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Effects of carnosine on prooxidant–antioxidant status in heart tissue, plasma and erythrocytes of rats with isoproterenol-induced myocardial infarction

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ABSTRACT

Rats were injected with isoproterenol (ISO; 110 mg/kg, *ip*, 2 doses, 24 h interval) to induce acute myocardial infarction (AMI) and were sacrificed 6 and 24 h after the last ISO injection. The heart tissue, plasma and erythrocytes of these rats were evaluated for cardiac markers and oxidative stress parameters. Levels of cardiac troponin T (cTnT) and the activities of creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) in plasma were increased 6 and 24 h after ISO treatment. The levels of malondialdehyde (MDA), diene conjugate (DC), and protein carbonyl (PC) were increased in heart tissue and plasma, while levels of erythrocyte MDA and glutathione (GSH) and plasma ferric reducing antioxidant power (FRAP) were also increased. However, GSH levels and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) decreased in heart tissue of rats with AMI. We also investigated the effects of carnosine (CAR) treatment on these parameters 24 h after the last ISO injection. CAR (250 mg/kg/day; *ip*) treatments were carried out either 10 days before ISO injection or 2 days concomitant with ISO. Pretreatment with CAR decreased plasma LDH and AST activities and ameliorated cardiac histopathological changes in ISO-treated rats. Cardiac MDA, DC and PC levels decreased, but GSH levels and SOD and GSH-Px activities increased. However, the increases in plasma MDA and PC levels as well as erythrocyte H_2O_2-induced MDA and GSH levels did not change due to CAR pretreatment. In conclusion, our findings indicate that CAR pretreatment may have protective effects on ISO-induced cardiac toxicity by decreasing oxidative stress.

Introduction

Acute myocardial infarction (AMI) is a prevalent ischemic heart disease that is one of the main causes of death from cardiovascular disease [40]. Ischemic cardiac tissue is known to generate reactive oxygen species (ROS) and causes oxidative damage in membrane lipids, proteins, and DNA [29,32,40,43].

Isoproterenol [L-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride; ISO] is a synthetic β-adrenergic agonist. When administered to animals in high doses, ISO causes infarct-like necrosis of the heart muscle, which morphologically resembles AMI in humans [18,29]. The generation of highly cytotoxic free radicals through auto-oxidation of catecholamines plays an important role in ISO-induced cardiac damage [18,29]. Therefore, many investigators have tested the preventive effects of several antioxidants on ISO-induced AMI [18,23,26,29,30,32,36,43].

Carnosine (β-alanyl-L-histidine; CAR) is an antioxidant dipeptide that is present in several mammalian tissues. It has several functions, which include protection of membrane, pH-buffering capacity, and metal-chelating ability [1,9,14]. CAR is also a potent scavenger of ROS and aldehydes, and it inhibits lipid peroxidation and protein oxidation [1,9,14]. CAR has been used to prevent oxidative stress-induced pathologies such as liver injury [3,22,24], atherosclerosis [33], diabetic complications [20], ischemia-reperfusion [13,39], neurodegeneration [15] and aging [4,15]. It has been reported that CAR may also have cardioprotective effects [35] and has been shown to ameliorate cardiac dysfunction induced by doxorubicin (DOX) [11,44]. However, to our knowledge, there have not been any

Abbreviations: AMI, acute myocardial infarction; AOA, antioxidant activity; AST, aspartate aminotransferase; CAR, carnosine; CAT, catalase; CK, creatine kinase; cTnI, cardiac troponin I; cTnT, cardiac troponin T; DC, diene conjugate; DOX, doxorubicin; FRAP, ferric reducing antioxidant power; GSH, glutathione; GSH-Px, glutathione peroxidase; GST, glutathione transferase; ISO, isoproterenol; LDH, lactate dehydrogenase; MDA, malondialdehyde; PC, protein carbonyl; ROS, reactive oxygen species; SOD, superoxide dismutase.

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studies investigating the in vivo effect of CAR on ISO-induced AMI.

In our study, we aimed to determine whether the prooxidant status of cardiac tissue effects plasma and erythrocytes and whether ISO-induced myocardial oxidative stress and tissue damage could be prevented by treatment with CAR.

Materials and methods

Chemicals

ISO (DL-15627) and L-CAR (C-9625) were purchased from Sigma Aldrich Chemical Company (USA). All other chemicals were of analytical grade.

Animals and experimental design

Sprague-Dawley male rats weighing 300–400 g were used in this study. They were obtained from the Center for Experimental Medical Research Institute of Istanbul University. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of Istanbul. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages.

Rats were divided into four groups.

a) Control rats ($n = 8$): Rats were given 0.9% NaCl as vehicle.
b) AMI-induced rats ($n = 8$): ISO (110 mg/kg, *ip*) was injected into the rats in two doses 24-h apart to produce AMI. Rats were anesthetized using sodium thiopental (50 mg/kg, *ip*) and were sacrificed 6 and 24 h after their last ISO treatment.
c) CAR-treated rats with AMI ($n = 8$): CAR (250 mg/kg/day for 2 days; *ip*) was administered to the rats 30 min prior to injection with ISO. These rats were sacrificed 24 h after their last ISO injection.
d) CAR-pretreated rats with AMI ($n = 8$): Rats were treated with CAR (250 mg/kg/day) for a period of 12 days. On the 11th and 12th days, ISO was given as two doses with a 24 h interval, and the rats were sacrificed 24 h after the last ISO treatment.

Blood samples were collected into heparinized tubes. Plasma and erythrocytes were separated by centrifugation at 1500 × *g* for 10 min. The hearts were dissected out immediately, washed with ice-cold saline, and were homogenized in ice-cold 0.15 M KCl (10%, w/v). Tissue samples and plasma were frozen at −80 °C until analyzed.

Analysis of plasma

The levels of plasma cardiac troponin T (cTnT) and the activities of creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were measured (Roche Diagnostics, Mannheim, Germany). The degree of lipid peroxidation in the plasma was assessed using thiobarbituric acid according to the method of Buege and Aust [10]. The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Protein carbonyl levels were determined by ELISA (OxiSelect, Cell Biolabs, Inc, CA, USA). Plasma antioxidant activity (AOA) was evaluated using a ferric reducing antioxidant power (FRAP)-assay [7]. This assay uses antioxidants as reductants in a redox-linked colorimetric method. At low pH, the ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex is reduced to the ferrous form, which is monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly proportional to the reducing power of the electron-donating antioxidants present in the plasma. The change in absorbance is translated into a FRAP value by relating the change in absorbance at 593 nm of a test sample to that of a standard solution with a known FRAP value.

Analysis of erythrocytes

Erythrocyte susceptibility to lipid peroxidation was determined according to the method of Stocks and Dormandy [38]. The final composition of the incubation mixture was 5 mM H_2O_2, 2 mM sodium azide, and an erythrocyte suspension in phosphate-buffered saline (30 mg Hb/mL incubation mixture). Lipid peroxidation was assayed by measuring MDA production over 2 h incubation period at 37 °C. Values were expressed as nanomoles of MDA per gram of Hb. Hb concentration of erythrocyte suspensions and whole blood were measured using Drabkin's reagent. Glutathione (GSH) levels in erythrocytes were determined according to Beutler et al. [8] This method is based upon the development of a relatively stable yellow color when 5,5′-dithiobis-(2-nitrobenzoate) is added to sulfhydryl compounds.

Analysis of heart tissue

MDA levels in cardiac homogenates were measured using the thiobarbituric acid test [28]. Diene conjugate (DC) formation was determined spectrophotometrically at 234 nm [10]. Cardiac lipids were extracted with a chloroform/methanol (2:1) mixture. The extracted lipids were re-dissolved in cyclohexane, and the approximate amounts of hydroperoxides were calculated using a molar extinction coefficient of $2.52 \times 10^{-4}\ M^{-1}\ cm^{-1}$.

PC levels were determined by ELISA. GSH levels were measured in heart homogenates by the method of Beutler et al. [8]. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined in the postmitochondrial fraction of the heart, which was separated by sequential centrifugation. In brief, heart homogenates were centrifuged at 600 × *g* for 10 min at 4 °C to remove crude fractions. Then, supernatants were centrifuged at 10,000 × *g* for 20 min to obtain the postmitochondrial fraction. SOD activity was determined by its ability to increase the riboflavin-sensitized photooxidation of o-dianisidine [25]. GSH-Px activity was measured using the method of Lawrence and Burk with cumene hydroperoxide as the substrate [19]. In this method, GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase and the oxidation of NADPH was followed spectrophotometrically at 340 nm at 37 °C. Results were calculated using an extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$. Protein levels were determined using bicinchoninic acid [37].

Histopathological observations

Heart tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin (H&E) for histological studies.

Statistical analysis

Results were expressed as mean ± SD. Experimental groups were compared using Student's *t*-test or Mann–Whitney *U* test, and $p < 0.05$ was considered to be statistically significant.

Results

Cardiac markers in plasma and oxidative stress parameters in heart tissue, plasma, and erythrocytes were analyzed in rats 6 and 24 h after ISO treatment. The results are as follows:

Table 1
Cardiac troponin T (cTnT) levels and creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities in plasma of rats 6 and 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	Control	ISO/6 h	ISO/24 h
cTnT (μg/L)	0.68 ± 0.27	2.95 ± 1.21[a]	1.73 ± 0.65[a,b]
CK (U/L)	203.7 ± 20.6	401.4 ± 129.1[a]	331.6 ± 79.0[a]
LDH (U/L)	89.2 ± 9.84	195.7 ± 29.5[a]	122.0 ± 36.2[a,b]
AST (U/L)	217.0 ± 46.7	582.1 ± 128.5[a]	317.5 ± 89.6[a,b]

[a] $p < 0.05$ as compared to controls.
[b] $p < 0.05$ as compared to ISO/6 h.

Table 2
Malondialdehyde (MDA), protein carbonyl (PC) and glutathione (GSH) levels and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in heart tissue of rats 6 and 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	Control	ISO/6 h	ISO/24 h
MDA (nmol/g)	127.0 ± 16.3	167.6 ± 28.7[a]	156.2 ± 32.0[a]
DC (μmol/g)	1.07 ± 0.17	1.66 ± 0.52[a]	1.46 ± 0.21[a]
PC (nmol/mg protein)	0.61 ± 0.19	1.21 ± 0.87[a]	1.03 ± 0.35[a]
GSH (μmol/g)	2.11 ± 0.18	1.61 ± 0.18[a]	1.63 ± 0.19[a]
SOD (U/mg protein)	24.3 ± 3.03	36.4 ± 4.02[a]	19.4 ± 2.10[a,b]
GSH-Px (nmol/min/mg protein)	248.7 ± 35.2	208.0 ± 37.9[a]	186.4 ± 33.7[a]

[a] $p < 0.05$ as compared to controls.
[b] $p < 0.05$ as compared to ISO/6 h.

a) Plasma cTnT levels and the activities of CK, LDH and AST significantly increased 6 and 24 h after ISO treatment (Table 1). cTnT levels and the activities of CK (not significant), LDH, and AST were higher at 6th h than at the 24th h following ISO.
b) Cardiac MDA, DC and PC levels increased 6 and 24 h after ISO injection. However, there were no differences between these parameters 6 h and 24 h after ISO treatment. Although decreased GSH levels and GSH-Px activities and increased SOD activities were observed in heart tissue 6 h after ISO, these parameters decreased at 24 h in ISO-treated rats (Table 2).
c) Plasma MDA and PC levels and erythrocyte H_2O_2-induced MDA and GSH levels also increased 6 and 24 h after ISO. MDA and PC levels were higher at the 6th h than at the 24th h. FRAP values increased at the 6th h, but not at the 24th h in ISO-treated rats (Table 3).
d) Normal myocardial structure was observed in the control group (Fig. 1A). Six hours after ISO, the pathological features of edema became apparent around the myocardial fibers and perivascular areas. Patchy lymphocytes, an infiltration of plasma cells, edema, and necrosis of scattered myocardial cells were observed. Contractile dysfunction (wavy appearance) was observed in a few areas (Fig. 1B). Twenty-four hours after ISO, the pathological features of the heart were normal in many areas. But myocardial fibers exhibited a wavy appearance due to contractile dysfunction in some fields. Edema was observed around the vessels. Patchy lymphocytes, an infiltration of plasma cells, edema, myxomatous degeneration, and necrosis of myocardial cells were observed, especially near the heart apex (Fig. 1C).

Table 3
Malondialdehyde (MDA) and protein carbonyl (PC) levels and ferric reducing antioxidant power (FRAP) values in plasma as well as H_2O_2-induced MDA and glutathione (GSH) levels in erythrocytes of rats 6 and 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	Control	ISO/6 h	ISO/24 h
Plasma			
MDA (μmol/L)	5.33 ± 0.41	7.14 ± 0.68[a]	6.22 ± 0.52[a,b]
PC (nmol/mg protein)	2.48 ± 0.21	3.22 ± 0.52[a]	2.86 ± 0.40[a,b]
FRAP (μmol/L)	239.7 ± 42.7	332.2 ± 44.9[a]	231.6 ± 22.2[b]
Erythrocyte			
MDA (nmol/g Hb/h)	739.4 ± 60.3	871.4 ± 31.7[a]	823.6 ± 58.7[a]
GSH (mmol/L)	3.00 ± 0.36	3.66 ± 0.49[a]	3.89 ± 0.64[a]

[a] $p < 0.05$ as compared to controls.
[b] $p < 0.05$ as compared to ISO/6 h.

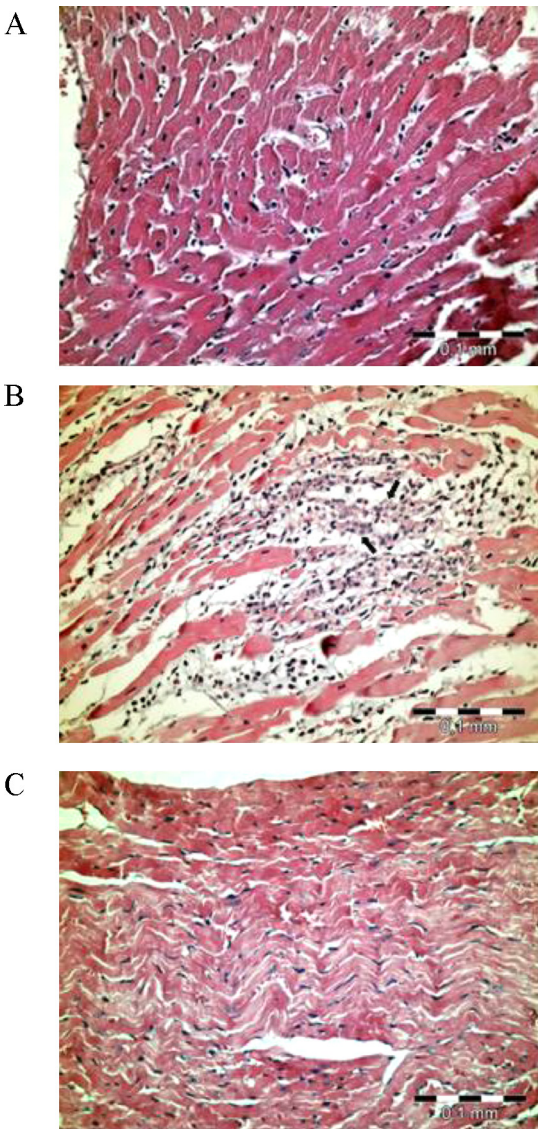

Fig. 1. Histopathological findings of the heart tissue 6 and 24 h after isoproterenol (ISO) treatment. (A) Normal myocardial histology. (B) Degenerated muscle cells and mild inflammatory cell infiltration among myocardial fibers 6 h after ISO. (C) Myocardial fibers with wavy appearance (contractile dysfunction) 24 h after ISO (H&E 400×).

Tables 4–6 show the changes in cardiac markers in the plasma and the prooxidant–antioxidant balance in heart tissue, plasma, erythrocytes of rats with ISO-induced AMI that were treated with CAR for 2 or 12 days. The results are as follows:

a) There were no changes in cardiac markers in the plasma or in the oxidative stress parameters in heart tissue, plasma, or erythrocytes in rats treated with the two doses of CAR and given ISO. However, plasma FRAP levels in these rats increased.

Although the 12 doses of CAR did not affect plasma cTnT or CK activities, AST and LDH activities decreased in the plasma of these rats (Table 4).

Table 4
Carnosine (CAR) treatment for 2 and 12 days on cardiac troponin T (cTnT) levels and creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities in plasma of rats 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	ISO	CARx2 + ISO	CARx12 + ISO
cTnT (μg/L)	1.73 ± 0.65	1.87 ± 0.72	1.79 ± 0.42
CK (U/L)	331.6 ± 79.0	358.0 ± 70.2	308.1 ± 69.2
LDH (U/L)	122.0 ± 36.2	137.5 ± 48.7	79.2 ± 16.7[a,b]
AST (U/L)	317.5 ± 89.6	322.0 ± 80.5	235.7 ± 43.4[a,b]

[a] $p < 0.05$ as compared to ISO.
[b] $p < 0.05$ as compared to CARx2 + ISO.

Table 5
Carnosine (CAR) treatment for 2 and 12 days on malondialdehyde (MDA), protein carbonyl (PC) and glutathione (GSH) levels and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in heart tissue of rats 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	ISO	CARx2 + ISO	CARx12 + ISO
MDA (nmol/g)	156.2 ± 32.0	142.8 ± 36.0	120.2 ± 28.9[a]
DC (μmol/g)	1.46 ± 0.21	1.38 ± 0.24	1.15 ± 0.15[a,b]
PC (nmol/mg protein)	1.03 ± 0.35	0.94 ± 0.22	0.71 ± 0.12[a,b]
GSH (μmol/g)	1.63 ± 0.19	1.70 ± 0.23	1.88 ± 0.24[a]
SOD (U/mg protein)	19.4 ± 2.10	20.6 ± 2.42	22.8 ± 2.82[a]
GSH-Px (nmol/min/mg protein)	186.4 ± 33.7	202.0 ± 37.2	226.7 ± 29.8[a]

[a] $p < 0.05$ as compared to ISO.
[b] $p < 0.05$ as compared to CARx2 + ISO.

Table 6
Carnosine (CAR) treatment for 2 and 12 days on malondialdehyde (MDA) and protein carbonyl (PC) levels and ferric reducing antioxidant power (FRAP) values in plasma as well as H_2O_2-induced MDA and glutathione (GSH) levels in erythrocytes of rats 24 h after isoproterenol (ISO) treatment (means ± SD; $n = 8$, each).

	ISO	CARx2 + ISO	CARx12 + ISO
Plasma			
MDA (μmol/L)	6.22 ± 0.52	5.80 ± 0.47	5.68 ± 0.52
PC (nmol/mg protein)	2.86 ± 0.40	2.78 ± 0.42	2.72 ± 0.48
FRAP (μmol/L)	231.6 ± 22.2	268.2 ± 42.1[a]	340.6 ± 38.4[a,b]
Erythrocyte			
MDA (nmol/g Hb/h)	823.6 ± 58.7	848.2 ± 60.7	865.6 ± 58.1
GSH (mmol/L)	3.89 ± 0.64	3.74 ± 0.62	3.39 ± 0.57

[a] $p < 0.05$ as compared to ISO.
[b] $p < 0.05$ as compared to CARx2 + ISO.

b) CAR pretreatment caused significant decreases in cardiac MDA, DC and PC levels, and increases in GSH levels and the activities of SOD and GSH-Px with CAR treatment for 12 days (Table 5).
c) CAR pretreatment had no effect on plasma MDA and PC levels, nor did it affect erythrocyte H_2O_2-induced MDA or GSH levels in ISO-treated rats. However, CAR pretreatment did increase FRAP values.
d) In ISO-treated rats injected with 2 doses of CAR, focal edema was observed in the myocardial tissue, particularly at the apex side. Patchy lymphocytes, infiltration of plasma cells, and a few degenerative and necrotic cells were observed in the myocardial tissue. There was perivascular edema and a wavy appearance of the fibers in a few areas (Fig. 2B). However, in ISO-treated rats injected with 12 doses of CAR, interstitial edema was observed among the myocardial fibers. Degenerative vacuoles were seen in a few myocardial cells (Fig. 2C).

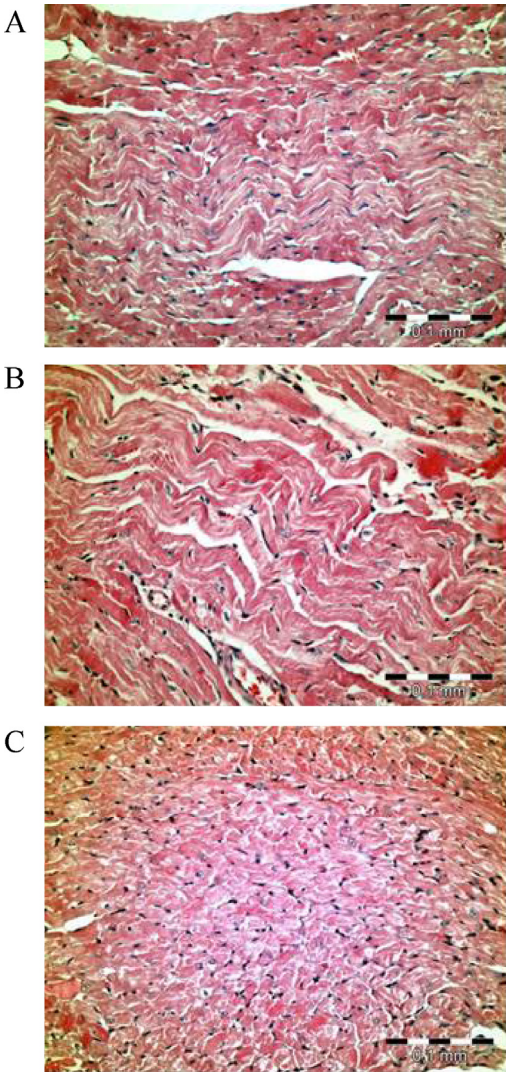

Fig. 2. Histopathological findings of the heart tissue of rats with acute myocardial infarction (AMI) treated with two or twelve doses of carnosine (CAR). (A) Myocardial fibers with wavy appearance (contractile dysfunction) 24 h after isoproterenol (ISO). (B) Myocardial fibers with wavy appearance in rats with AMI treated with 2 doses of CAR. (C) Interstitial edema and mild degeneration in myocardial cells of rats with AMI treated with 12 doses of CAR (H&E 400×).

Discussion

Animal models are very useful when investigating new approaches to the prevention and therapy of human AMI. Experimental AMI in rodent models can be induced by the injection of one or two doses of ISO 24-h apart. Electrocardiographic (ECG) abnormalities are generally used for the diagnosis of AMI. Animals treated with ISO show changes in ECG, which indicate myocardial ischemia [18,43]. Cardiac troponins such as cTnI, cTnT, and CK-MB are very sensitive and specific indicators of AMI [27]. ISO treatment has been reported to result in significant increases in serum cTnI [18,27,32] and cTnT [23,30,32] levels and has increased the activities of CK-MB [23,30–32], CK, AST and LDH in experimental animals [29,30,36,43]. In our study, serum cTnT levels and the activities of CK, AST and LDH were determined 6 and 24 h after two doses of ISO. Plasma cardiac markers increased significantly when compared to controls. Increases in cTnT levels and the activities of CK (not significant), AST and LDH as well as cardiac histopathological changes were higher at the 6th h than at the 24th h following ISO treatment, as previously reported in mice and rats [12,27].

Oxidative stress is considered to be one of the mechanisms of ISO-induced AMI. Several authors have studied cardiac prooxidant and antioxidant parameters after ISO treatment in rats, and have

found significant increases in lipid peroxide [18,29,30,32,36,43] and PC [23] levels as well as DNA damage [31] in heart tissue. In addition, levels of non-enzymatic antioxidants such as GSH, vitamin E, and vitamin C as well as the activities of enzymatic antioxidants such as SOD, catalase (CAT), GSH-Px and glutathione transferase (GST) decreased in the heart tissue of ISO-treated rats [18,23,29,30,32,36,43]. In our study, we also compared prooxidant and antioxidant parameters in heart tissue 6 and 24 h after ISO treatment. Cardiac MDA, DC and PC levels increased 6 h after ISO. Although GSH levels and GSH-Px activity decreased, cardiac SOD activity was observed to increase 6 h after ISO. It has been reported that ROS can directly increase SOD expression [41,42]. Therefore, the increase in SOD activity may be related to increased superoxide radical formation, and an imbalance between SOD and GSH-Px might be one of the responsible factors for increased oxidative stress in the heart tissue. Furthermore, 24th h following ISO, tissue levels of MDA, DC and PC continued to increase. However, GSH levels as well as SOD and GSH-Px activities were reduced at this time as compared to control values. These findings clearly indicate that ISO treatment disturbed the prooxidant–antioxidant balance in favor of prooxidation in heart tissue.

Plasma MDA and PC levels as well as erythrocyte MDA and GSH levels were also determined in rats with AMI. Increases in lipid peroxide levels [26,32,43] and decreases in nonenzymatic [18,23,26,32] and enzymatic [43] antioxidants have been found in plasma after ISO treatment. In our study, plasma MDA and PC levels increased 6 and 24 h after ISO treatment. However, these values were higher at the 6th h than at the 24th h following ISO treatment. Interestingly, although plasma FRAP values increased 6 h after ISO, these values remained unchanged 24 h after ISO. Our results indicate that there is increased passage of oxidized products from damaged cardiac tissue to plasma in ISO-treated rats.

Since erythrocyte membranes are rich in polyunsaturated fatty acids, they are very sensitive to oxidative stress. They are continuously exposed to high concentrations of oxygen and contain a powerful transition metal catalyst [5,38]. In the literature, there are two studies that have investigated oxidative stress in the erythrocytes of ISO-treated rats. ISO treatment was reported to cause significant decreases in the activities of antioxidant enzymes such as SOD, CAT, GSH-Px and GST [32]. In addition, one group [34] reported that erythrocyte MDA levels and SOD activity increased 6 and 12 h after ISO, while those of the control group did not. They found that GSH levels remained unchanged at the 6th h, but were increased at the 12th h after ISO treatment. In our study, MDA and GSH levels increased 6 and 24 h after ISO. The increase in erythrocyte GSH levels may be due to an adaptive response toward oxidative stress. These results clearly indicate that ISO treatment may cause peroxidative damage in erythrocytes.

Antioxidant therapy may be an important strategy to improve ISO-induced oxidative stress and cardiac damage. Several antioxidants such as ginkgo biloba [29], gallic acid [30], fluvastatin [43], lycopene [23], thymol [26], ellagic acid [18], ursolic acid [32] and taurine [36] have been found to attenuate cardiac damage in ISO-treated rats. CAR has antioxidant properties. Its antioxidant potential may be dependent on its ability to inactivate ROS, scavenge free radicals, and chelate prooxidant metals [1,9,14]. Since L-CAR has a short half-life in humans (due to its rapid inactivation by serum and tissue carnosinase), the potential antioxidant activity of L-CAR in vivo is limited in humans. Therefore, L-CAR derivatives, such as D-CAR and N-acetyl-CAR, which are resistant to carnosinase, have been used by some investigators [2,6,15]. While ineffective in humans, L-CAR has a long half-life in rodents, since they lack serum carnosinase activity [2,6,15]. CAR levels were found to increase in the plasma and tissues of rodents following CAR administration [21,24].

To our knowledge, there has not been an in vivo study investigating the effect of CAR treatment in ISO-induced AMI. However, CAR treatment has been reported to attenuate ischemia-reperfusion heart damage [39]. Increases in cardiac lipid peroxide levels, decreases in antioxidant enzyme activities, and cardiac dysfunction were found to improve in DOX-treated rats treated with CAR [11]. In addition, CAR treatment also ameliorated disturbances in hemodynamic parameters and cardiac damage in DOX-treated rabbits [44].

In our study, we investigated the effects of CAR on two different treatment protocols (two doses of CAR together with ISO or ten doses of CAR before ISO and two doses of them together with ISO) on cardiac markers and prooxidant–antioxidant balance in heart tissue, plasma and erythrocytes 24 h after two doses of ISO. The dose of CAR was chosen according to our previous studies, which defined as the effective concentration [4,16,22]. When CAR was given together with ISO for 2 days, it had no positive effects. However, CAR pretreatment for 12 days resulted in decreased plasma LDH and AST activities, but did not alter cTnT levels and CK activities in the plasma of ISO-treated rats. We also observed a slight decrease of cardiac histopathological findings. CAR pretreatment for 12 days also resulted in significant decreases of MDA, DC and PC and increases in GSH, SOD and GSH-Px in the heart tissue of ISO-treated rats. These results indicate that CAR, which is known to be a good scavenger of superoxide and hydroxyl radicals, spares SOD molecules [17] and compensates for the deficits in the antioxidant system in the heart tissue due to ISO. Interestingly, although CAR treatment increased the serum antioxidant power in ISO-treated rats, there were no changes in the levels of oxidative stress parameters in plasma and erythrocytes.

In conclusion, our findings indicate that CAR pretreatment may have protective effects on ISO-induced cardiac toxicity by decreasing oxidative stress.

Conflict of interest

No conflict of interest.

Funding

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References

[1] Aldini G, Facino RF, Beretta G, Carini M. Carnosine and related dipeptides as quenchers of reactive carbonyl species: from structural studies to therapeutic perspectives. Biofactors 2005;24:77–87.
[2] Aldini G, Orioli M, Rossoni G, Savi F, Braidotti P, Vistoli G, et al. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. J Cell Mol Med 2011;15:1339–54.
[3] Artun BC, Küskü-Kiraz Z, Güllüoğlu M, Çevikbaş U, Koçak-Toker N, Uysal M. The effect of carnosine pretreatment on oxidative stress and hepatotoxicity in binge ethanol administered rats. Hum Exp Toxicol 2010;29:659–65.
[4] Aydın AF, Küçükgergin C, Özdemirler-Erata G, Koçak-Toker N, Uysal M. The effect of carnosine treatment on prooxidant–antioxidant balance in liver, heart and brain tissues of male aged rats. Biogerontology 2010;11:103–19.
[5] Aydın AF, Küskü-Kiraz Z, Doğru-Abbasoğlu S, Uysal M. Effect of carnosine treatment on oxidative stress in serum, apo-B containing lipoproteins fraction and erythrocytes of aged rats. Pharmacol Rep 2010;62:733–9.
[6] Bellia F, Vecchio G, Rizzarelli E. Carnosine derivatives: new multifunctional drug-like molecules. Amino Acids 2012;43:153–63.
[7] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
[8] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882–8.
[9] Boldyrev AA. Protection of proteins from oxidative stress. Ann NY Acad Sci 2005;1057:1–13.
[10] Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302–10.

[11] Dursun N, Taşkın E, Oztürk F. Protection against adriamycin-induced cardiomyopathy by carnosine in rats: role of endogenous antioxidant. Biol Trace Elem Res 2011;143:412–24.
[12] Engle SK, Jordan WH, Pritt ML, Chiang AY, Davis MA, Zimmermaa JL, et al. Qualification of cardiac troponin I concentration in mouse serum using isoproterenol and implementation in pharmacology studies to accelerate drug development. Toxicol Pathol 2009;37:617–28.
[13] Fouad AA, El-Rehany MAA, Maghraby HK. The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. Eur J Pharmacol 2007;572:61–8.
[14] Hipkiss AR, Brownson C. Carnosine react with carbonyl groups: another possible role for the antiageing peptide. Biogerontology 2000;1:2217–23.
[15] Hipkiss AR. Carnosine and its possible roles in nutrition and health. Adv Food Nutr Res 2009;57:87–154.
[16] Kalaz EB, Evran B, Develi-İş S, Vural P, Doğru-Abbasoğlu S, Uysal M. Effect of carnosine on prooxidant–antioxidant balance in several tissues of rats exposed to chronic cold plus immobilization stress. J Pharmacol Sci 2012;120:98–104.
[17] Kang JH, Kim KS, Choi SY, Kwon HY, Won MH, Kang TC. Protective effects of carnosine, homocarnosine and anserine against peroxyl radical-mediated Cu,Zn-superoxide dismutase modification. Biochim Biophys Acta 2002;1570:89–96.
[18] Kannan MM, Quine SD. Ellagic acid ameliorates isoproterenol induced oxidative stress: evidence from electrocardiological, biochemical and histological study. Eur J Pharmacol 2011;659:45–52.
[19] Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium deficient rat liver. Biochem Biophys Res Commun 1976;71:952–8.
[20] Lee YT, Hsu CC, Lin MH, Liu KS, Yin MC. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. Eur J Pharmacol 2005;513:145–50.
[21] Maynard LM, Boissonneault GA, Chow CK, Bruckner GG. High levels of dietary carnosine are associated with increased concentrations of carnosine and histidine in rat soleus muscle. J Nutr 2001;131:287–90.
[22] Mehmetçik G, Özdemirler G, Koçak-Toker N, Cevikbaş U, Uysal M. Role of carnosine in preventing thioacetamide-induced liver injury in the rat. Peptides 2008;29:425–9.
[23] Mohamadin AM, Elberry AA, Mariee AD, Morsy GM, Al-Abbasi FA. Lycopene attenuates oxidative stress and heart lysosomal damage in isoproterenol induced cardiotoxicity in rats: a biochemical study. Pathophysiology 2012;19:121–30.
[24] Mong MC, Chao CY, Yin MC. Histidine carnosine alleviated hepatic steatosis in mice high saturated fat diet. Eur J Pharmacol 2011;653:82–8.
[25] Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986;82:512–20.
[26] Nagoor Meeran MF, Mainzen Prince PS. Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. J Biochem Mol Toxicol 2012;26:368–73.
[27] O'Brien PJ, Smith DEC, Knechtel TJ, Marchak MA. Cardiac troponin I is a sensitive, specific biomarker of cardiac injury in laboratory animals. Lab Anim 2006;40:153–71.
[28] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
[29] Panda VS, Naik SR. Cardioprotective activity of Gingko biloba phytosomes in isoproterenol-induced myocardial necrosis in rats: a biochemical and histoarchitectural evaluation. Exp Toxicol Pathol 2008;60:397–404.
[30] Priscilla DH, Prince PSM. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction. Chem Biol Interact 2009;179:118–24.
[31] Radhiga T, Rajamanickam C, Senthil S, Pugalendi KV. Effect of ursolic acid on cardiac marker enzymes, lipid profile and macroscopic enzyme mapping assay in isoproterenol-induced myocardial ischemic rats. Food Chem Toxicol 2012;50:3971–7.
[32] 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;94:1135–42.
[33] Rashid I, Van Reyk DM, Davies MJ. Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation in vitro. FEBS Lett 2007;581:1067–70.
[34] Rathore N, Kale M, John S, Bhatnagar D. Lipid peroxidation and antioxidant oxidative stress in rat erythrocytes. Indian J Physiol Pharmacol 2000;44:161–6.
[35] Roberts PR, Zaloga GP. Cardiovascular effects of carnosine. Biochemistry (Moscow) 2000;65:856–61.
[36] Shiny KS, Kumar SHS, Farvin KHS, Anandan R, Devadasan K. Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. J Pharm Pharmacol 2005;57:1–5.
[37] Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85.
[38] Stocks J, Offerman L, Modell CB, Dormandy TL. The susceptibility to autoxidation of human red cell lipids in health and disease. Br J Haematol 1972;23:713–24.
[39] Stvolinsky SL, Dobrota D. Anti-ischemic activity of carnosine. Biochemistry (Moscow) 2000;65:849–55.
[40] Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. Circulation 2007;116:2634–53.
[41] Warner BB, Stuart L, Gebb S, Wispé JR. Redox regulation of manganese superoxide dismutase. Am J Physiol 1996;271:L150–8.
[42] Yoo HY, Chang MS, Rho HM. The activation of the rat copper/zinc superoxide dismutase gene by hydrogen peroxide through the hydrogen peroxide-responsive element and by paraquat and heat shock through the same heat shock element. J Biol Chem 1999;274:23887–92.
[43] 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;586:244–50.
[44] Zieba R, Wagrowska-Danilewicz M. Influence of carnosine on the cardiotoxicity of doxorubicin in rabbits. Pol J Pharmacol 2003;55:1079–87.