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The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats

Amr A. Fouad [a,*], Mahmoud Abdel-Aziz El-Rehany [b], Hala K. Maghraby [c]

[a] Department of Pharmacology, Faculty of Medicine, Minia University, El-Minia, Egypt
[b] Department of Biochemistry, Faculty of Medicine, Minia University, El-Minia, Egypt
[c] Department of Pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt

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Abstract

The potential protective effect of the natural antioxidant, carnosine was evaluated against ischemia/reperfusion liver injury in rats. Ischemia was induced by clamping the pedicle supplying the left hepatic lobe for 60 min followed by reperfusion for 2 h. Untreated rats exposed to ischemia/reperfusion showed significant elevation of serum aspartate aminotransferase and alanine aminotransferase levels, and malondialdehyde level and caspase-3 activity in liver homogenates associated with significant reduction in hepatic nitrite level, catalase and glutathione peroxidase activities as compared with sham-operated group. Pre-treatment with a single i.p. dose of carnosine (250 mg/kg), 30 min prior to the ischemic episode significantly attenuated the deterioration in the measured biochemical parameters observed with ischemia/reperfusion-induced liver injury. Also, light and electron microscopic examinations in ischemia/reperfusion untreated group revealed severe hepatic damage, such as cytoplasmic vacuolation, necrotic and apoptotic cell death, which was markedly ameliorated by pre-ischemic treatment with carnosine. These results strongly emphasize that carnosine can be useful as a prophylactic treatment to protect the liver against hypoxia-reoxygenation damage.

Keywords: Carnosine; Antioxidant activity; Liver; Ischemia-reperfusion; (Rats)

1. Introduction

Ischemia/reperfusion injury is a major determinant in many clinical conditions such as liver resections, hemorrhagic and other types of shock, and liver transplantation. Generation of reactive oxygen species and exhaustion of oxidative defense mechanisms are the main factors implicated in the pathogenesis of ischemia/reperfusion-induced liver damage (Bilzer and Gerbes, 2000; Galaris et al., 2006). Also, several studies have demonstrated the beneficial effect of antioxidants in protecting the liver against ischemia/reperfusion injury (Giakoustidis et al., 2006; Zhang et al., 2006; Vali et al., 2007).

Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide in humans and many animal species. It is involved in various physiological functions including antioxidant, membrane-stabilizing and pH-buffering activities (Gariballa and Sinclair, 2000). The antioxidant effect of carnosine is related to its ability to inactivate reactive oxygen species, scavenge free radicals and chelate prooxidant metals (Kang et al., 2002). In previous studies, carnosine inhibited Fe^{2+}-induced oxidation of membrane lipids, oxidative modification of proteins and DNA fragmentation caused by reactive oxygen species (Gariballa and Sinclair, 2000; Stvolinsky and Dobrota, 2000). Carnosine also protected both *in vitro* and *in vivo* activity of antioxidant enzymes as ceruplasmine (Kang et al., 2002) and superoxide dismutase (Choi et al., 1999). Also, carnosine exerted neuroprotective and cardioprotective effects in PC12 cells and cardiomyoblasts exposed to hypoxia/reoxygenation, respectively (Bharadwaj et al., 2002; Tabakman et al., 2002). In addition, carnosine attenuated ischemia/reperfusion-induced renal dysfunction in rats (Fujii et al., 2003, 2005), and compensated deficit in antioxidant defense system of brain of rats and Mongolian gerbils in the post-ischemic period (Dobrota et al., 2005). However, the protective effect of carnosine in ischemia/reperfusion liver injury is not yet studied.

* Corresponding author. Tel.: +20 106872356.
E-mail address: amrfouad65@yahoo.com (A.A. Fouad).

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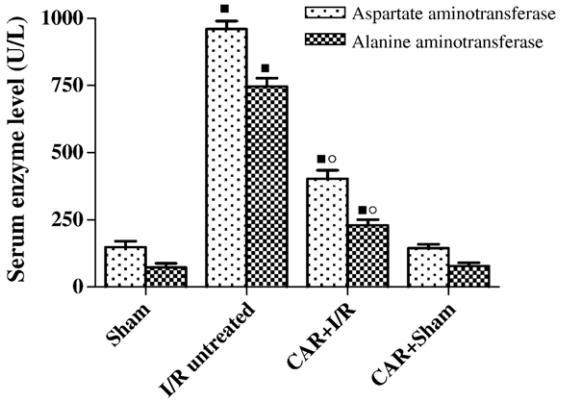

Fig. 1. Effect of ischemia/reperfusion (I/R) liver injury on serum aspartate aminotransferase and alanine aminotransferase levels in rats treated and untreated with carnosine (CAR). Data are expressed as mean±S.E.M., ■$P<0.05$ with respect to sham-operated (sham) group, °$P<0.05$ with respect to I/R untreated group.

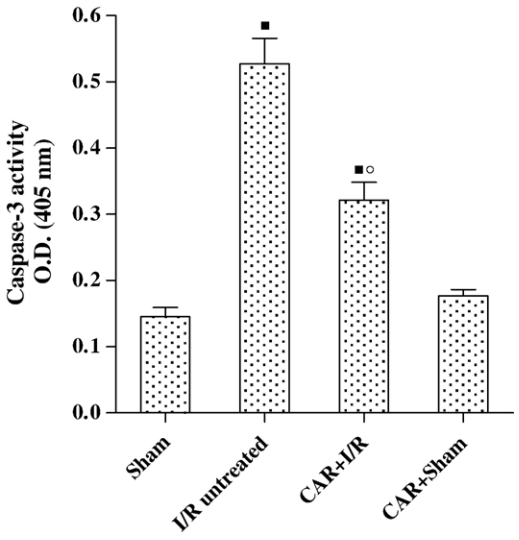

Fig. 2. Activity of caspase-3 in the liver following ischemia/reperfusion (I/R) injury in rats treated and untreated with carnosine (CAR). O.D.: optical density. Data are expressed as mean±S.E.M., ■$P<0.05$ with respect to sham-operated (sham) group, °$P<0.05$ with respect to I/R untreated group.

The present study aimed to evaluate the hepatoprotective effect of pre-ischemic treatment with a single i.p. bolus dose of carnosine in rats exposed to ischemia/reperfusion liver injury. For this purpose, serum levels of aspartate aminotransferase and alanine aminotransferase, hepatic malondialdehyde and nitrite (the stable end product of nitric oxide) levels, and catalase and glutathione peroxidase activities were measured. Also, caspase-3 protease activity was determined as a marker of hepatocellular apoptosis. In addition, light and electron microscopic examinations of liver tissue were performed.

2. Materials and methods

2.1. Animals

Thirty male Sprague–Dawley rats, weighing 180–200 g, were obtained from Othman Animal House (Abu-Rawash, Giza, Egypt). The animals were kept in a standard housing facility and were supplied with ordinary laboratory chow and water *ad libitum*. The experimental protocol was approved by the Local Animal Care Committee, and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

2.2. Chemicals

L-carnosine and cadmium granulated (Fluka, Switzerland). Cupric sulfate, zinc sulfate, potassium dichromate and hydrogen peroxide (El-Nasr Pharmaceutical Chemicals, Egypt). Glacial acetic acid, potassium phosphate and pyridine (Prolabo, France). Sulfanilamide, *N*-naphthylethylenediamine, glutathione, glutathione reductase, nicotinamide adenine dinucleotide hydrogen phosphate (NADPH), sodium azide, sodium dodecyl sulphate, thiobarbituric acid and 1,1,3,3-tetramethoxypropane (Sigma Chemical Company, USA). Tris–hydrochloric acid buffer and urethane (BDH, England).

2.3. Experimental design

The rats were randomly assigned to four groups. The first group ($n=6$) was sham-operated and served as control, while the second group ($n=8$) received a single i.p. injection of 1 ml normal saline (vehicle of carnosine) and subjected to hepatic ischemia/reperfusion injury 30 min later. The third group ($n=8$)

Table 1
Changes in liver biochemical parameters following ischemia/reperfusion (I/R) injury in rats treated and untreated with carnosine (CAR)

Groups	Measured parameters			
	Malondialdehyde (nmol/g tissue)	Nitrite (nmol/g tissue)	Catalase (U/mg protein)	Glutathione peroxidase (U/g tissue)
Sham ($n=6$)	93.04±4.15	87.25±4.63	46.27±2.87	17.12±0.94
I/R untreated ($n=8$)	190.36±7.57[a]	58.53±3.06[a]	28.35±1.08[a]	11.29±0.46[a]
CAR+I/R ($n=8$)	127.42±3.10[a, b]	71.96±2.14[a, b]	38.77±1.66[b]	15.44±0.81[b]
CAR+Sham ($n=8$)	96.45±3.11	92.69±3.24	41.49±2.26	18.76±0.69

Data are expressed as mean±S.E.M.
[a] $P<0.05$ with respect to sham-operated (sham) group.
[b] $P<0.05$ with respect to ischemia/reperfusion untreated group.