Damar Cerrahi Dergisi 2018;27(3):142-149

DOI: 10.9739/tjvs.2018.187
www.turkishjournalofvascularsurgery.org

Original Article

The effect of L-carnitine on cardiac injury in an experimental aortic ischemia-reperfusion injury model

Deneysel aortik iskemi reperfüzyon modelinde kardiyak hasar üzerinde L-karnitinin etkisi

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ABSTRACT

Objectives: The aim of this study was to examine the effect of L-carnitine (3 hydroxy-4 N trimethylammonium butyrate) on ischemia-reperfusion (IR) injury in cardiomyocytes following occlusion-reperfusion of the rat infrarenal abdominal aorta.

Patients and methods: Twenty-four female Sprague-Dawley rats weighing 200 to 250 g were used in this study. The rats were randomly divided into three equal groups (n=8). L-carnitine was administered intraperitoneally 200 mg/kg/day to the treated group (IR+L-carnitine), while the control and IR groups were given intraperitoneal injection of physiological saline three days before the surgical procedure. In the control group, laparotomy and infrarenal abdominal aorta preparation were performed; however, infrarenal abdomianal aorta occlusion was not provided. In the IR and the treated groups (IR+L-carnitine), infrarenal abdominal aorta was clamped with a cross-clamp for 45 min. Removal of the cross-clamp was followed by reperfusion for 10 h. In the treated group, five min before the removal of the aortic clamp, intraperitoneal 200 mg/kg L-carnitine was administered. At the end of the reperfusion period, the rats were sacrificed, and the samples were taken for biochemical and histopathological examinations. Creatine kinase-myocardial band (CK-MB), myoglobin, and troponin I levels were measured at 0, 4, and 10 h. Heart-type fatty acid binding protein (H-FABP) as the biomarker of cardiac ischemia and myeloperoxidase as an indicator of inflammatory response after I/R injury were measured at 10 h. Heart tissue samples were taken for histopathological examination.

Results: There was no statistically significant difference among the control, IR, and IR/L-carnitine groups in terms of the plasma levels of myoglobin, CK-MB, troponin I, myeloperoxidase, and H-FABP levels at 0, 4, and 10 h ($p>0.05$).

Conclusion: Our study results showed that L-carnitine failed to promote a significant effect on the reduction of ischemic injury in the experimental infrarenal aortic cross-clamp-induced I/R model in rats.

Keywords: Aortic occlusion; cardiac damage; ischemia-reperfusion injury; L-carnitine.

ÖZ

Amaç: Bu çalışmada sıçanlarda L-karnitinin infrarenal abdominal aortun oklüzyon-reperfüzyon sonrası kardiyomiyositlerde oluşan iskemi-reperfüzyon (IR) hasarına etkisi incelendi.

Hastalar ve Yöntemler: Bu çalışmada ağırlığı 200-250 g olan 24 adet dişi Sprague-Dawley cinsi sıçan kullanıldı. Sıçanlar rastgele üç eşit gruba ayrıldı (n=8). Cerrahi işlemden üç gün önce tedavi grubuna (IR+L-karnitin) intraperitoneal yoldan 200 mg/kg/gün L-karnitin verilirken, kontrol ve IR gruplarına intraperitoneal serum fizyolojik uygulandı. Kontrol grubunda, laparotomi ve infrarenal abdominal aort hazırlığı yapıldı; ancak infrarenal abdominal aorta oklüzyon uygulanmadı. İskemi-reperfüzyon ve tedavi (IR+L-karnitin) gruplarında, infrarenal abdominal aort çapraz klemp ile 45 dk. süreyle klemplendi. Çapraz klempin kaldırılması sonrasında 10 saat süreyle reperfüzyon uygulandı. Tedavi grubunda, aorttaki klempin kaldırılmasından beş dk. önce, 200 mg/kg intraperitoneal L-karnitin uygulandı. Reperfüzyon süresinin sonunda, sıçanlar sakrifiye edildi ve biyokimya ve histopatolojik incelemeler için örnekler alındı. Kreatin kinaz-miyokardiyal bant (CK-MB), miyoglobin ve troponin I düzeyleri 0, 4. ve 10. saatte ölçüldü. I/R hasarından sonra 10. saatte kardiyak iskeminin biyobelirteci olarak kalp tipi yağ asidi bağlayıcı protein (H-FABP) ve enflamatuvar yanıtın göstergesi olarak miyeloperoksidaz ölçüldü. Histopatolojik inceleme için kalpten doku örnekleri alındı.

Bulgular: Kontrol, IR ve IR/L-karnitin grupları arasında miyoglobin, CK-MB, troponin I, miyeloperoksidaz ve H-FABP düzeyleri açısından 0, 4. ve 10. saatte istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: Çalışma sonuçlarımız sıçanlarda infrarenal aort çapraz klemplenmesi ile oluşturulan deneysel I/R modelinde L-karnitinin iskemik hasarı azaltmada anlamlı bir etkisinin olmadığını gösterdi.

Anahtar sözcükler: Aort oklüzyonu; kardiyak hasar; iskemi-reperfüzyon hasarı; L-karnitin.

Received: May 07, 2018 ***Accepted:*** June 05, 2018

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Citation:
Umaroğlu Öztürk S, Tireli E, Yılmazbayhan D, Ergen A, Ugurlucan M. The effect of L-carnitine on cardiac injury in an experimental aortic ischemia-reperfusion injury model. Damar Cer Derg 2018;27(3):142-149

Ischemia leads to depletion of the cellular stores, accumulation of toxic metabolites, and eventually cell death.[1] Reperfusion injury occurs following reinitiation of the blood flow into the ischemic tissue. Several elements held responsible for this phenomenon include free radicals, renin-angiotensin-aldosterone system, thrombocytes, and contractures.[2]

The severity of inflammatory response following ischemia may be similar in remote organs. These remote injuries most commonly involve the lung and cardiovascular system and may lead to systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndrome (MODS) both of which have 30 to 40% mortality rate in the intensive care unit setting.[3] Myocardial stunning, reperfusion arrhythmias, myocyte necrosis, and coronary endothelial and microvascular dysfunction of the heart may ensue from reperfusion injury.[4,5]

In this study, we aimed to investigate ischemia-reperfusion (IR) injury-related cardiac injury which may develop following cardiovascular interventions requiring aortic cross-clamping, and the use of L-carnitine (3 hydroxy-4 N trimethylammonium butyrate) which can preclude the accumulation of carnitine and acetyl-CoA's (acetyl coenzyme A) toxic esters, thereby, preventing the injury. Creatine kinase-myocardial band (CK-MB), troponin I, and myoglobin levels were biochemically measured, whereas myeloperoxidase (MPO) and free fatty acid binding protein (FABP) levels were evaluated through immunoassay methods with kits specially designed for rats.

PATIENTS AND METHODS

This study was conducted by İstanbul University Faculty of Medicine, Departments of Cardiovascular Surgery, Molecular Medicine and Pathology between December 2010 and October 2011 at the İstanbul University Faculty of Medicine Experimental Research and Application Institute. Ethical approval was obtained from the İstanbul University Faculty of Medicine Local Ethics Committee's (HADYEK) prior to the experimental procedures. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Experimental animals

In this study, a total of 24 female Sprague-Dawley rats weighing 200 to 250 g were used. They were randomly separated in three equal groups (n=8), kept in cages with temperature and humidity rates of 24 to 26˚C and 50 to 60%, respectively for three days; as 12 hour-12 hour daytime-nighttime circadian rhythm. Commercially available standard pellet food and tap water were used to feed the rats. The rats were treated according to the Laboratory Animals Care and Use Guide reorganized by the Experimental Animals Care Principals and Laboratory Sources Institute and received humane care.

Anesthesia

The rats were not fed for 12 hours prior to surgery, although they were allowed drinking water. A total of 50 mg/kg ketamine hydrochloride (Ketalar®, Parke-Dawis, Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Rompun®, Bayer AG, Leverkusen, Germany) were administered intraperitoneally for induction of anesthesia. One-third of the full dosage was repeated intramuscularly, if needed. Surgical interventions, injections and blood collection were performed under anesthesia. There was no need for respiratory support during anesthesia. Hypothermia was prevented via heat lamps.

Study design

Twenty-four rats were divided in three quantitatively equal random groups. Group 1 (controls, n=8) and Group 2 (IR, n=8) were intraperitoneally administered isotonic sodium chloride with the dose of 1 mL/day. Group 3 (IR+L-carnitine, n=8) was intraperitoneally treated with 200 mg/kg/day L-carnitine diluted with isotonic saline to make 1 mL.

To prevent bacterial translocation prior to laparotomy, all rats were treated with intramuscular 25 mg/kg cefazolin (Iespor® vial, I.E. Ulagay, Istanbul, Turkey). Abdominal aortic dissection following laparotomy was performed on the controls (Group 1) with the same stress level and surgery length as in other groups. Group 2 (IR) and Group 3 (IR+L-carnitine) also underwent laparotomy and abdominal aortic dissection, and infrarenal aorta was occluded by an atraumatic microvascular clamp for 45 min (Figure 1). To minimize heat and liquid loss, abdominal incision was temporarily closed with 4.0 prolene sutures. Microvascular clamp was removed following another laparotomy 45 min later and reperfusion was maintained for the following 10 h. Aortic clamping and reperfusion were confirmed by the loss of pulsations in the distal aorta and reinitiation of pulsations in the distal aorta

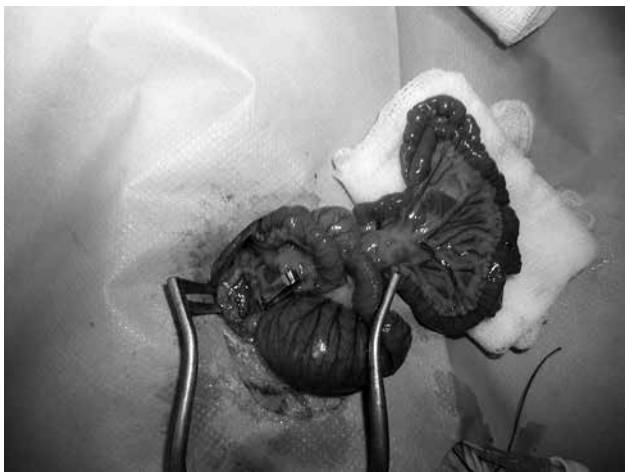

Figure 1. Infrarenal abdominal aortic cross-clamping.

after the removal of clamp, respectively. A total of 200 mg/kg L-carnitine diluted with isotonic saline to make a total of 1 mL was administered intraperitoneally 5 min before clamp removal in Group 3 (IR+L-carnitine).

Furthermore, CK-MB, myoglobin, and troponin I values were obtained from a 0.3 mL of blood sample taken from the inferior vena cava at 0 and 4 h following the clamp removal. At 10 h, the rats were sacrificed and dissected to take the heart tissue samples to be examined histopathologically, for enzyme immunoassay evaluation of myeloperoxidase and free FABP levels, and measurement of CK-MB, myoglobin, and troponin I values. Further blood samples were obtained from the ascending aorta, after the rats were sacrificed.

Blood and tissue sampling

Triage meter device (serial no: 46565, Biosite) (Figure 2) was used to measure CK-MB, myoglobin, and troponin I levels taken from 0.3 mL samples at 0, 4, and 10 h. In addition, 5 mL blood samples were taken from the inferior vena cava of all three groups after the reperfusion period. For biochemical evaluations, blood samples were centrifuged at a rate of 4,000 g rpm for five min to separate plasma. The remaining part of samples were put into the Eppendorf tubes and kept at -80°C to be used for enzyme-linked immunosorbent assay (ELISA) studies.

Median sternotomy was performed for heart tissue sampling and, then, all the hearts were taken out.

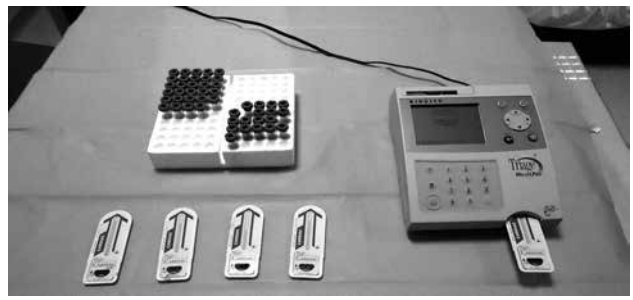

Figure 2. Biosite triage meter device and measurements.

All samples taken for histopathological examination were fixed in formaldehyde solutions (10%) and sent to İstanbul University Faculty of Medicine Pathology Division.

ELISA studies

Plasma heart-type fatty acid binding protein (H-FABP)

Plasma H-FABP levels were measured through the rat H-FABP ELISA kit (highly sensitive) (Life Diagnostics Inc., West Chester PA, USA; Catalog No: 2310-2-HS) by solid phase sandwich ELISA technique. Plates were coated with antibodies specific for H-FABP. Standard solutions (2.5 ng/mL, 1.25 ng/mL, 0.313 ng/mL, 0.156 ng/mL, 0.078 ng/mL, 0 ng/mL) and experiment samples were added to plates. Then, a secondary antibody bound to peroxidase enzyme was added. After the first incubation, H-FABP antigens were seen to be bound to immobilized and peroxidase-bound antibodies. The tetrametilbenzidin (TMB) enzyme was added following wash-away of excess antibodies. A second incubation was performed

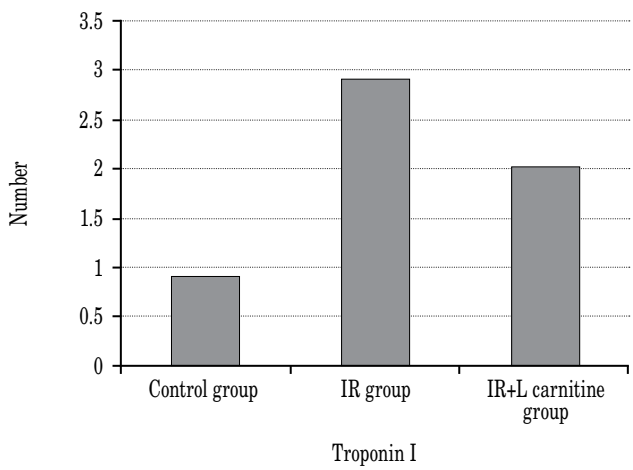

Figure 3. Graphical analysis of Troponin I values among groups.

Table 1. Mean and standard deviations of biochemical values of the groups

	Troponin I (ng/mL)	Myoglobin (ng/mL)	CK-MB (ng/mL)	FABP (ng/mL)	MPO (ng/mL)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control group	1.21±0.97	0.05±0	0.05±0	4.66±1.13	56.2±2.91
IR group	3.55±5.55	0.05±0	0.05±0	4.66±1.94	58.43±5.47
IR /L-carnitine group	2.39±2.62	0.05±0	0.05±0	5.08±1.75	59.16±4.97

CK-MB: Creatine kinase-myocardial band; MPO: Myeloperoxidase; FABP: Fatty acid binding protein; IR: Ischemia-reperfusion.

and unbound enzymes were removed. Citric acid which colorizes the enzyme by binding was also added. The results were analyzed by comparing the outcomes with concentration-absorbance curve obtained from standard solutions and organized into units in ng/mL.

Plasma myeloperoxidase (MPO)

Plasma MPO levels were measured by the MASSAY rat MPO ELISA kit (Catalog No: 23831) ELISA technique. Plates were covered with rat MPO polyclonal antibodies. Standard solutions (50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.125 ng/mL, 1.56 ng/mL) and samples were added into the plates. The rat MPO antigens were bound to immobilized antibodies. A second biotinized antibody was added into the plates and TBS were washed away by a buffer solution. Avidin-biotin-peroxidase complex was transferred into the plates. The TMB enzyme was added following the removal of excess antibodies. Citric acid was also added as the colorizer. The results were matched to the data obtained from standard concentration-absorbance curve and organized into units in ng/mL.

Histopathological studies

All heart tissue samples were evaluated by a single pathologist who was blind to the study groups. At least two sections were examined. At the end of the study, the hearts were completely excised. The remaining blood inside the heart chambers was washed away with ringer lactate solution. An incision was made along atrium, from the apex to the base, and the heart was fixed in formalin solution. Tissues were cut into pieces of 0.5 cm and fixed into paraffin and, then, cut into sections of 3-4 μm thickness parallel to atrioventricular groove. These sections were stained with hematoxylin and eosin (H-E) and examined under light microscope in terms of interstitial edema, myocardial cell swelling, loss of cross striation, myofibril fluctuation, bleeding into tissue, loss of nucleus, pyknosis, karyorrhexis, contraction band necrosis, neutrophil infiltration, intensely stained eosinophilic cytoplasm, separation of muscle fibrils, and necrosis. The Heart Histological Injury Score was used for pathological grading: 0: no injury or minimal injury, 1: mild injury, 2: moderate injury, and 3: severe injury.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 14.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed in frequency, ratio and mean ± standard deviation. The Shapiro-Wilk normality test was used to analyze whether the outcome data were equally distributed. One-way Analysis of Variance (ANOVA) was used to analyze whether there was a statistically significant difference among the normally distributed groups. The Kruskal-Wallis test and Mann-Whitney U test were used to analyze whether there was a statistically significant difference among the abnormally distributed groups and whether there was a significant difference between two groups, respectively. A p value of less than 0.05 was considered statistically significant.

RESULTS

Biochemical findings

The CK-MB, myoglobin, and troponin I levels at 0, 4, and 10 h were measured. The CK-MB, myoglobin, and troponin I levels at 0, 4, and 10 h were found to be identical (for all, $p \geq 0.05$). There was no statistically significant difference between the control group, IR and IR+L-carnitine groups in terms of myoglobin, CK-MB, troponin I levels ($p > 0.05$), (Figure 3, Table 1).

ELISA findings

There was no statistically significant difference between the control group, IR and IR+L-carnitine groups in terms of MPO and H-FABP (Figure 4, 5).

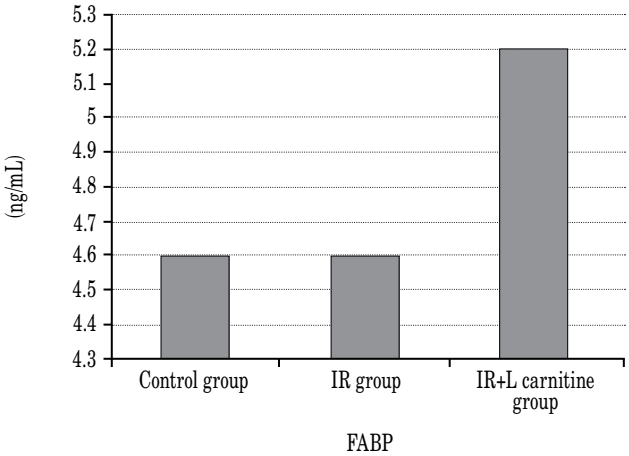

Figure 4. Graphic analysis of FABP values among groups. FABP: Fatty acid binding protein.

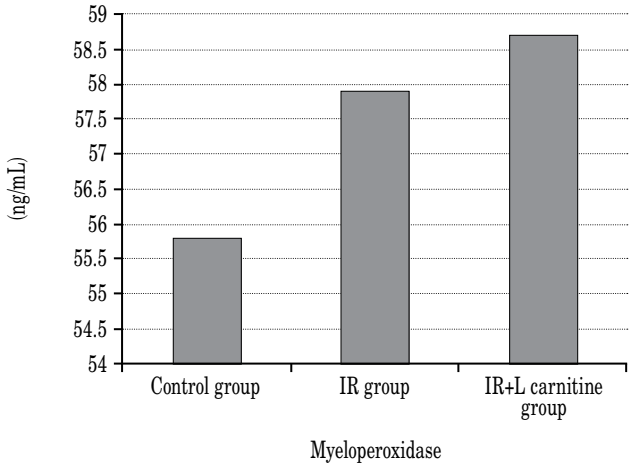

Figure 5. Graphic analysis of myeloperoxidase values among groups.

Histopathological myocardial injury evaluation

There was no histopathologically statistically significant difference when all groups were examined microscopically, and all groups had an injury score of 0.

DISCUSSION

Multiple organ dysfunction is a major cause of mortality and morbidity after abdominal aortic aneurysm surgery. Aneurysm surgery may cause I/R injury, leading to translocation of bacteria and endotoxemia with the systemic release of reactive oxygen radicals and inflammatory response including heart, lung, and kidney damage.[6]

In cardiovascular surgery, aortic and peripheral vascular clamping may lead to IR injury. Cross-clamping of the infrarenal aorta and removal of the clamp is inevitable in abdominal aortic surgery. Hemodynamic effects of aortic cross-clamping varies by preoperative coronary circulatory and myocardial functions, level of cross-clamping, intravascular volume, anesthesia technique, anesthetics used, and surgical pathology.[7]

In abdominal aortic surgery, local and systemic impact of IR injury is a major concern. While local effects are commonly encountered distal to clamp site and muscle tissue, systemic effects are more prominent in all organs, particularly, in the brain, heart, lungs, and kidneys. Loss of microvascular functions during IR periods indirectly leads to leukocyte extravasation, formation of post-capillary venullary plasma protein loss, increased infiltration in capillary beds and leukocyte plugs, and dilatation in arterioles in remote organs. Increased oxygen radical production in every level of microcirculation is responsible for the effects mentioned previously.[6]

Cardiac-related mortality is common in the early and late periods of elective aortic aneurysm repair surgery.[8] Aortic cross-clamping leads to an increased afterload by increasing vascular resistance, a decrease preload by decreasing venous return, and a decreased heart rate by shortening in myocyte fibrils. Serious consequences of aortic cross-clamping were shown by various experimental and clinical trials. Studies showed that it caused a 15 to 30% decline in the stroke volume and cardiac index, as well as over a 40% increase in systemic resistance, thereby, resulting in increased arterial blood pressure.[9]

Hemodynamic response following aortic cross-clamping is drastically influenced by preoperative cardiac state and reserves. Attia et al.[10] showed that there were two different hemodynamic responses after cross-clamping. In patients with no underlying atherosclerotic heart valve disease, aortic cross-clamping decreased pulmonary artery, pulmonary capillary wedge and central venous pressures, whereas it increased the ventricular filling pressure in patients with clinically proven cardiac diseases.

In a study conducted by Gooding et al.,[11] in addition to increased pulmonary capillary wedge pressure, cross-clamping led to severely decreased cardiac index in patients with coronary artery disease, compared to those without any underlying coronary pathology.

L-carnitine, which is an endogenous compound obtained through synthesis of essential amino acids lysine and methionine, is the essential cofactor of carnitine palmitoyltransferase I (CPT I).[12] It mediates the transportation of fatty acids to mitochondria and acetyl CoA formation by combining long-chain-fatty-acids. Along with fatty acid metabolism, L-carnitine also plays an important role in glucose metabolism, regulation of intra-mitochondrial acetyl CoA/CoA ratio, and pyruvate dehydrogenase complex.[13,14]

Energy obtained from L-carnitine is crucial for cardiac tissues.[12] More importantly, heart failure is deteriorated by low myocardial adenozin trifosfat (ATP) levels in cardiovascular diseases, such as heart failure and ischemia. Thus, ATP required to maintain cell and tissue integrity and cardiac functions may be supplied by L-carnitine and its derivatives.[15,16] Aoyagi et al.[17] showed that carnitine infusion was effective in decreasing myocardial injury. Onem et al.[18] showed L-carnitine infusion proved to be protective in IR injury in rats.

The CK-MB, myoglobin, troponin T, and troponin I are markers which can be analyzed in blood following cardiac injury. Myoglobin, which is also present in the skeletal muscles, is the earliest marker in blood (1 to 3 h); however, it has low specificity.[19] It peaks within five to eight h and turns back to normal levels after 24 h. Troponin I, despite having the highest sensitivity and specificity,[20] rises much later (3 to 6 h). It generally peaks at about 12 h and, then, turns back to normal levels in 3 to 10 days.

In a study conducted by Plebani and Zaninotto,[21] sensitivity and specificity of myoglobin in the early period were found to be 92% and 55%, respectively. This study was performed in unselected patient groups. Low specificity of myoglobin in the early period can be explained by other causes leading to a rise in myoglobin such as skeletal muscle injury, neuromuscular disorders, renal insufficiency, intramuscular injection, heavy exercise, and intake of various drugs and toxins. As this is a disadvantage for myoglobin, the measurement of cardiac specific markers such as troponin I and T along with myoglobin could may be useful.[22]

Cardiac troponin T (cTnT) is a necrosis-sensitive marker. However, its sensitivity is yet to be determined. It increases in response to musculoskeletal injury in rats. It cannot be detected in plasma of healthy adults and athletes. Adams et al.[22] found increased cTnT levels in patients with polymyositis without myocardial injury.

In patients with acute coronary syndrome, increased troponin I levels were found to be associated with increased mortality and morbidity.[20] It was reported that troponin I levels were high during myocardial infarction and related to increased mortality in the first postoperative six months.[20] In our study, there was no statistically significant difference between the three groups in terms of CK-MB, myoglobin, and troponin I measured from the blood samples taken at 0, 4, and 10 h. Termination of our study at 10 h might explain why CK-MB and troponin levels did not increase.

Free FABP is an important intracellular low-molecular-weight protein that is abundant in cytosol. Heart-type free fatty acid binding protein is most commonly present in myocardium; however, it is also found in renal and musculoskeletal tissues.[23] It is an early marker of myocardial injury and also used to evaluate the size of infarct. Following myocardial injury, H-FABP is found in plasma and urine, and only in urine of humans and rats, respectively. First spike and peak times are earlier than CK-MB. The H-FABP was found to be more sensitive after post-thrombolytic reperfusion injury.[23] Hasegawa et al.[24] showed that, in addition to being an early marker of myocardial injury, H-FABP might also be used to predict clinical prognoses in pediatric heart surgery. Our study could not identify any statistical difference, when H-FABP levels from IR and IR/L-carnitine and controls were compared. This could be explained by variations in the FABP metabolism, half-life, and critical levels in rats.

Myeloperoxidase is a hemoprotein secreted by activated neutrophils and monocytes. It has microbicidal activity through formation of hypochlorous acid from hydrogen peroxide.[25] Several retrospective studies showed that high free MPO levels could be a determinant in early myocardial infarction, and an early marker in myocardial necrosis.[19,26] Marshall et al.[26] showed that acute myocardial infarction was associated with neutrophil activation and MPO secretion in systemic circulation, which supports the previously conducted studies and their findings.

According to a study conducted by Inoue et al.,[27] in the diagnosis of acute coronary syndrome, heart-specific troponin T was prone to false-positive results, despite having a high sensitivity, and H-FABP had a similar relationship. Hence, it was proposed H-FABP and MPO together would be an ideal diagnostic

method to assess patients with acute coronary syndrome who apply to emergency service with chest pain. In our study, MPO levels obtained from IR and IR/L-carnitine, and control groups were statistically identical.

The IR model we used in our study was also inspired by other studies performed in different centers and times by Ekim et al.,[28] Oz Oyar et al.,[29] and Kiriş et al.[30] L-carnitine dosing was adapted from the studies done by Onal et al.[31] and Scafidi et al.[32] and modified to be 200 mg/kg.

In the histological examinations, dense muscle fibrils, scarce connective tissue, and normal heart tissue was noted. Ischemia of cardiac tissue is histologically characterized by myofibrils fluctuation at 0 to 30 min, coagulation necrosis, edema, hemorrhage, neutrophilic infiltration at 4 to 12 hours, nuclear pyknosis, densely eosinophilic cytoplasm, marginal contraction band necrosis at 18 to 24 hours, total coagulation necrosis, intense neutrophilic infiltration, striations and loss of nucleus at 24 to 72 hours.[33]

There are some limitations to this study. First, myocardial histopathological changes caused by IR injury are seen in the late period. In our study, 45 min of ischemia and 10 h of reperfusion may not be long enough for cellular changes to occur, and this could explain why there was no pathological finding in all three groups. Second, electron microscopy might have been a more useful tool to show heart injury scores.

As an inevitable outcome of heart surgery, ischemia and ensuing reperfusion injury might develop. L-carnitine infusion increases tissue carnitine levels, thus improves systolic and diastolic functions. In many studies carnitine was administered before or after ischemia as continuous infusion. Since this way of application is not clinically practical, its use is restricted, so there's no consensus on the application route and dosing. When looking at the current studies on local and systemic effects of cross-clamping during aortic surgery, particularly, mechanism of cardiac injury in cross-clamping is still uncertain. Number of studies on L-carnitine use following vascular reconstruction-related cardiac IR injury is small.

In conclusion, in this experimental IR model, following infrarenal abdominal aortic clamping, there was no significant difference in myoglobin, troponin I and CK-MB levels taken from the blood samples of controls, IR and IR+L-carnitine groups at 0, 4 and 10 hours. There was also no statistically significant difference in MPO and H-FABP levels measured at the 10th hour among all three groups. There wasn't any significant histological difference in the cardiac tissue samples taken from the sacrificed rats in all three groups. No negative effect was observed in IR period in terms of dosage and carnitine use. Ineffectiveness of L-carnitine might be related to the route, duration of administration and dosage of the drug in our study. Another factor affecting the outcome might be the grade of ischemic injury. Forty five min of ischemia and lack of direct clamping to the heart might be insufficient to induce an observable damage. Thus, favorable effects of L-carnitine could be hindered by a low level of ischemic injury. As a result; we observed L-carnitine had no significant effect in decreasing ischemic injury in the experimental IR model. A deeper understanding into the mechanisms of L-carnitine's benefits in decreasing ischemic injury, particularly, local and remote organ IR injury after aortic surgery might lead to a quicker and more proper management in future. Further research is needed to validate L-carnitine's efficacy on prophylactic treatment of reperfusion induced injury.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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