

Protective effect of carnosine on adriamycin-induced oxidative heart damage in rats

Sıçanlarda adriamisininin neden olduğu oksidatif kalp hasarı üzerine karnozinin koruyucu etkisi

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ABSTRACT

Objective: Oxidative stress is one of the major factors involved in the pathogenesis of adriamycin (ADR)-induced cardiac dysfunction. The present study examined the antioxidant protective effects of carnosine (CAR) on adriamycin-induced cardiac damage in rats.

Methods: Female Sprague Dawley rats were divided into four groups. Control (CONT, n=8, saline only i.v.); carnosine (CAR, n=8, 10 mg/kg/day, i.v.); adriamycin (ADR, n=10, 4 mg/kg four times every 2 days for 8 days, i.v.) alone and carnosine with adriamycin (CAR+ADR, n=10). Carnosine was given one week before adriamycin treatment and following one week with adriamycin treatment. After measurement of physiological functions, blood samples were collected for biochemical assays. The hearts were excised for hemodynamic study. Comparisons between different groups were made using ANOVA and posthoc Tukey test.

Results: Adriamycin produced evident cardiac damage revealed by; hemodynamic changes - decreased left ventricular developed pressure ($p<0.01$), the maximum-minimum rates of change in left ventricular pressure ($\pm dP/dt$, $p<0.01$), electrocardiogram (ECG) changes (elevated ST, decreased R-wave, $p<0.001$), cardiac injury marker changes (increased creatine kinase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase), plasma antioxidant enzymes activity changes (decreased superoxide dismutase, glutathione peroxidase, catalase activities, $p<0.03$) and lipid peroxidation (elevated malondialdehyde, $p<0.05$) to the control and carnosine groups. Carnosine treatment caused significant attenuation ($p<0.05$) of cardiac dysfunction induced by adriamycin (CAR+ADR), revealed by normalization of the ventricular function, ECG and biochemical variables.

Conclusion: An increase in oxidative stress, superoxide dismutase, glutathione peroxidase levels, catalase inactivation and cardiac dysfunction induced by adriamycin were prevented by carnosine. (*Anadolu Kardiyol Derg 2011 1: 3-10*)

Key words: Adriamycin, carnosine, cardiac damage, Langendorff, antioxidant

ÖZET

Amaç: Oksidatif stres, adriamisininin (ADR) neden olduğu kardiyak fonksiyon bozukluğu patogenezinde, önemli faktörlerden birisidir. Bu çalışmada sıçanlarda adriamisin ile oluşturulan kalp hasarı üzerine karnozinin antioksidan savunma etkisi araştırılmıştır.

Yöntemler: Dişi Sprague Dawley sıçanlar 4 gruba ayrıldı; kontrol (KONT, n=8, serum fizyolojik i.v.); karnozin (KAR, n=8, 10 mg/kg/gün, i.v.) sadece adriamisin (ADR, n=10, 4 mg/kg dört defa, iki gün ara ile toplam 8 gün, i.v.); karnozin ile adriamisin (KAR+ADR, n=10). Karnozin, adriamisinden bir hafta önce verilmeye başlandı ve sonraki bir hafta adriamisinle birlikte verildi. Fizyolojik fonksiyon değerlendirmelerinden sonra biyokimyasal tayinler için kan örnekleri alındı. Kalpler hemodinamik çalışma için izole edildi. Gruplar arası farklılıkların belirlenmesi için ANOVA ve posthoc Tukey testi kullanıldı.

Bulgular: Adriamisin, belirgin bir şekilde kalp hasarı yapmış olup; karnozin ve kontrol grubuna göre, hemodinamik değişiklikler [azalmış sol ventrikül basınç gelişimi ($p<0.01$), maksimum-minimum sol ventrikül basınç değişim oranları ($\pm dP/dt$, $p<0.01$)], elektrokardiyogram (EKG) değişiklikleri (artmış ST ve azalmış R-dalgası, $p<0.001$), kardiyak hasar belirleyicilerindeki değişiklikler (artmış kreatin kinaz, laktat dehidrogenaz, aspartat aminotransferaz, alanin aminotransferaz), plazma antioksidan aktivite değişiklikleri (azalmış süperoksit dismutaz, glutatyon peroksidaz, katalaz aktiviteleri, $p<0.03$) ve lipid peroksidasyonuna (artmış malondialdehit, $p<0.05$) neden olmuştur. Karnozin tedavisi (KAR+ADR); ventriküler

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fonksiyon, EKG ve biyokimyasal değişkenleri normal değerlerine yaklaştırarak, adriamisinin neden olduğu kardiyak fonksiyon bozukluklarının önemli derecede azalmasına sebep olmuştur.

Sonuç: Adriamisinin oksidatif streste yaptığı artış, süperoksit dismutaz, glutatyon peroksidaz, katalaz gibi antioksidan enzim aktivasyon baskılayıcı etkileri ile oluşan kalp fonksiyon bozuklukları, karnozin ile engellenmiştir. (*Anadolu Kardiyol Derg 2011 1: 3-10*)

Anahtar kelimeler: Adriamisin, karnozin, kalp hasarı, Langendorff, antioksidan

Introduction

Anthracyclines are among the most active and broad spectrum antineoplastic agents used in the treatment of several cancers, such as solid tumors, leukemias, and lymphomas. Unfortunately, the conventional and cardiac toxicities of anthracyclines are among the main factors that limit their use (1). Clinically, cardiotoxicity results in cardiomyopathy with irreversible congestive heart failure and high mortality. Adriamycin-induced cardiotoxicity has been attributed to a number of effects, including the direct inhibition of key transporters involved in ion homeostasis, alterations in cellular iron and calcium metabolism, disruption of sarcoplasmic reticulum function, mitochondrial dysfunction, and apoptotic cell loss (2). The mechanism underlying these events seems to be linked to an increased production of reactive oxygen species (ROS) and increased oxidative damage (3). Oxidative stress results from an imbalance between the generation of ROS and reactive nitrogen species and removal by the cellular antioxidant system (4). Typical changes in the adriamycin-induced heart failure are increased free radicals accompanied by a decrease of endogenous antioxidants; the subsequent increase in oxidants results in enhanced oxidative stress leading to a slow loss of myofibrils and vacuolization of myocardial cells. So, adriamycin-induced heart failure models are currently used in different experimental settings for research on therapeutic strategies for heart failure (5).

First, isolated and characterized in 1900 by Gulewitsch, carnosine (β -alanyl-L-histidine) is a dipeptide, which commonly presents in mammalian tissue, and in particular, in skeletal muscle cells; it is responsible for a variety of activities related to the detoxification of the body from free radical species and the by-products of membrane lipids peroxidation, but recent studies have shown that this small molecule also has membrane protecting activity, proton buffering capacity, formation of complexes with transition metals, and regulation of macrophage function (6).

Two studies determined the protective effect of carnosine (CAR) on the cardiotoxicity of adriamycin (ADR) (7, 8). These studies evaluated the influences of adriamycin on the hemodynamic parameters and on the degree of cardiac muscle cell alterations.

This is the first study that designed to show the ability of carnosine to protect cardiac tissue from adriamycin-induced oxidative cardiac damage using enrichment antioxidant enzyme activities analysis.

Methods

Study Protocol

This study was approved by our Institutional Animal Research Committee. Female Sprague-Dawley rats (body weight, 160-243g)

were housed individually in polypropylene cages under hygienic and standard environmental conditions ($24\pm 1^\circ\text{C}$, humidity 60-70%, 12 h light/dark cycle). The animals were allowed a standard diet and water *ad libitum*. Adriamycin, physiological saline and carnosine were administered into the jugular vein. Catheterization was made under ketamin (39.35 mg/kg, i.m.) plus xylazine (4.96 mg/kg, i.m.) anesthesia. A polyethylene catheter (PE-50, Intramedic, Clay Adams, MD) was implanted in the jugular vein with distal end tunneled subcutaneously to an exit in the nape of neck.

Administration of CAR (white powder, dissolved in physiological saline, Sigma Chemical Co, USA,) given one week prior to the first dose of ADR. Three important factors were considered in determining the amount of carnosine administered to animals. First, Lee et al. (10) reported that very low dose carnosine did not reduce lipid peroxidation. Second, they also showed that high and low doses of carnosine affected the catalase or glutathione peroxidase activities at the same rate. Generally, the cumulative dose of CAR administered is approximately 250-300 mg/kg (9-11). Cardiac damage was induced in rats by four intravenous infusions of adriamycin (Adriamycin HCl, Adriblastina vial 10 mg, as a freeze-dried powder to be dissolved in physiological saline, Pharmacia) at cumulative dose of 16 mg/kg. When chronic use of adriamycin exceeds 500 mg/m², it produces characteristic cardiomyopathy findings in some humans (12). Robert et al. reported that when Sprague Dawley rats, aged 10-12 weeks, were treated every other day with adriamycin, it caused damage to cardiomyocytes, and the functional efficiency of the myocardium would be affected before the morphological alterations became detectable (13). In order to avoid the long delay in long-term models, we chose this short-term model, and we pre-tested the dose and schedule (for 4 mg/kg i.p. every 2 days) before the study was completed. This pretest provided data that was expected from clinical experience namely functional and biochemical alterations. The duration and amount of adriamycin to be given to the rats were modified with minor changes by taking into consideration the above information in these studies (12). All efforts were made to minimize animal suffering and to reduce the number of animals used in this experiment. The animals were divided into the four groups of rats randomly allocated to every group. The first group was treated with normal saline (CONT, n=8). Carnosine was administered to the second group at a dose 10 mg/kg in each infusion for two weeks (CAR, n=8). Adriamycin was administered to the third group at a dose of 4 mg/kg i.v. every 2 days for 8 days (ADR, n=10). Carnosine was given one week before adriamycin treatment and following one week with adriamycin treatment. (CAR+ADR, n=10). Four animals died before the determination (Fig. 1). Rats in each group were observed for 2 weeks for general appearance, behavior, mortality and body weights.

Arterial blood pressure

Mean, systolic, diastolic blood pressures and heart rate were directly measured through the femoral artery. Each rat was briefly anesthetized with ketamin (39.35 mg/kg, i.m.) plus xylazine (4.96 mg/kg, i.m.). A polyethylene catheter (PE-50, Intramedic, Clay Adams, MD, USA) was implanted in the femoral artery. The catheter was filled with heparinized solution of normal saline (10 IU/ml). The measurement of blood pressure was begun 20-30 minutes after connecting the catheter to the transducer. The transducer output was amplified and the arterial pressure signal passed to an analog-digital converter installed in a per second and the mean arterial pressure and heart rate (beats/min) were determined from the pulse wave. After measurement of blood pressure and heart rate, blood samples were collected, and plasma was separated by centrifugation and used for biochemical assays. The hearts were rapidly excised, washed with cold saline and hemodynamic study was done.

Hemodynamic and electrocardiographic (ECG) parameters

Twenty four hours after adriamycin injection, the animals were anesthetized with ketamin (39.35 mg/kg, i.m.) plus xylazine (4.96 mg/kg, i.m.). The heart was cannulated via the aorta and perfused at a constant perfusion rate. All hearts were perfused with modified Krebs-Henseleit buffer at 37°C, containing (mmol/l) 118.5 NaCl, 25 NaHCO₃, 3.2 KCl, 1.19 MgSO₄, 1.25 CaCl₂, 1.2 KH₂PO₄, 11 glucose and bubbled with 95% O₂, 5% CO₂ mixture. The pH was maintained at 7.4. After the heart began spontaneous contraction, a small incision was made in the left atrium; a latex balloon connected to a pressure transducer via polyethylene cannula was inserted through the left atrium and mitral valve into the left ventricle. The balloon was filled with enough water to increase end-diastolic pressure to approximately 10 mmHg. The left ventricular pressures were recorded by a computer that allows continuous monitoring of left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximal and minimal first derivatives of left ventricular developed pressure (LVDP) as a function of time, +dP/dt and -dP/dt. LVDP was calculated by subtracting end-diastolic pressure from left ventricular systolic pressure (LVDP= LVSP-LVEDP). At the same time, ECG was recorded, especially ST, R-R intervals and R-wave changes in-vitro.

Biochemical assays

Creatine kinase assay

The creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were estimated by kinetic determination using the commercial kits of Bechman by Bechman Coulter LX-2000 (Brea, CA, USA).

Thiobarbituric acid reactive substances assay

Measurement of lipid peroxidation by determining TBARS was performed by using a modified thiobarbituric acid (TBA) method by Jain (14) as described previously by Stocks and Dormandy (15): 1.5 ml of 30% (w/v) of TBA was promptly added

to 0.6 ml plasma, which was diluted with 2.4 ml serum physiologic containing phosphate buffer. Contents were vortexed briefly and cooled on ice for 2 hours. The tubes were centrifugated for 10 min. at 2000 rpm 0.225 ml of 0.1 M EDTA and 0.75 ml of 1% TBA were added to 3 ml supernatant. Contents were boiled for 15 min. The color developed was assayed by measuring absorbance at 532 nm, and expressed in µmol/l by comparing to known malondialdehyde (MDA) standards.

Glutathione peroxidase (GSH-Px) assay

Plasma GSH-Px activity was assayed in a 3 ml cuvette containing 2.63 ml of phosphate buffer (0.05 M, pH=7.4, containing 5 ml EDTA), 0.10 ml NADPH (8.4 mM), 0.01 ml NaN₃ (112.5 mM), 0.10 ml reduced glutathione (0.15 M) and 0.05 ml plasma. The conversion of NADPH to NADP was assayed by measuring the absorbance at 340 nm at 1 min. intervals for 5 min. Specific activity of the enzyme was expressed in U/ml (16).

Superoxide dismutase (SOD) assay

Plasma of 0.5 ml was added to a cuvette containing 1 ml xyantin (0.3 mM/l), 0.5 ml EDTA (0.6 nM), 0.5 ml NBT (150 mM), 0.3 ml Na₂CO₃ (400 mM), 0.15 ml bovine serum albumin (BSA). After heating until 25°C, 50 µl xyantin oxydase was added and incubated for 20 min. 1 ml (0.8 mm) CuCl₂ added and the reaction was stopped. The color that developed was assayed by measuring absorbance 560 nm and expressed U/l (17).

Catalase (CAT) activity

One ml of plasma was added to a cuvette containing 0.5 ml of 37.5 mM H₂O₂ solution prepared in a potassium phosphate buffer. The disappearance of H₂O₂ was monitored at 240 nm wave length at 1 min. intervals for 5 min. Specific activity of the enzyme was expressed in U/ml (18).

Statistical analysis

Statistical analyses were made by using Excel and SPSS (SPSS Inc., Chicago, IL, USA) system for Windows, version 11.0. All results were expressed as mean±S.D. Normality tests are used to determine Shapiro-Wilk and Kolmogrov-Smirnov (p>0.05). Comparisons between different groups were made using one way ANOVA multiple comparison analysis and posthoc Tukey test. In all cases, p<0.05 was considered to be significant.

Results

Blood pressure and heart rate

Mean, systolic, diastolic blood pressures were decreased by adriamycin administration compared to the CONT and CAR groups (p<0.01, Table 1). These variables in the ADR group were maintained by carnosine in the CAR+ADR group (p<0.05). Adriamycin led to a decrease in heart rate compared to the CONT and CAR groups (p<0.05), while carnosine treatment produced its significant increase in the CAR+ADR group (p<0.05).

Hemodynamic and electrocardiographic variables

LVDP was decreased seven fold by the adriamycin administration compared to the CONT ($p<0.01$) (Fig. 2A). This variable in the ADR group was increased by carnosine in the CAR+ADR group ($p<0.001$). The $+dP/dt$ (Fig. 2B) and $-dP/dt$ (Fig. 2C) variables were decreased significantly ($p<0.05$) by adriamycin administration compared to the CONT and CAR groups. These altered variables in the ADR group were increased by carnosine in the CAR+ADR group ($p<0.001$).

Rats treated by adriamycin alone showed many ECG changes such as increased ST, R-R intervals and low R-wave compared to the CONT and CAR groups (respectively; $p<0.01$ and $p<0.001$, Table 2). These altered variables in the ADR group were attenuated by carnosine treatment in the CAR+ADR group ($p<0.001$).

Biochemical changes

Adriamycin treatment led to an increase in the plasma CK, LDH, AST and ALT levels compared to the CONT and CAR groups ($p<0.05$ for all). Carnosine treatment produced significant reduction of this changes induced by adriamycin in the CAR+ADR group ($p<0.01$ vs ADR, Table 3).

Adriamycin treatment resulted in 40% increase in lipid peroxidation compared to the control. Carnosine prevented this increase in the CAR+ADR group. There was a significant decrease ($p<0.05$) in the CAR+ADR group compared to the ADR group (55%, Fig.3A).

Adriamycin treatment resulted in 51%, 34%, and 55% decreases of SOD (Fig. 3B), GSH-Px (Fig. 3C) and CAT (Fig. 3. D) activities to the control values respectively ($p<0.03$ vs CONT). In

Table 1. Effect of carnosine on adriamycin-induced changes in blood pressure and heart rate of rats

Variables	Groups				*F	*p
	CONT (n=8)	CAR (n=8)	ADR (n=10)	CAR+ADR (n=10)		
MAP, mmHg	83±4	91±12	62±18 ^{a,b}	83±15 ^c	8.966	<0.0001
SAP, mmHg	94±5	100±13	69±19 ^{a,b}	89±18 ^c	8.374	<0.0001
DAP, mmHg	73±4	83±13	54±15 ^{a,b}	76±13 ^c	8.841	<0.0001
HR, Beats/min	350±10	342±21	283±17 ^{a,b}	333±6 ^c	32.901	<0.0001

Data are presented as mean±S.D.
*One way ANOVA and posthoc Tukey test: significantly differ ($p<0.05$) from; ^a:CONT, ^b:CAR, ^c:ADR
ADR - adriamycin, CAR - carnosine, CONT - control, DAP - diastolic arterial pressure, HR - heart rate, MAP - mean arterial pressure, SAP - systolic arterial pressure

Table 2. Effect of the carnosine on adriamycin-induced ECG changes in rats

Variables	Groups				*F	*p
	CONT (n=8)	CAR (n=8)	ADR (n=10)	CAR+ADR (n=10)		
ST segment, ms	17±1.51	16.5±2.39	52±1.52 ^{a,b}	14.8±10.23 ^c	15.153	<0.0001
R-R interval, s	1.25±0.07	1.07±0.12	2.10±0.21 ^{a,b}	1.06±0.68 ^c	2.987	0.046
R-wave, mV	3.07±0.55	3.49±0.48	1.07±0.63 ^{a,b}	4.08±0.23 ^c	15.153	<0.0001

Data are presented as mean±S.D.
*One way ANOVA and posthoc Tukey test: significantly differ ($p<0.05$) from; ^a:CONT, ^b:CAR, ^c:ADR
ADR - adriamycin, CAR - carnosine, CONT - control, ECG - electrocardiogram

Table 3. Effect of the carnosine on adriamycin-induced changes in plasma levels of cardiac enzymes in rats

Variables	Groups				*F	*p
	CONT (n=8)	CAR (n=8)	ADR (n=10)	CAR+ADR (n=10)		
CK, U/l	263±58	207±65	531±47 ^{a,b}	209±93 ^c	24.955	<0.0001
LDH, U/l	258±69	169±75	499±78 ^{a,b}	151±86 ^c	12.511	<0.0001
AST, U/l	64.5±5	65±11	99±23 ^{a,b}	58±10 ^c	5.879	0.005
ALT, U/l	38±3	26.5±6	55±16 ^b	27.5±11 ^c	8.453	0.001

Data are presented as mean±S.D.
*One way ANOVA and posthoc Tukey test: significantly differ ($p<0.05$) from; ^a:CONT, ^b:CAR, ^c:ADR.
ADR - adriamycin, ALT - alanine amino transferase, AST - alanine amino transferase, CAR - carnosine, CK - creatine kinase, CONT - control, LDH - lactate dehydrogenase

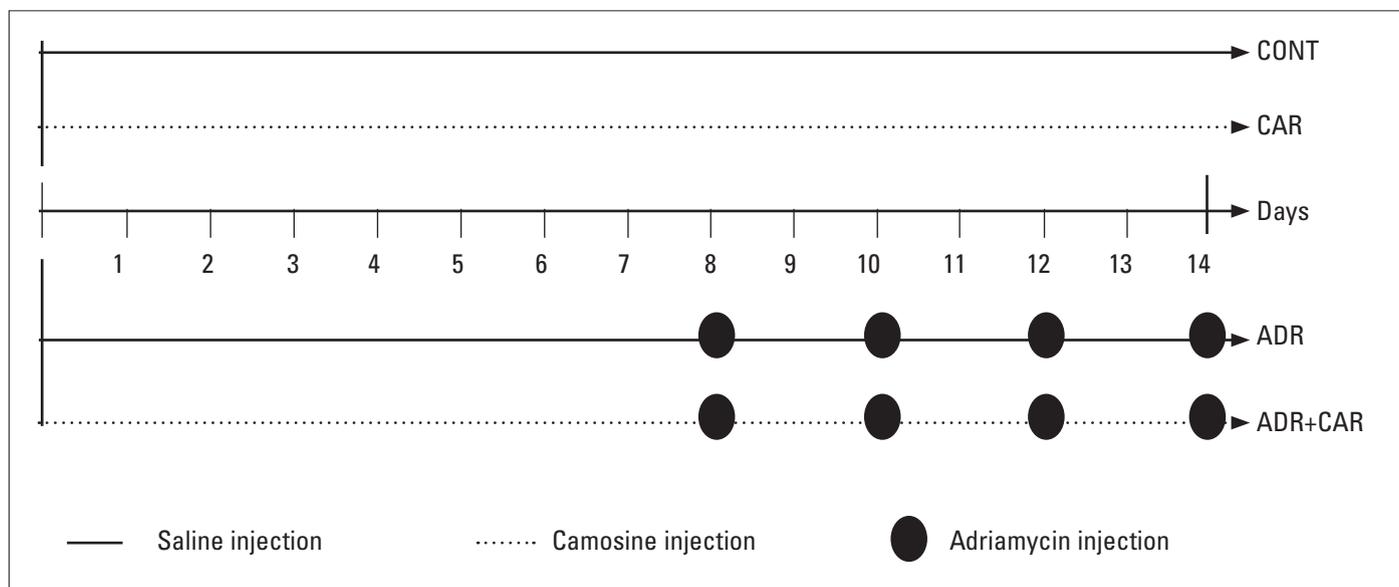


Figure 1. Experimental protocol. Rats were divided into four groups, control (CONT, injected saline, 14 days, straight line), carnosine (CAR, injected carnosine, 14 days, dotted line), adriamycin (ADR, injected adriamycin four times every 2 days, black circle) and adriamycin co-administered carnosine (ADR+CAR, injected carnosine, 14 days and adriamycin four times every 2 days)

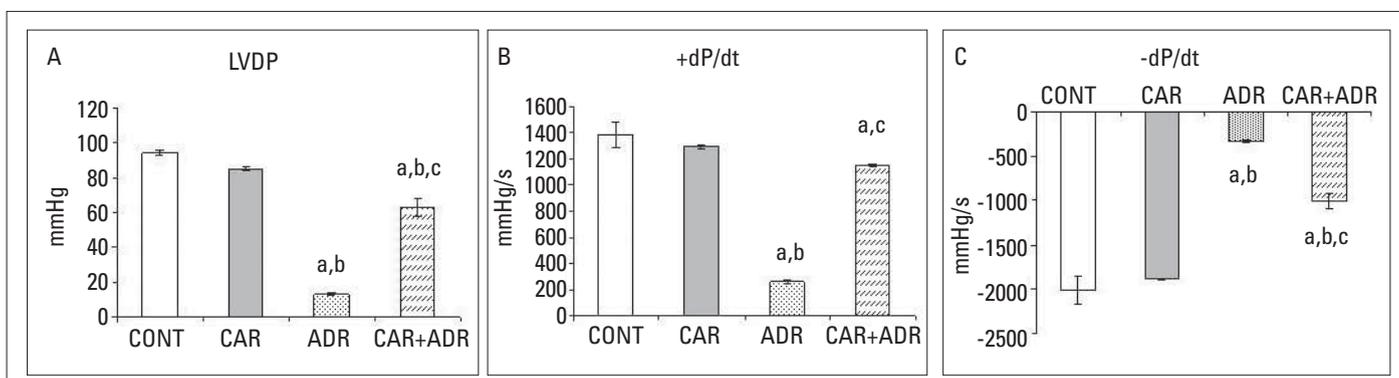


Figure 2. Effect of the carnosine on adriamycin-induced changes of cardiac function in rats
 LVDP - left ventricular developed diastolic pressure (A), +dp/dt - the maximum rate of change in left ventricular pressure (B), -dp/dt - the minimum rate of change in left ventricular pressure (C)

Data are presented as mean±S.E.M. One way ANOVA and posthoc Tukey test: a: $p < 0.01$ vs CONT, b: $p < 0.001$ vs CAR, c: $p < 0.001$ vs ADR. ADR - adriamycin, CAR - carnosine, CONT - control, LVDP -left ventricular developed pressure

the CAR+ADR group, SOD, GSH-Px and CAT activities were maintained normal and they were significantly higher than in the ADR group ($p < 0.01$).

Discussion

Our initial hypothesis behind preconditioning was that;

Oxidative stress is the result of excessive production of oxidant species and/or depletion of intracellular antioxidant defenses, leading to an imbalance in the redox status of the myocardial cells. In this study, we demonstrated that adriamycin caused a significant increase in plasma MDA and decrease in plasma SOD, CAT and GSH-Px activities indicating increased oxidative stress by adriamycin. An increase in lipid peroxidation following acute adriamycin administration was significantly prevented by carnosine.

Adriamycin-induced cardiac damage is believed to be related to the generation of highly reactive oxygen species, which by means of membrane lipid peroxidation, cause direct damage on cardiac myocyte membranes. Another important factor may be the relatively poor antioxidant defense system of the heart as they are in other tissues such as kidney and liver, which is reflected by the resistance of the liver and kidney to anthracycline-induced damage from free radicals.

Carnosine is a scavenger of hydroxyl and superoxide radicals and a strong quencher of singlet molecular oxygen.

In this study, adriamycin treatment (cumulative dose 16 mg/kg, i.v) produced evident cardiac damage in rats, manifested by cardiac dysfunction, ECG changes, altered plasma levels of cardiac injury markers. The observed cardiac function changes included decreased LVDP and $\pm dp/dt$. Li et al. (19) showed that mice receiving adriamycin alone had significant cardiac func-

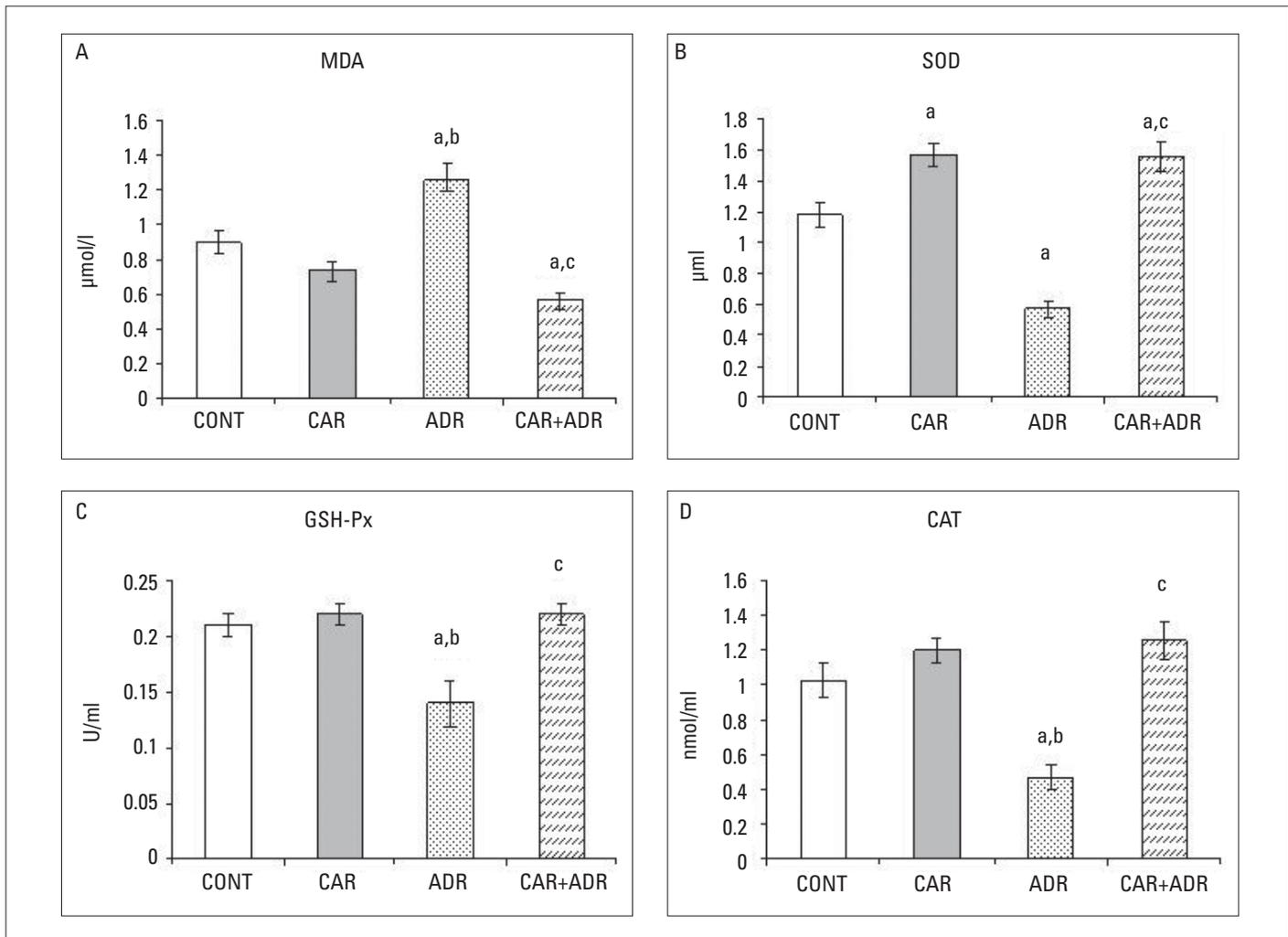


Figure 3. Effect of the carnosine on adriamycin-induced in malondialdehit (MDA: A), superoxide dismutase (SOD: B), glutathione peroxidase (GSH-Px: C), catalase (CAT: D) activities of rats

Data are presented as mean \pm S.E.M. One way ANOVA and posthoc Tukey test: a: $p < 0.03$ vs CONT, b: $p < 0.01$ vs CAR, c: $p < 0.01$ vs ADR
ADR - adriamycin, CAR - carnosine, CONT - control

tional deterioration characterized by enlargement of the LV cavity and signs of decreased cardiac function, i.e. increased left ventricular (LV) diameter, end-diastolic pressure and decreased LV fractional shortening and $\pm dP/dt$, compared to sham animals (19). The result of this study showed that, in comparison to the group treated with adriamycin-alone by treated carnosine and adriamycin showed nearly normal cardiac functions. When carnosine was perfused through the aorta of isolated rat hearts to determine effects on cardiac function, in a concentration-dependent manner, carnosine increased $\pm dP/dt$ values, left ventricular systolic pressures, and heart rate (20). Adriamycin-induced cardiac damage does not only affect mechanical functions of the heart, but also results in inhomogeneity of ventricular depolarization and repolarization reflected by the occurrence of changes in the ECG. The observed ECG changes included prolongation ST segment, R-R and QRS intervals and low R-wave in adriamycin treated rats. Venkasetan et al. (21) has reported ST segment elevation, increased heart rate, flattening of the T-wave, prolongation of the

QT interval and loss of the R-wave voltage in rats 48 hours after adriamycin injection. Fisher et al. (22) demonstrated a strong correlation between ST segment duration and adriamycin-induced cardiotoxicity. Le March et al. (23) observed an increase in action potential duration in Purkinje fibers after incubation with adriamycin. Jabr and Cole (24) observed action potential duration prolongation resulting from adriamycin-generated ROS. Results of this study showed that, in comparison to the group treated with adriamycin alone, rats treated by carnosine and adriamycin have nearly normal ECG data. Carnosine significantly prevented ST segment prolongation throughout the study period.

The degree of adriamycin-induced cardiac damage was assessed chemically by determining of plasma levels of CK, LDH, AST and ALT of cardiac injury. Adriamycin administration produced significant elevation of CK, LDH, AST and ALT compared with the control group. Elevation in the serum levels of CK and LDH enzyme activities, 48 hours after adriamycin administration, has been reported by several investigators (21, 25, 26).

In this study, we demonstrated that an increase in lipid peroxidation (significant increase in plasma MDA and decrease in plasma SOD, CAT and GSH-Px activities) following acute adriamycin administration was significantly prevented by carnosine. Superoxide dismutase converts superoxide radicals into hydrogen peroxide and molecular oxygen. Since hydrogen peroxide is still toxic to the cell, superoxide dismutase works in conjunction with two other enzymes, catalase and glutathione peroxidase, to convert hydrogen peroxide into water (27). Daosukho et al. (28) myocardial antioxidant defense system was operating at a lower rate despite a higher level of oxidative stress in an adriamycin-induced cardiotoxicity condition. The adriamycin-induced generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging enzymes to the radicals, resulting in myocyte lesions and reduction of scavengers, as evident from the present study. Prior study showed that GSH-Px, SOD and CAT enzyme activities were depressed by adriamycin (29). However, Li et al. (30) showed that SOD and CAT enzyme activities were not changed, but GSH-Px activity was depressed by adriamycin. Kang et al. (31) showed that the protective effect of carnosine protects against the loss of Cu, Zn-SOD activity incubation with salsolinol, an endogenous neurotoxin. Carnosine and related compounds are reported to be the active free radical scavengers (32). Carnosine protects the Cu, Zn-SOD activity against oxidative stress (31). In this study, SOD activity was increased by carnosine administration to control levels in the CAR+ADR group. However, CAT and GSH-Px activity also increased. It has been reported that a rise in SOD activity, without a concomitant rise in the activity of CAT and/or GSH-Px might be detrimental (33), because SOD generates hydrogen peroxide as a metabolite, which is cytotoxic and has to be scavenged by CAT or GSH-Px. Thus, a simultaneous increase in CAT and/or GSH-Px activities is essential for an overall beneficial effect of an increase in SOD activity (29). Carnosine is present in high concentrations [up to 20 mM] in skeletal muscles and the brain, where it is supposed to play a role as a biological antioxidant, and protect these tissues against oxidative damage induced by different factors (34). Carnosine can scavenge peroxy radicals, scavenge singlet oxygen (35), and bind hydrogen peroxide and is an efficient chelating agent for copper and other transition metals such as iron and cobalt, preventing them from participating in the deleterious Fenton reaction with peroxides (36).

Study limitations

We should have measured the serum CK-MB levels, because CK-MB isoenzyme is more specific than total CK enzyme in detecting myocardial injury. The protective effect of carnosine on adriamycin-induced injury should have been confirmed by the histopathological findings.

Conclusion

Adriamycin-induced oxidative stress with reduced endogenous antioxidant enzyme activities (SOD, GSH-Px and CAT),

increased lipid peroxidation and left ventricular dysfunction might be prevented by the increase in myocardial SOD, CAT and GSH-Px activities, following carnosine administration.

Conflict of interest: None declared.

References

1. Iqbal M, Dubey K, Anwer T, Ashish A, Pillai KK. Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. *Pharmacol Rep* 2008; 60: 382-90.
2. Toko H, Oka T, Zou Y, Sakamoto M, Mizukami M, Sano M, et al. Angiotensin II type 1a receptor mediates doxorubicin-induced cardiomyopathy. *Hypertens Res* 2002; 25: 597-603.
3. Berthiaume JM, Wallace KB. Adriamycin-induced oxidative mitochondrial cardiotoxicity. *Cell Biol Toxicol* 2007; 23: 15-25.
4. Chen Y, Jungsuwadee P, Vore M, Butterfield DA, St Clair DK. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol Interv* 2007; 7: 147-56.
5. Christiansen S, Autschbach R. Doxorubicin in experimental and clinical heart failure. *Eur J Cardiothorac Surg* 2006; 30: 611-6.
6. Guiotto A, Calderan A, Ruzza P, Borin G. Carnosine and carnosine-related antioxidants: a review. *Curr Med Chem* 2005; 12: 2293-315.
7. Assir AMA, Abdel-Wahab AFI. Carnosine protects against doxorubicin-induced cardiotoxicity. *SPJ* 2003; 11: 23-31.
8. Zieba R, Wagrowska-Danilewicz M. Influence of carnosine on the cardiotoxicity of doxorubicin in rabbits. *Pol J Pharmacol* 2003; 55: 1079-87.
9. Baykara B, Tekmen I, Pekçetin C, Ulukuş C, Tuncel P, Sağol O, et al. The protective effects of carnosine and melatonin in ischemia-reperfusion injury in the rat liver. *Acta Histochem* 2009; 111: 42-51.
10. 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.
11. Mikami K, Otaka M, Watanabe D, Goto T, Endoh A, Miura K, et al. Zinc L-carnosine protects against mucosal injury in portal hypertensive gastropathy through induction of heat shock protein 72. *J Gastroenterol Hepatol* 2006; 21: 1669-74.
12. Dimitrov NV, Hay MB, Siew S, Hudler DA, Charamella LJ, Ullrey DE. Abrogation of adriamycin-induced cardiotoxicity by selenium in rabbits. *Am J Pathol* 1987; 126: 376-83.
13. Robert J. Preclinical assessment of anthracycline cardiotoxicity in laboratory animals: predictiveness and pitfalls. *Cell Biol Toxicol* 2007; 23: 27-37.
14. Jain SK. Evidence for membrane lipid peroxidation during the in vivo aging of human erythrocytes. *Biochim Biophys Acta* 1988; 937: 205-10.
15. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br J Haematol* 1971; 20: 95-111.
16. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-69.
17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; 34: 497-500.
18. Beers RF Jr, Sizer IW. A spectrophotometric methods for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; 95: 133-40.
19. Li L, Takemura G, Li Y, Miyata S, Esaki M, Okada H, et al. Preventive effect of erythropoietin on cardiac dysfunction in doxorubicin-induced cardiomyopathy. *Circulation* 2006; 113: 535-43.

20. Roberts PR, Zaloga GP. Cardiovascular effects of carnosine. *Biochemistry (Mosc)* 2000; 65: 856-61.
21. Venkatesan N. Curcumin attenuation of acute adriamycin myocardial toxicity in rats. *Br J Pharmacol* 1998; 124: 425-7.
22. Fisher PW, Salloum F, Das A, Hyder H, Kukreja RC. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. *Circulation* 2005; 111: 1601-10.
23. le Marec H, Spinelli W, Rosen MR. The effects of doxorubicin on ventricular tachycardia. *Circulation* 1986; 74: 881-9.
24. Jabr RI, Cole WC. Alterations in electrical activity and membrane currents induced by intracellular oxygen-derived free radical stress in guinea pig ventricular myocytes. *Circ Res* 1993; 72: 1229-44.
25. Pacher P, Liaudet L, Bai P, Virag L, Mabley JG, Hasko G, et al. Activation of poly(ADP-ribose) polymerase contributes to development of doxorubicin-induced heart failure. *J Pharmacol Exp Ther* 2002; 300: 862-7.
26. Saad SY, Najjar TA, Al-Rikabi AC. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. *Pharmacol Res* 2001; 43: 211-8.
27. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 1991; 91: 14S-22S.
28. Daosukho C, Chen Y, Noel T, Sompol P, Nithipongvanitch R, Velez JM, et al. Phenylbutyrate, a histone deacetylase inhibitor, protects against Adriamycin-induced cardiac injury. *Free Radic Biol Med* 2007; 42: 1818-25.
29. Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK. Protection against acute adriamycin-induced cardiotoxicity by garlic: role of endogenous antioxidants and inhibition of TNF-alpha expression. *BMC Pharmacol* 2003; 3: 16.
30. Li T, Danelisen I, Bello-Klein A, Singal PK. Effects of probucol on changes of antioxidant enzymes in adriamycin-induced cardiomyopathy in rats. *Cardiovasc Res* 2000; 46: 523-30.
31. Kang JH. Protective effects of carnosine and N-Acetylcarnosine on salsolinol-mediated Cu, Zn-superoxide dismutase inactivation. *Bull Korean Chem Soc* 2007; 28: 1881-4.
32. Hartman PE, Hartman Z, Ault KT. Scavenging of singlet molecular oxygen by imidazole compounds: high and sustained activities of carboxy terminal histidine dipeptides and exceptional activity of imidazole-4-acetic acid. *Photochem Photobiol* 1990; 51: 59-66.
33. Harman D. The aging process: major risk factor for disease and death. *Proc Natl Acad Sci U S A* 1991; 88: 5360-3.
34. Sharonov BP, Govorova NJu, Lyzlova SN. Carnosine as a potential scavenger of oxidants generated by stimulated neutrophils. *Biochem Int* 1990; 21: 61-8.
35. Hartman PE. Interception of toxic agents/mutagens/carcinogens: some of nature's novel strategies. *Basic Life Sci* 1986; 39: 169-79.
36. Kang JH, Kim KS, Choi SY, Kwon HY, Won MH, Kang TC. Protective effects of carnosine, homocarnosine and anserine against peroxy radical-mediated Cu,Zn-superoxide dismutase modification. *Biochim Biophys Acta* 2002; 1570: 89-96.