Carnosine Treatment Attenuates Testicular Toxicity Induced by Arsenic in Rats

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Abstract: The protective effect of carnosine was investigated in rats exposed to testicular toxicity induced by sodium arsenite (10 mg/kg/day, p.o., for 2 consecutive days). Carnosine (50 mg/kg/day, i.p.) was given for 5 consecutive days, starting three days before sodium arsenite administration. Carnosine significantly attenuated the arsenic-induced decreases of serum testosterone and testicular reduced glutathione levels, and significantly decreased the elevations of testicular malondialdehyde, and nitric oxide resulted from sodium arsenite administration. Carnosine also significantly decreased the arsenic-induced increases of testicular nuclear factor-κB, tumor necrosis factor-α, inducible nitric oxide synthase, cyclooxygenase-2, and caspase-3. It was concluded that carnosine represents a potential candidate to protect against the deleterious effects of arsenic on testicular tissue.
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Keywords: Carnosine; arsenic; testes; rats

1. Introduction:

Inorganic arsenic is a ubiquitous environmental pollutant with multiple toxic effects in both animals and humans. Ground water containing high arsenic levels is the major source of exposure. The permissible arsenic limit in the drinking water recommended by the WHO is 10 ppb. However, this limit is exceeded in many countries all over the world [1]. Industrial production of agricultural pesticides, wood preservatives, and glass production is another source for arsenic contamination [2]. The testes are highly susceptible to arsenic exposure whether acute or chronic. It was reported that arsenic intoxication leads to reduction of testicular weight, impairment of spermatogenesis and androgenesis, and male reproductive dysfunction [3, 4]. The mechanisms by which arsenic causes male reproductive toxicity are not fully elucidated. Growing evidence supports the role of oxidative/nitrosative stress, inflammation, and apoptosis in the pathophysiology of tissue injury mediated by arsenic [5-7]. Oxidative/nitrosative stress up-regulates inflammatory cascades, and necrotic and apoptotic pathways leading eventually to cell death (Chen et al. 2012). It was also reported that arsenic-induced testicular toxicity can be ameliorated by antioxidants and anti-inflammatory agents [9-11].

Carnosine is an endogenous dipeptide involved in various physiological functions, including free radical scavenging, membrane-stabilizing and pH-buffering activities [12]. Carnosine was proved effective in preventing oxidative tissue injury in different experimental models [13, 14]. Carnosine also has anti-inflammatory and metal ion-chelating properties which contribute to its protective effects [15]. Recent studies demonstrated that carnosine significantly protected against testicular injury induced by gamma irradiation in mice, and ischemia-reperfusion testicular injury in rats [16, 17]. However, the protective effect of carnosine against arsenic-induced testicular toxicity was not yet investigated.

2. Materials and Methods:

Drugs and chemicals

Carnosine powder (Sigma-Aldrich Company, USA), and sodium arsenite powder (Loba Chemie, India) were dissolved in normal saline. The doses used in the present study were selected in accordance with previous reports [5, 16].

Animals and treatments

Male Sprague-Dawley rats, weighing 250 ± 10 g were housed at standard facilities (24 ± 1ºC, 45 ± 5% humidity, and 12 h light-dark cycle). They were supplied with standard laboratory chow and water *ad libitum*, and left to acclimatize for seven days before the experiments. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

The rats were randomly allocated to 3 groups (n = 10, each). The first (control) group received normal saline, orally, for two consecutive days. The rats of the second and third groups received sodium arsenite (10 mg/kg/day, p.o., for 2 consecutive days). The animals of the second and third groups received a daily i.p. injection of the vehicle of carnosine or carnosine (50 mg/kg), respectively, for 5 consecutive days starting three days before arsenite administration.

Sampling and biochemical analysis

The rats were euthanized 24 h following the last administration of sodium arsenite. Blood samples were collected through a puncture in the left ventricle,

left for 60 min to clot, and centrifuged for 10 min at 5000 rpm. The obtained clear sera were stored at −80°C and subsequently serum testosterone level was measured using rat testosterone enzyme-linked immunosorbent assay (ELISA) kit following the instructions of the manufacturer (DRG Diagnostics, GmbH, Germany).

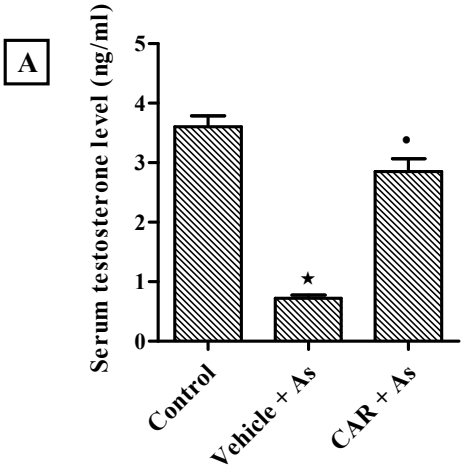
The right testes obtained were homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The testicular homogenates were centrifuged at 5000 rpm for 10 min at 4ºC. The resulting supernatant was used for determination of malondialdehyde (MDA) and reduced glutathione (GSH) levels using colorimetric assay kits according to the recommendations of the manufacturer (BioVision, Inc., USA). The testicular level of nitric oxide (NO) was assayed using colorimetric assay kit following the manufacturer's instructions (Sigma-Aldrich Co., USA). In addition, ELISA kits were used to measure testicular tissue levels of nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and caspase-3 (R &D Systems, USA).

Statistical analysis

The data are expressed as mean ± S.E.M. The results were analyzed by one-way ANOVA followed by Tukey test for post hoc comparisons using SPSS for Windows (version 21). $P < 0.05$ was selected as the criterion for statistical significance.

3. Results:

Sodium arsenite resulted in significant decreases of serum testosterone, and testicular GSH, and significant increases in testicular MDA, and NO as compared to the control group. Carnosine-treated rats showed significantly higher serum testosterone, and testicular GSH, and significantly lower MDA, and NO in testes as compared to the arsenic group non-treated with carnosine (Figure 1A and Figure 1B).



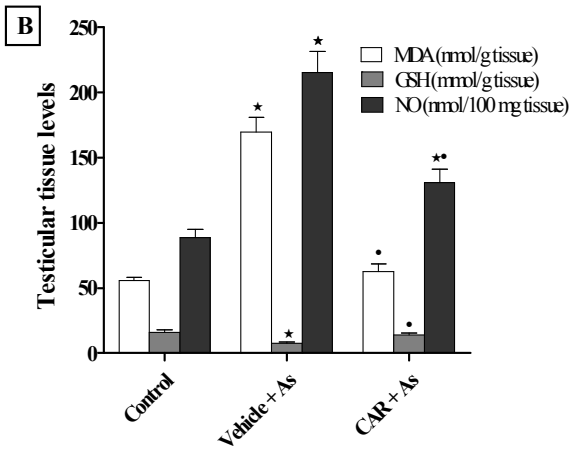


Figure 1. Effects of carnosine (CAR) on: (A) serum testosterone level; (B) testicular tissue levels MDA, GSH, and NO of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control group, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

In addition, significant increases of NF-κB and TNF-α levels, and iNOS, COX-2, and caspase-3 activities were observed in testes of rats received sodium arsenite as compared to the control animals. Carnosine significantly decreased the arsenic-induced elevations of NF-κB and TNF-α levels, and iNOS, COX-2, and caspase-3 activities in the testes as compared to the arsenic group non-treated with carnosine (Figures. 2-6).

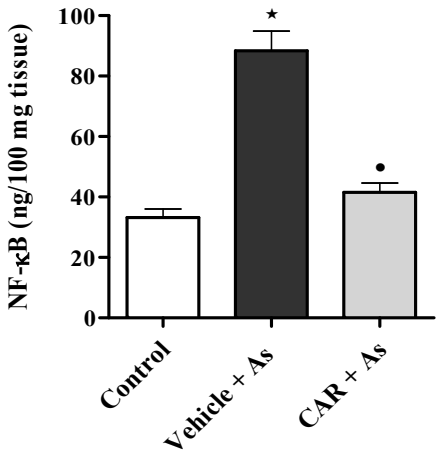


Figure 2. Effects of carnosine (CAR) on NF-κB in testes of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

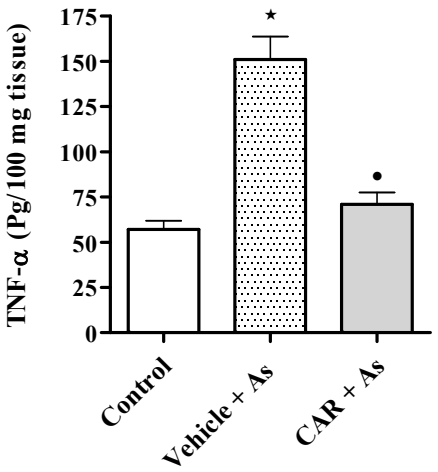


Figure 3. Effects of carnosine (CAR) on TNF-α in testes of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

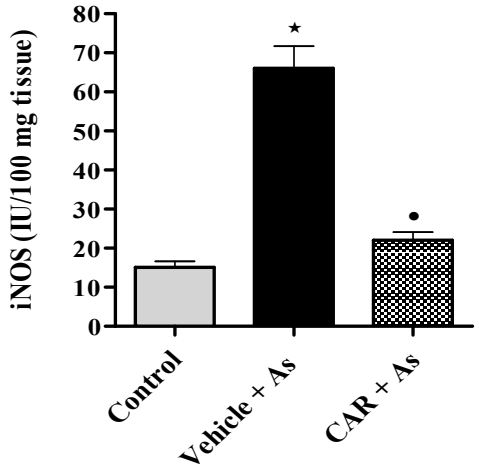


Figure 4. Effects of carnosine (CAR) on iNOS in testes of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

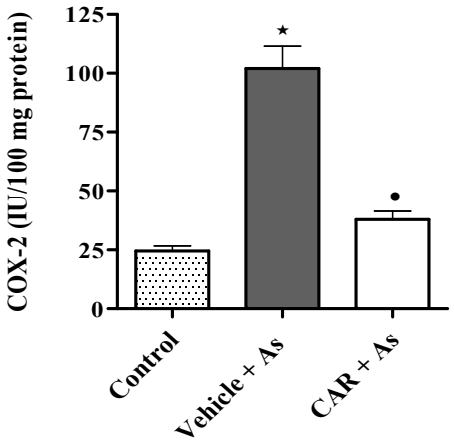


Figure 5. Effects of carnosine (CAR) on COX-2 in testes of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

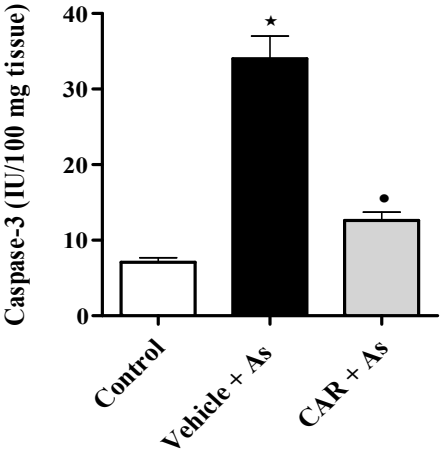


Figure 6. Effects of carnosine (CAR) on caspase-3 in testes of rats exposed to arsenic (As) toxicity. Data are mean ± S.E.M. of 10 rats, $^{*}P < 0.05$ vs. control, $^{\bullet}P < 0.05$ vs. vehicle + arsenic group.

4. Discussions:

The present study showed that carnosine treatment significantly protected against arsenic-induced testicular toxicity in rats as indicated by the improvement in the disturbed biochemical parameters and amelioration of testicular tissue damage observed by histopathological and immunohistochemical examinations. The present work, in agreement with previous studies, confirmed that oxidative stress, increased lipid peroxidation, depletion of antioxidant defenses, and increased production of pro-inflammatory cytokines are implicated in the pathogenesis of testicular injury due to arsenic exposure. In addition, it has been demonstrated that increased NO production is involved in the pathogenesis of arsenic-mediated cytotoxicity and oxidative damage [18]. This can be explained by the ability of TNF-α to up-regulate the iNOS, which catalyzes the production of large amounts of NO. Excess NO reacts with superoxide anion to generate peroxynitrite radical that causes further cell damage by nitrating cellular macromolecules [19]. Excess NO also depletes intracellular GSH increasing the susceptibility to oxidative stress [20]. Moreover, arsenic induces the expression of COX-2, which

increases the production of inflammatory prostaglandins implicated in tissue injury [21].

Carnosine is a natural water-soluble antioxidant that functions in the cytosol where the oxidation mediators are abundantly located. It suppresses the generation of reactive oxygen species and scavenges lipid peroxidation products during free radical reactions [22]. The anti-inflammatory activitiy of carnosine is also responsible for its tissue protective effect [23]. Carnosine also inhibits iNOS activity and decreases NO production, and therefore prevents nitrosative tissue stress [24]. In addition, carnosine reduces the release of TNF-α [25], and inhibits COX-2, which is capable of producing large amounts of inflammatory prostaglandins [26]. This in accordance with the present results which revealed that carnosine treatment significantly suppressed lipid peroxidation, prevented the depletion of GSH, decreased NO production, and reduced the expression of iNOS, TNF-α, and COX-2 in the testes of rats exposed to arsenic toxicity.

It was reported that arsenic administration caused NF-κB activation with subsequent cascade of events responsible for tissue injury [27]. Elevated TNF-α is known as an important step for activation of the NF-κB signaling pathway [28]. Previous studies showed that agents which inhibit TNF-α production and NF-κB significantly ameliorated arsenic-induced tissue injury [5, 7]. This is in accordance with previous results which demonstrated that carnosine provided a significant anti-inflammatory effect by inhibiting the activity of NF-κB [29]. Therefore, the testicular protective effect of carnosine can be attributed to its ability to inhibit NF-κB signaling pathway, which promotes the transcription of TNF-α, COX-2, and iNOS [30-32].

Also, it was demonstrated that arsenic toxicity resulted in cell apoptosis by activating the caspase family of proteases [33]. The present study revealed that carnosine significantly decreased the arsenic-induced increase activity of caspase-3, an executioner of cell apoptosis, in the testicular tissue. This is in agreement with previous studies which showed that carnosine provided a significant anti-apoptotic effect by inhibiting caspase-3 activity [34]. Therefore, it could be stated that carnosine protected against arsenic-induced testicular cell apoptosis.

It was concluded that carnosine significantly ameliorated arsenic-induced testicular injury and dysfunction in rats. The antioxidant and anti-inflammatory activities of carnosine can be considered the main factors responsible for the testicular protective effect. Therefore, carnosine may represent a potential therapeutic option to protect the testes against the detrimental effect of arsenic exposure.

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