin precursor translated as a 77-amino acid preproapelin, has several proteolytic cleavage sites, formed C-terminal peptides, including apelin-36, apelin-13, and [Pyr1] apelin-13 [1,4,8,26]. They are synthesized from a single gene and activate the G-protein coupled receptor (GPCR, APJ) that has high homology with angiotensin II receptor-1 (AT-1) but does not bind to angiotensin II [4,9]. It has been shown that apelin signaling pathways are widely represented in the cardiovascular system and it is an important regulator of cardiac function [10]. Apelin is expressed in the endothelium, acts via paracrine and endocrine signaling to activate APJ on myocardial cells, endothelial cells, and some smooth muscle cells [1,38]. Apelin has a functional role in cardiovascular development and may also participate in cardiovascular pathological processes [16,25,42]. The apelin-APJ system may be involved in cardioprotection during an acute myocardial ischemia [36]. Some studies investigated the post-ischemic protection and infarct size limitation by apelin [33]. Apelin-13 has cardioprotective effects only when given as a post-conditioning agent after ischemia, whereas it is not effective when administered before ischemia [33]. Its cardioprotection against I/R injury occurs through activation of PI3K/Akt/eNOS and ERK signaling pathways [17,23,31,40]. Foussal et al. has been reported that apelin preserved cardiac function via decreasing oxidative stress and increasing catalase activity, suggesting that apelin is a potent regulator of cardiomyocyte antioxidant defense against oxidative stress in the hypertrophic myocardial remodeling [10]. Apelin peptides cause endothelium-dependent vasorelaxation via releasing of NO [53]. In addition, apelin phosphorylates Akt and increases intracellular calcium, thereby it will cause endothelial NO synthetase (eNOS) phosphorylation and increase NO release [4,23,24,53].

Therefore, the aim of the present study was to investigate the pharmacological post-infarct treatment with [Pyr1]-apelin-13 and its capability to exert long-term cardioprotection. Furthermore, we investigated the effect of post-infarct treatment with [Pyr1]-apelin-13 on myocardial infarct size, NO production, oxidative stress and ischemia/reperfusion injury for 2 weeks after MI.

2. Materials and methods

2.1. Animals

The present study was performed in accordance with the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). The experimental protocol was approved by the institutional care and use committee of Tehran University of Medical Sciences (Tehran, Iran). 22 male Wistar rats (weighing 250–300 g at the beginning of surgery) were housed in controlled environment conditions ($22 \pm 2^\circ\text{C}$; light–dark cycle 7 AM–7 PM). Rats were allowed to access water and standard laboratory food ad libitum. Animals were adapted for at least 7 days before the experiments.

2.2. Rat model of myocardial ischemia and reperfusion

Myocardial infarction was induced by ligation of the left anterior descending coronary artery (LAD). After induction of anesthesia

(with thiopental; 60 mg/kg i.p.), the animals were placed in the supine position and body temperature was maintained as close as possible to 37°C by means of thermal pad and heating lamp. Then, they were intubated and ventilated by room air using a rodent ventilator (tidal volume 2–3 mL, respiratory rate 65–70 per min, Harvard rodent ventilator model 683, Holliston, MA, USA). An intercostal thoracotomy in the left fourth intercostal space was performed under sterile conditions. The heart was exposed and pericardium incised. MI was produced by ligation of the LAD coronary artery with 6–0 polypropylene suture approximately 1–2 mm distal from its origin. Both ends of the silk suture were then passed through a small vinyl tube, and the LAD was occluded by pulling the snare, which was fixed by clamping the tube with a mosquito hemostat. Successful constriction of LAD was characterized by ECG changes consisting of ST segment elevation immediately after ligation and cyanosis of the affected myocardium. After 30 min of LAD occlusion, the occluder was removed and restoration of blood flow was verified. After completion of all surgical procedures, the chest was closed in layers. The lungs were inflated by increasing positive end expiratory pressure and the animals were removed from the ventilator and allowed to recover. The sham operated rats underwent the same procedure of thoracotomy, without the ligation of the coronary artery. This protocol resulted in the creation of three groups: control sham-operated group (sham, $n=6$), control MI group (MI, $n=6$), and apelin-treated MI group (MI-AP, $n=6$). Post-operative, rats were hydrated with normal saline (s.c) and received appropriate post surgery analgesia. Tetracycline was used as post-operative antibiotic. [Pyr1]-apelin-13 (Sigma) was dissolved in normal saline and administered i.p. 24 h after induction of MI (10 nmol/kg/day, once a day) [7,40,45] for 5 days. Sham and MI animals received normal saline.

2.3. Blood sampling

Blood samples were collected from tail vein at days 1, 3, 5 and 7 after surgery for biochemical analysis (CK-MB, LDH, MDA and NO). Blood sampling in apelin treated animals were collected 1 h after administration of apelin. The samples were centrifuged at 5,000 rpm, 4°C , for 15 min; the serum was removed and stored at -70°C until biochemical analysis.

2.4. Hemodynamic studies

We assessed hemodynamic function 14 days following reperfusion. The rats were anesthetized with thiopental and ventilated. A small incision was made to the right of the midline in the neck. The right common carotid artery was identified and cannulated with a PE50 catheter that was connected to the PowerLab data acquisition system via pressure transducer (AD Instrument Pty Ltd, Mountain View, CA, USA) to monitor systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). Heart rate (HR) was monitored and recorded by PowerLab data acquisition system (AD Instrument) via lead II ECG.

2.5. Assessment of infarct size

At day 14 of reperfusion, after hemodynamic recording, LAD was reoccluded, and 2% Evans blue dye (2 ml, Sigma) was infused through femoral vein to differentiate the ischemic area from non-ischemic area. Hearts were excised quickly, frozen at -20°C for 24 h and then sliced into 2 mm transverse sections. Slices were then incubated in 1% 2,3,5 triphenyltetrazolium chloride (TTC in 0.1 M phosphate buffer, pH 7.4 Sigma) solution for 15–20 min at 37°C . TTC reacts with the viable tissue, producing a red formazan derivative, which is distinct from the white necrotic area when placed in 10% formalin for 24–48 h. The area at risk and infarcted area were

calculated by Photoshop program (Ver. 7.0, Adobe System, San Jose, CA, USA). Area at risk was expressed as a percentage of left ventricle (AAR/LV) and infarct size was expressed as a percentage of area at risk (IS/AAR).

2.6. Measurement of LDH and CK-MB

Serum levels of CK-MB and LDH (markers of myocyte necrosis) were measured by a colorimetric method, with specific CK-MB and LDH kits (Pars Azmoon, Tehran, Iran), using an auto analyzer (Roche Hitachi Modular DP Systems; Mannheim, Germany) according to the manufacturer's instructions. The recorded values are presented in IU/l.

2.7. Assessment of oxidative stress by TBARS estimation

To evaluate the oxidative damage, MDA level (marker of lipid peroxidation) was assessed by reaction with thiobarbituric acid (TBA) at 90–100 °C [37]. MDA or MDA-like substances, and TBA react to produce a pink pigment with maximum absorption at 532 nm. In brief, 50 μ l of serum, 1 ml of TBA: 1 ml of trichloroacetic acid (TCA) [0.75% TBA: 30% TCA] were mixed and placed in boiling water bath for 90 min, cooled and centrifuged at 4 °C for 10 min at 3000 rpm. The absorbance of the supernatant was measured against a reference blank at 532 nm by spectrophotometer. 1,1,3,3-Tetramethoxypropane (Sigma Chemicals, USA) was used as a standard. The results were expressed as nmol/ml of serum.

2.8. Measurement of NO

Serum NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-) were measured as an index of NO production, based on the Griess reaction [22]. The principle of the assay is the conversion of nitrate into nitrite by copperized cadmium granules and followed by color development with Griess reagent (sulfanilamide and N-naphthyl ethylenediamine) in acidic medium. The assay was calibrated with standard solutions of sodium nitrite. A 50 μ l sample of serum was used to determine NOx and the results were expressed as nmol/ml. Optical densities were measured at 540 nm in a 96-well microplate reader after 3 h incubation time.

2.9. Statistical analysis

All data are presented as means \pm SEM ($n=6$ per group). All statistical analyses were performed by SPSS software (Version 15.0, SPSS Inc., Chicago, IL). *T*-test was used for infarct size analysis between MI and MI-AP groups. One-way ANOVA was used to compare differences among the sham, MI and MI-AP groups followed by Tukey post hoc test for hemodynamic parameters. Two-way ANOVA was done for biochemical parameters between groups in different days followed by Tukey post hoc test. Significant differences were considered as $P<0.05$.

3. Results

3.1. Mortality and survival

There was no mortality in sham group. Totally four animals died during protocols; three animals in MI group (3/9) and one animal in apelin treated MI group (1/7).

3.2. Hemodynamic studies

Hemodynamic data obtained at day 14 after reperfusion are summarized in Table 1. SBP (107.606 ± 4.41), DBP (86.85 ± 6.154), MAP (94.868 ± 5.058) and HR (296.294 ± 14.65) clearly diminished

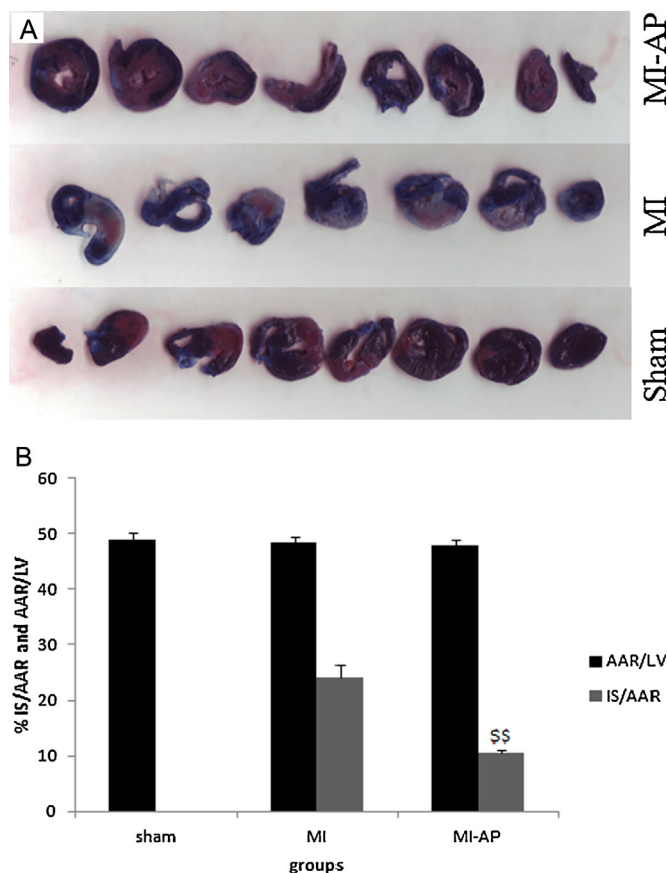


Fig. 1. Morphometric analysis of infarct size at day 14 after myocardial infarction ($n=6$). (A) Transverse sections of the hearts from apex to base stained with Evans blue dye and TTC. (B) Quantitative analysis of infarct size. AAR, area at risk; IS, infarct size; LV, left ventricle; MI, myocardial infarction; AP, apelin; data are presented as means \pm SEM. $^{**}P<0.01$ vs. MI group.

in MI compared to sham-operated rats [(SBP, 131.37 ± 9.246), (DBP, 101.129 ± 7.174), (MAP, 115.494 ± 7.352) and (HR 370.48 ± 12.925) $P<0.001$ for all of them]. Our results showed that five days treatment with apelin after LAD ligation (MI-AP group) significantly increased HR (390.405 ± 7.865) compared to MI ($P<0.001$) and there were no significant differences with sham. Post-infarct treatment with apelin could not change SBP (110.867 ± 8.63), DBP (89.33 ± 4.124) and MAP (101.73 ± 4.215) in comparison with MI group (Table 1).

3.3. Infarct size

To evaluate the effect of apelin in the post-infarct cardioprotection, we measured left ventricular infarct size at day 14 after reperfusion as a ratio of infarct size to area at risk (%IS/AAR). As shown in Fig. 1A and B, 5 days treatment with apelin significantly reduced infarct size compared to MI group ($23.98 \pm 2.39\%$ vs. $10.49 \pm 0.59\%$ in MI-AP group, $P<0.01$). Moreover, no significant differences were observed in the size of the area at risk as a ratio of area at risk to total left ventricular area (%AAR/LV), which represented approximately 47% of the LV in all groups (Fig. 1B).

3.4. Effect of apelin on CK-MB levels

Induction of MI significantly increased serum levels of CK-MB in comparison with sham group at days 1, 3 and 5 after reperfusion ($P<0.05$, Fig. 2), but there were no significant differences at day 7 after reperfusion among MI and sham groups. Our results

Table 1
Hemodynamic parameters.

Groups	SBP	DBP	MAP	HR
Sham	131.37 ± 9.246	101.129 ± 7.174	115.494 ± 7.352	370.48 ± 12.925
MI	107.607 ± 4.41***	86.85 ± 6.154***	94.868 ± 5.058***	296.294 ± 14.65***
MI-AP	110.867 ± 8.63***	89.33 ± 4.124***	101.73 ± 4.215***	390.405 ± 7.865 ^{sss}

HR, heart rate (beats/min); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial pressure (mmHg); MI, myocardial infarction; AP, apelin. data are presented as means ± SEM.

*** $P < 0.001$ compared to sham group.

^{sss} $P < 0.001$ compared to MI group.

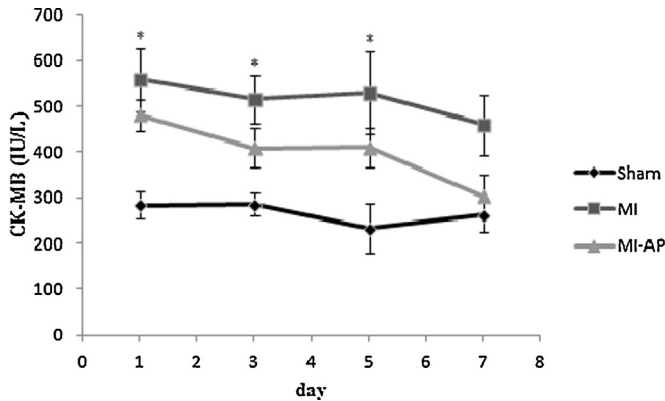


Fig. 2. Serum levels of CK-MB at days 1, 3, 5 and 7 after reperfusion ($n=6$). MI, myocardial infarction; AP, apelin; data are presented as means ± SEM. * $P < 0.05$ vs. sham group.

showed that apelin administration 24 h after induction of MI gradually decreases CK-MB levels to that in sham group in the consecutive days. There were also no significant differences between MI-AP and MI groups. As shown in Fig. 2, CK-MB levels remained relatively constant at the various days in sham group until day 7.

3.5. Effect of apelin on LDH levels

Induction of MI significantly increased LDH level ($P < 0.001$) in comparison with sham operated animals. Its level in MI group peaked at day 5 and then decreased at day 7. Fig. 3 shows that apelin administration 24 h after induction of MI significantly decreases serum levels of LDH in comparison with MI group at days 3, 5 ($P < 0.001$) and 7 ($P < 0.01$) after reperfusion, but there were no significant differences at day 1. LDH levels in apelin treated MI rats at day 1 after reperfusion showed significant differences with sham group and then decreased to sham levels at days 3, 5 and 7 after

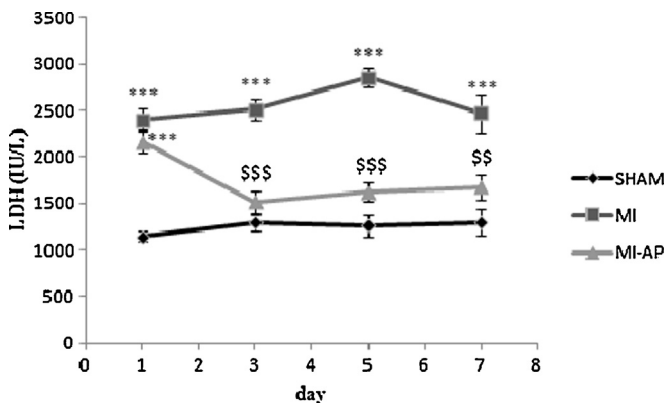


Fig. 3. Serum levels of LDH at days 1, 3, 5 and 7 after reperfusion ($n=6$). MI, myocardial infarction; AP, apelin; data are presented as means ± SEM. *** $P < 0.001$ vs. sham group, ^{\$\$} $P < 0.01$ and ^{\$\$\$} $P < 0.001$ vs. MI group.

treatment. LDH levels remained relatively constant at the various days in sham group until day 7.

3.6. Effect of apelin on MDA levels

MDA, a biomarker of cardiac oxidative injury, is a product of lipid peroxidation that can be produced by a variety of oxidative damages. As shown in Fig. 4, induction of MI significantly increased MDA levels at days 1, 3, 5 ($P < 0.001$) and 7 ($P < 0.01$) in comparison with sham group. Apelin administration 24 h after induction of MI significantly decreased MDA levels in comparison with MI group at days 1, 3, 5 ($P < 0.001$) and 7 ($P < 0.05$) after reperfusion, suggesting the possible antioxidant role of apelin against myocardial oxidative injury induced by reperfusion. Fig. 4 shows that there are no significant differences among apelin treated and sham operated animals, except at day 1 ($P < 0.01$). So apelin could decrease MDA levels to that in sham operated animals in the consecutive days.

3.7. Effect of apelin on NO levels

Post-infarct treatment with apelin 24 h after induction of MI significantly increased serum levels of NO in comparison with MI group at days 1 ($P < 0.05$), 3 ($P < 0.01$), 5 ($P < 0.001$) and 7 ($P < 0.01$) after reperfusion (Fig. 5). Our results also showed that MI and apelin significantly increased NO levels in comparison with sham group at all days of experiment. NO peaked at day 5 after reperfusion in apelin treated animals and then declined.

4. Discussion

In the present study, we provide an evidence for the role of apelin in the long term cardioprotection after induction of myocardial infarction. In addition, we have demonstrated that post infarct treatment with [Pyr1]-apelin-13 significantly reduces infarct size and prevents the progression of myocardial damages by decreasing

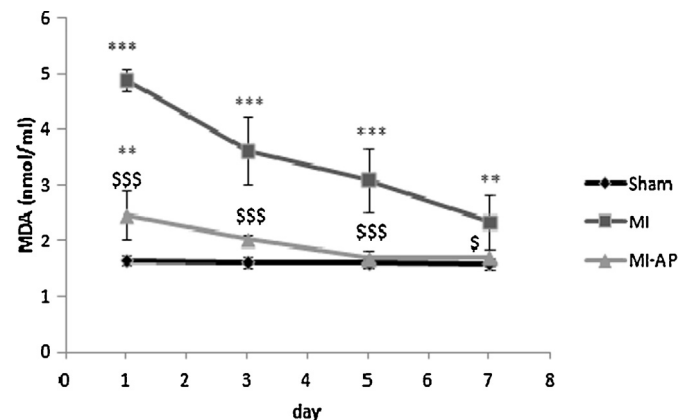


Fig. 4. Serum levels of MDA at days 1, 3, 5 and 7 after reperfusion ($n=6$). MI, myocardial infarction; AP, apelin; data are presented as means ± SEM. ** $P < 0.01$ and *** $P < 0.001$ vs. sham group, ^{\$} $P < 0.05$ and ^{\$\$\$} $P < 0.001$ vs. MI group.

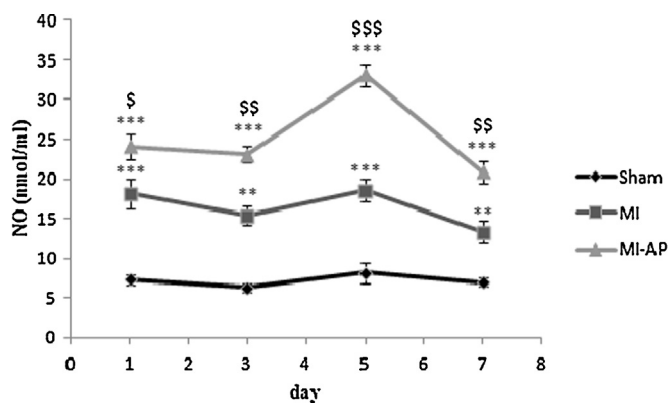


Fig. 5. Serum levels of NO at days 1, 3, 5 and 7 after reperfusion ($n=6$). MI, myocardial infarction; AP, apelin; data are presented as means \pm SEM. ** $P < 0.01$ and *** $P < 0.001$ vs. sham group, \$ $P < 0.05$, \$\$ $P < 0.01$ and \$\$\$ $P < 0.001$ vs. MI group.

LDH, CK-MB, MDA and increasing NO levels in serum; suggesting that apelin has cardioprotective function against I/R injuries after induction of myocardial infarction.

Myocardial injuries caused by I/R, comprise three phases: (a) acute oxidative injuries during ischemic phase, (b) acute oxidative injuries during early reperfusion, and (c) chronic injuries following long term reperfusion. Post-I/R cell death is associated with both acute and chronic phase [33]. The chronic cell death likely induces by the post-I/R oxidative stress and inflammatory events. It has been reported that administration of some pharmacological agents before myocardial ischemia may not be an effective approach for prevention of the heart tissue from I/R-induced damages, so it may be not a practical approach in the attenuation and prevention of myocardial ischemic disease. However, administration of the efficient cardioprotective agents at the reperfusion phase would be a useful strategy in the clinical setting [33,48]. In the present study, apelin was given 1 day after myocardial reperfusion for 5 consecutive days to examine its protective role on I/R-induced chronic damages. Adipose tissue secretes different bioactive factors such as peptides and cytokines which they can modulate cardiovascular function by direct or indirect mechanisms [10,40,51]. Some studies have investigated the post-ischemic protection and infarct size limitation by apelin [33]. They have shown that apelin has an acute antioxidant cardioprotective functions only when given as a post-conditioning agent [33]. Post-infarct treatment with apelin significantly protected the heart from I/R-induced damages in vivo and in vitro models by preventing oxidative damages [10,24,51]. Studies have shown that there was progressive increase of ROS production and decrease of antioxidants in the viable myocardium in post-MI rats [54]. In physiologic levels, ROS act as signaling molecules in several cellular functions; on the other hand, in pathologic conditions overproduction of ROS have deleterious effects by damages to the several cellular components [49,54]. In recent years, it has been reported that antioxidants protect myocardial tissue against I/R injury by reducing infarct size [43]. Reperfusion also resulted in an elevated lipid peroxidation, as evidenced by enhanced levels of MDA. In this study we showed that apelin post-infarct treatment for five days after induction of MI could decrease infarct size at day 14 and MDA at days 1, 3, 5 and 7 after reperfusion, which has a long term antioxidant cardioprotective effect against myocardial infarction. Therefore, antioxidant actions of apelin might play critical role in the prevention of I/R-induced cardiac oxidative damage. In addition, apelin reduced CK-MB and LDH, which are also important metabolic myocardial enzymes, released from the myocardium into the plasma with progression of cardiomyocytes damages [28]. Increased serum level of CK-MB is considered as an important marker of myocyte injury [28] and

we observed that when apelin administrated 24 h after induction of MI, it reduced this enzyme to that in the sham group. Elevation of LDH level in serum, as marker of systemic tissue damages [28], was also attenuated by apelin post-infarct treatment. In agreement with our results, other studies showed that cardioprotective agents could decrease CK-MB and LDH levels in the infarcted myocardium [31,33,51]. Indeed, we found out that cardioprotective effects of apelin may be due to reduction of MDA level and CK-MB and LDH release from damaged myocardium. Foussal et al showed that in the mice model of pressure-overload-induced left ventricular hypertrophy, chronic treatment with apelin decreases oxidative stress damages through increased myocardial catalase activity and decreased plasma lipid hydroperoxide and by these mechanisms it prevents cardiac ROS-induced hypertrophy [10]. Our results are in line with these studies and shows that apelin post-infarct treatment decreases LDH and CK-MB leakage and MDA level in the consecutive days after treatment. In fact, our study and results from others support the notion that apelin plays a direct cardioprotective role against I/R injury. It has also been reported that in the rat model of isoproterenol induced myocardial damage, administration of apelin reduces MDA in plasma and myocardium and decreases plasma levels of LDH [19]. In this study apelin 1 h after injection could reduce some markers of myocyte injury, but its effects increased with time. Apelin, like ghrelin and erythropoietin [3,5,13,21,47] could induce rapid and long term cardioprotective effects. It is also possible that apelin could decrease cardiomyocytes apoptosis and increase myocardial angiogenesis, thereby could limit myocardial infarct size and decrease cell death in long term. Despite we did not investigate such effects; we suppose that these may also be due to its antiapoptotic and angiogenic actions. Further studies will be necessary to dissect the molecular mechanisms involved in antioxidant, antiapoptotic and angiogenic activity of apelin.

It has been well recognized that NO participates in the control of myocardial contractility and heart rate, limits remodeling following MI and has a cardioprotective role in the ischemic pre and post conditioning. Many studies suggest that endogenous and exogenous NO is one of the most prominent defense systems against myocardial I/R injuries, which could limit tissue cardiac damages [34]. Following I/R, production of ROS increases because of imbalance between oxidant and antioxidant defense systems. Superoxide radical is one of the oxidant molecules, which is involved in ROS metabolic pathways, decreases bioavailability of NO, thus increases cardiac load. NO could abrogate mitochondrial oxidative injury and calcium overload, and decrease synthesis of superoxide anion through suppression of NAD(P)H oxidase activity [11,32,52]. Thus, preservation of NO levels is essential for protection of the heart following ischemia. Chao et al. have been reported that kallistatin increases NO levels via eNOS activation in the infarcted myocardium 1 day after induction of acute I/R [6]. In the present study, our results showed that at days 1, 3, 5 and 7 after induction of MI, apelin also significantly increases NO levels in the serum in comparison with sham operated and MI animals. Since it has been shown that long term treatment with L-NAME causes unwanted side effects in vivo, we did not use L-NAME for determining the effects of NO in the protective effect of apelin, but instead we measured its changes in different days after reperfusion. Studies have been reported that in the setting of I/R injury, cardioprotective effects of apelin occur through activation of PI3K/Akt/eNOS signaling pathway [17,23,31,40]. Apelin phosphorylates Akt and increases intracellular calcium; thereby it will cause eNOS phosphorylation and increase NO release [4,23,24,53]. It has been reported that NO generated from iNOS protects cells from apoptosis under chronic situations [11,34]. In iNOS knockout mice, resveratrol, the main flavanol of red wine, could not decrease myocardial injury, whereas it shows protection against myocardial

oxidative damages in wild-type mice, suggesting that NO is a protective molecule [15]. Thus, from reported studies and our results on the changes of serum levels of NO following apelin post-infarct treatment, and with knowledge of the apelin signaling pathways in the activation of PI3K/Akt/eNOS and phosphorylation of eNOS, we suggest that NO formation via decreasing oxidative stress and MDA production might be an important protective mechanism of apelin after induction of MI.

Our present study also demonstrated that apelin acts as a chronotropic agent and has not any effect on blood pressure in comparison with MI animals. As shown in Table 1, apelin could not reverse the effect of MI on blood pressure reduction. MI reduced MAP and since apelin has hypotensive effect, we cannot suggest that this hypotension in MI-AP group is due to MI or apelin. Previous studies reported that apelin increases heart rate and cardiac output and reduces peripheral vascular resistance with or without blood pressure changes [1,17,50]. In support of other findings, we demonstrated that in vivo subchronic treatment of MI rats with apelin could significantly increase heart rate. Yao et al. showed that microinjection of apelin-13 into the rostral ventrolateral medulla (RVLM) could increase the activity of neurons. Its positive chronotropic effect is associated with superoxide radical derived from NAD(P)H oxidase in the neurons of RVLM [50]. Tatemoto et al. showed that intravenous administration of apelin in anesthetized rats decreases blood pressure and HR, and these effects are inhibited by NOS inhibitor [44]. Cheng et al. demonstrated that the intravenously applied apelin decreased MAP and increased HR in conscious rats [7]. The reason for these discrepancies is not clear but may relate to animal species, different doses and structures of apelin peptides used in those studies. It is also possible that apelin may have an effect on electrophysiology of the heart. It may have direct action on the sinus node and/or AV node, or may be its effects are mediated via the central nervous system. In the MI animals we observed that HR decreased. The effect of MI on HR is different so that some investigators show its increase, others show decrease of HR and some others show no changes. In this study the decrease of HR may be due to remodeling and hypertrophy of myocardium and increase the time of impulse conduction in the hypertrophic cardiomyocytes. HR reduction may be a compensatory mechanism to increase end diastolic time and end diastolic volume to increase ejection fraction, so with this mechanism the heart could balance oxygen demand and contractility. However there are no reports to indicate these effects of apelin. Further studies are needed to indicate more precisely the chronotropic and blood pressure effects of apelinergic system.

The limitations of this study were measurement of ejection fraction, left ventricular function and determination of serum apelin level. The quality of data can be improved by other methods for evaluating cardiac function such as echocardiography. In addition, we suggest evaluating the long term antiapoptotic and angiogenic effects of apelin and its signaling pathways to elucidate protective mechanisms of apelin.

5. Conclusion

Taken together, these findings indicate that long term post-infarct treatment by [Pyr1]-apelin-13 inhibits ROS injury, lipid peroxidation and myocardial damages through NO formation. Furthermore, our results suggest that effects of apelin on myocardium are not related to blood pressure changes. The present study delivers important new insights to the mechanisms of long term antioxidant cardioprotective effect of apelin. Apelin should be recognized as an important regulator in protection of post-MI injury and myocardial damages.

Conflict of interest

The authors declare no conflict of interest.

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