Pharmacological Reports 66 (2014) 81–86

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

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

Betül Evran [a], Hande Karpuzoğlu [a], Seval Develi [a], Esra Betül Kalaz [a], Merva Soluk-Tekkeşin [b], Vakur Olgaç [b], Semra Doğru-Abbasoğlu [a,*], Müjdat Uysal [a]

[a] Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
[b] Department of Pathology, The Institute of Oncology, Istanbul University, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 5 December 2012
Received in revised form 22 July 2013
Accepted 13 August 2013
Available online 1 February 2014

Keywords:
Carnosine
Myocardial infarction
Isoproterenol
Oxidative stress
Rats

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.

* Corresponding author.
E-mail address: sdabbasoglu@yahoo.com (S. Doğru-Abbasoğlu).

1734-1140/$ – see front matter © 2014 Institute of Pharmacology, Polish Academy of Sciences. Published by Elsevier Urban & Partner Sp. z o.o. All rights reserved.
http://dx.doi.org/10.1016/j.pharep.2013.08.008

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: