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Aerobic training and L-arginine supplement attenuates myocardial infarction-induced kidney and liver injury in rats via reduces oxidative stress

Kamal Ranjbar[a], Farzad Nazem[b], Reyhaneh Sabrinezhad[c], Afshin Nazari[d,*]

[a] Department of Physical Education and Sport Science, Bandar Abbas, Islamic Azad University, Bandar Abbas, Iran
[b] Department of Sport Physiology, Faculty of Physical Education and Sport Science, Bu Ali Sina University, Hamedan, Iran
[c] Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
[d] Razi Herbal Medicines Research Center, Department of Physiology, Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Introduction: The aim of the present study was to determine the effect of exercise training and l-arginine supplementation on kidney and liver injury in rats with MI.
Material and methods: Four weeks after MI, 50 male wistar rats randomly divided into five followed groups: sham surgery without MI (Sham, n = 10), Sedentary-MI (Sed-MI, n = 10) 3: L-Arginine-MI (La-MI, n = 10) 4: Exercise training-MI (Ex-MI, n = 10) and 5: Exercise and l-arginine-MI (Ex + La-MI). Ex-MI and Ex + La-MI groups running on a treadmill for 10 weeks with moderate intensity. Rats in the L-arginine-treated groups drank water containing 4% L-arginine. Tissues oxidative stress and kidney and liver functional indices were measured after treatments.
Result: Urea as a kidney function indexes, increased in Sed-MI group in compared to sham group and decreased significantly in Ex-MI and Ex + La-MI groups. The level of Cat and GSH of kidney were significantly lower in the MI-groups compared with the Sham group and kidney MDA levels increased after MI and significantly decreased in response to aerobic training and L-arginine. As well as, AST and ALT as liver injury indices, increased in MI-groups and decreased by training and L-arginine. In this regards, liver MDA and Cat respectively increased and decreased in MI-groups, but aerobic training and L-arginine increased liver GPX and decreased liver MDA.
Conclusion: These results demonstrated that kidney and liver function impaired 14 weeks after MI and aerobic training and L-arginine supplementation synergistically ameliorated kidneys and liver injury in myocardial infarction rats through oxidative stress reduction.

1. Introduction

The World Health Organization (WHO) has been reported that myocardial infarction (MI) is the most common cause of mortality in the world.[1] Left ventricular systolic dysfunction is the most important complication of MI. MI caused cardiac insufficiency affects not only reduces heart function but also damage to the other dependent organs such as kidneys and liver. Almost 45% of the cardiac output flows to the kidneys and liver.

It was shown that, 6 months after MI, considerable changes were observed in the rat liver.[2] MI-induced cardiac output reduction with consecutive reduction in kidneys and liver blood flow can lead to oxygen partial pressure drop in nephrons and hepatocytes.[3,4] In these regards previous studies showed that 4 weeks after induction of MI by ligation of the left coronary, the renal blood flow and the percent of cardiac output perfusing the kidneys were reduced by 18% and 14% respectively.[5] These findings suggest close interactions between the heart and the kidney, which is known as the "cardio-renal syndrome".[6]

Once the decrease in oxygen delivery crosses a critical threshold, a cascade of events is initiated in kidneys and liver that ultimately leads to cell deaths. It has been shown that limited cell supply and slow removal of metabolic products in the liver causes fibrosis in central vein zones, bridging fibrosis between adjacent central veins, and regeneration nodes.[2] Also, Tubular atrophy, renal vasoconstriction, formation of granulation tissue, interstitial fibrosis, inflammation and oxidative stress are the most important outcomes of kidney tissue after myocardial infarction.[4,6]

* Corresponding author at: Herbal Medicines Research Center, Department of Physiology, Lorestan University of Medical Sciences, Khorramabad, Iran.
E-mail address: nazari257@yahoo.com (A. Nazari).

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This process has been less considered in the literature and only a few case reports are documented in the scientific literature.[4,7,8] The molecular mechanism of cell deaths after MI is not yet clear. Inflammation and oxidative stress are the most common cause of damaged to the nephron and hepatocytes cells after MI.[4] Ultimately, damaged cells resulting in decreased kidneys and liver function.

In this regards, it has been shown that L-arginine as a precursor nitric oxide (NO) has various physiological properties including vasodilatation, scavenging superoxide (O^{2-}) formation and suppression xanthine oxidase (XO).[9] Previous studies showed that preservation of NO bioavailability leads to renal efferent arteriolar vasodilation, diuresis, natriuresis and increase glomerular filtration by reducing oxidative stress and maintaining renal function.[10–12] Lucas et al. showed that L-arginine attenuated hepatocellular damage induced by hepatic ischemia-reperfusion in rats.[13] On the other hand, it has been shown that exercise training (ET) promotes antioxidant capacity and attenuates oxidative stress in skeletal muscle,[14] kidneys and liver[15] of rat.

In light of this information, this study was designed to evaluate the protective effect of exercise training and L-arginine in protection from oxidative stress caused by MI in kidneys and liver of rats.

2. Material and methods

2.1. Animals

Male Wistar rats (6 weeks old) weighing between 150 and 180 g were maintained in a 12 h light/dark cycle at constant room temperature 22 ± 1 °C and relative humidity 55 ± 3%. They were fed ad libitum on standard laboratory rat chow and had free access to tap water. Animals used in these experiments were treated in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the study protocols were approved by the Institutional Animal Care and Use Committee at Lorestan University of medical science. The number of approval of the ethical committee was LUMS.REC.1394.27.

2.2. Experimental myocardial infarction

Animals underwent experimental myocardial infarction due to permanent left anterior decending (LAD) artery ligation or sham surgery as previously described.[16] Briefly, Rats were initially anesthetized by intraperitoneally injecting of 60 mg/kg sodium thiopental, intubated via gray angiocath and initiation of ventilation (Small Animal Ventilator, Model 683, Harvard Apparatus). The heart was exposed through a left lateral thoracotomy, and ligation of the LAD coronary artery was performed using 6-0 polyethylene thread just below the tip of the left auricle. Proximal LAD artery ligation in a rat model creates a reproducibly large lateral wall infarction.

A standard limb lead-II electrocardiogram (ECG) was continuously monitored and recorded throughout the experiment, using a computerized data acquisition system (ML750 Power Lab/4sp, ADInstruments). Proper ligation of the LAD was confirmed by ST elevation and increase in R-wave amplitude in ECG. Cefazolin (25 mg/kg i.p.) was administered as preoperative antibiotic cover. After completion of all surgical protocols, the chest was re-closed with separate purse-string silk sutures (size 6–0), and the lungs were fully expanded. Body temperature was measured by rectal thermometer and maintained at 37 ± 1 °C. To ensure complete healing of the infarct zone, all rats recovered in their cages for 4 week after the operation before beginning the exercise program. The rats in the sham group underwent thoracotomy and pericardiectomy without MI.

2.3. Experimental design

Four week after the operation, 50 rats that survived randomly distributed to the following experimental groups: Sham (n = 10, Sham); sedentary-MI (n = 10, Sed-MI); exercise-MI (n = 10, Ex-MI); sedentary + L-arginine-MI (n = 10, La-MI); exercise + L-arginine-MI (n = 10, Ex + La-MI). The rats assigned to the exercise group started exercising at 4 week post-MI using a motorized rodent treadmill, while the sham and sed groups remained sedentary throughout the experiment period. Initially, all mice were habituated on a ten-channel motor-driven treadmill (Razi Rad, Iran), at a speed of 10 m/min for 10 min/day for 1 week to reduce their stress in response to the new environment. After the adaptation period, the two groups of exercised rats performed an incremental running program to obtain progressive levels of intensity (10–17 m/min, 20–50 min/day, no incline). The exercise intensity was moderate and 55–60% of maximal oxygen consumption.[17,18] To determine VO2max, as described previously,[19,20] the treadmill was placed into a metabolic chamber. Ambient air was pumped through the metabolic chamber at a flow rate of 4.5 L min^{-1}, and samples of extracted air (200 mL min^{-1}) were directed to an oxygen analyzer that was based on a paramagnetic oxygen transducer (Servomex type 1155, Servomex, UK) and a carbon dioxide analyzer (LAIR 12, M&C Instruments, The Nether-lands). The VO2max protocol involved step-wise increases in the treadmill speed as follows: a 15-min period of acclimation, after which the treadmill was started at 10 m/min and then the speed was incrementally increased 5 m/min every 3 min until the rat reached exhaustion. VO2max was measured for each animal by using three criteria: (i) no change in VO2 when speed was increased, (ii) rats could no longer keep their position on the treadmill, and (iii) respiratory quotient (RQ = VCO2/VO2) > 1. Then, based on the level of VO2max, the speed corres-ponding to 60% VO2max was determined and used for daily training for 50 min, five times a week for 10 weeks. The VO2max was measured every other week, and running speed was adjusted to maintain 60% VO2 max.

2.4. L-arginine treatment

In the entire period of investigation, subjects in the L-arginine-treated groups drank water containing 4% (w/v) L-arginine (A5006, Sigma-Aldrich, USA).[21]

2.5. Assessment of liver and kidney functions

The rats were anaesthetized and sacrificed with an overdose of anesthesia 48 h after the last exercise session. Blood samples collected from the vena cava were centrifuged at 3000 rpm for 10 min for sera preparation. The sera were then stored at −80 °C and later the following parameters were measured: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Creatinine and Urea. ALT and AST are markers for hepatocyte injury, ALP is sensitive markers to investigate biliary function, while creatinine and urea reflects kidney function.[22] This biochemical markers play an important role in accurate diagnosis of hepatic and renal function. The activities of blood serum marker enzymes, such as alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), creatinine and urea, were measured using a Roche kit (Penzberg, Germany) and analyzed spectrophotometrically using the Hitachi Analytical Instrument (Roche Diagnostic GmbH, Mannheim, Germany).[23]

2.6. Tissue processing and homogenate preparation

The kidney and liver tissues were quickly collected and frozen in liquid N_2. Tissue homogenates were prepared at 4 °C. In brief,

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