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Effect of carnosine supplementation on apoptosis and irisin, total oxidant and antioxidants levels in the serum, liver and lung tissues in rats exposed to formaldehyde inhalation

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ABSTRACT

The main objective of the study has been to show whether carnosine has positive effects on liver and lung tissues of rats exposed to a range of formaldehyde concentrations, and to explore how irisin expression and antioxidant capacity are altered in these tissues by carnosine supplementation. *Sprague-Dawley* type male rats were divided into 8 groups with 6 animals in each: (I) Control; no chemical supplementation); (II) sham (100 mg/kg/day carnosine); (III) low dose formaldehyde (LDFA) for 5 days/week; (IV) LDFA for 5 days/week and carnosine); (V) moderate dose formaldehyde (MDFA) for 5 days/week); (VI) MDFA for 5 days/week and carnosine; (VII) high dose formaldehyde (HDFA) for 5 days/week; (VIII) and HDFA for 5 days/week and carnosine. Sham and control groups were exposed to normal air. Irisin levels of the serum, liver and lung tissue supernatants were analyzed by ELISA, while the REL method was used to determine total oxidant/antioxidant capacity. Irisin production by the tissues was detected immunohistochemically. Increasing doses of FA decreased serum/tissue irisin and total antioxidant levels relative to the controls, as also to increases in TUNEL expressions, total oxidant level, oxidant and apoptosis index. Irisin expression was detected in hepatocyte and sinusoidal cells of the liver and parenchymal cells of the lung. In conclusion, while FA exposure reduces irisin and total oxidant in the serum, liver and lung tissues in a dose-dependent manner and increases the total antioxidant capacity, carnosine supplementation reduces the oxidative stress and restores the histopathological and biochemical signs.

Introduction

Formaldehyde (CH_2O; FA), a normal metabolite found in all mammals, is required in the biosynthesis of purines, thymidine and some amino acids [29]. FA is a widely used substance that has both acute and chronic effects on humans [22]. Although it converts to gas state, it is water-soluble and can damage mucous membranes of the eyes, nose, and upper respiratory tract, as well as the lung and liver tissues [16–18]. Animal experiments also show adverse effects of FA on the skin, eyes, menstrual cycle, nerves, and the reproductive, digestive and respiratory systems [30,31,34].

Liu and Bai [27] showed the detrimental effects of FA on energy metabolism in cerebral cortical neurons of post-natal rats. Many peptide hormones are involved in the regulation of energy metabolism [4,14], the most recently discovered hormone being irisin [10]. It was discovered in 2012 by Boström et al. [10] and is a peptide of 112 amino acids. Although mainly synthesized in the muscle tissue, it can also be synthesized in other tissues, including the liver, pancreas, and salivary and sweat glands [4,5]. After exercise, circulated and skeletal tissue irisin were raised in some studies, decreased in others and remained unchanged in some; these anomalies needs to be resolved (reviewed, [4]). Irisin's cardinal function is to covert white fat tissue to brown fat tissue to generate heat [10].

If FA does impair the normal energy metabolism, as Liu and Bai [27] suggested, it can be hypothesized that irisin expression and FA exposure should in some way be associated, but this possible correlation has yet to be explored. Many studies with FA have shown that it causes oxidative stress, also indicated by the fact that therapeutic

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antioxidant administration reduces the damage [6,39,48]. It is now essential to offset the harmful effects of FA, which is commonly used not only in medicine, but also in daily life. Thus, there are now many groups examining various substances that might eliminate its toxicity. In 2004, Zararsiz et al. [47] administered 10% FA i.p. to male rats every other day for 14 days, supplementing them in the same way with 25 mg/kg melatonin. FA administration caused oxidative damage in the prefrontal cortex, which was reversed by melatonin. Also, Zararsiz et al. [49] noted that oxidative damage in the kidneys tissue of rats due to FA exposure was reduced by omega 3 fatty acids. Oxidative damage caused by FA in the hippocampus was also suppressed by omega 3 fatty acid in another study [20].

Overall the information above suggests that FA damages a number of tissues, reduces their antioxidant capacity [6,19,20,39,47–49], and impairs energy metabolism regulation [9]. Antioxidant molecules can help prevent or elimination this damage [6,19,20,39,47–49]. Carnosine (C), an antioxidant dipeptide, beta-alanyl-L-histidine, is found in muscle and brain tissues [11]. It scavenges reactive oxygen radicals, hinders the oxidation of the cell membrane [2,11] and prevents diabetic nephropathy [32]. Carnosine prevents the development of cancers, protects against the oxidative stress caused by alcohol [11], and eliminates the hepatic injury associated with ethanol in animal models [7]. Thus it is clear that carnosine can reverse the detrimental effects of FA. Although carnosine was discovered in 1900 by the Russian chemist Gulevich [8,11], its effects on liver and lung tissues – to which formaldehyde inflicts the greatest damage have not been seriously studied until recently. Although FA suppresses the energy balance, the effects of carnosine on irisin hormone have not been explored regarding energy balance, nor have the effects on total antioxidant capacity. Thus, the main objectives in this study have been:

1. To discover whether carnosine has favorable effects on liver and lung tissues of rats exposed to various doses (zero, low, moderate and high) of FA and measure the changes in irisin expression in these tissues and serum responses to carnosine at 100 mg/kg/day).
2. To follow the effects carnosine on antioxidant capacity that has been reduced by FA exposure.

Materials and methods

The study had the approval of the Animal Experiments Ethics Committee of Firat University Committee Resolution (number 62) on 09.05.2013 and given the number 2013/04. It included 48 Sprague-Dawley male rats of 260–280 g aged 8–10 weeks. The animals were randomly allocated to 8 groups, with 6 rats in each, for an experiment lasting 4 weeks. They were fed with a previously described rat diet [12]. (I) control group consisting of rats exposed to normal atmosphere air in glass cages measuring 100 cm × 50 cm × 20 cm for the 4 weeks. (II) shams; carnosine (C)-supplemented group of rats kept in a cage of the same way and given orally 100 mg/kg carnosine/day. Carnosine concentrations were established as previously described [45]. (III) low-dose FA (LDFA) exposure given to rats kept similarly caged and exposed to 5.27 ± 0.24 ppm FA by inhalation. (IV) LDFA + C group were rats which were kept caged as above and exposed to LDFA through inhalation, but supplemented with 100 mg/kg oral carnosine/day). (V) moderate-dose formaldehyde (MDFA) exposure to 10.02 ± 0.16 ppm formaldehyde through inhalation. (VI) MDFA + C group exposed to moderate-dose ppm formaldehyde through inhalation, but supplemented with 100 mg/kg oral carnosine/day. (VII) high-dose formaldehyde (HDFA) exposure to 15.2 ± 0.19 ppm formaldehyde through inhalation. (VIII) (HDFA + C group) were rats exposed to HDFA through inhalation, but supplemented with 100 mg/kg oral carnosine/day. The procedure of FA exposure has been described previously [33], but with a slight modification. 5 ppm FA concentrations were previously accepted as a low dose [33]. The formaldehyde levels used were based on the fact that such levels could also occur in the human work place. Also, Kimbell et al. exposed male rats to 0, 0.7, 2, 6, 10, and 15 ppm 6 h/day, 5 days/week for 6 months [21]. The paraformaldehyde (Merck KGaA, 64271 Darmstadt, Germany) exposure experiment in brief was as follows: 8 glass cages of 20 cm × 50 cm × 100 cm were prepared. Two holes of 0.5 cm in diameter were drilled in each cage for air circulation. To ensure the equal distribution of formaldehyde, the cage was divided into 2 by a mesh of 0.1 cm diameter so that one side of the mesh is 70 cm and the other 30 cm. On top of each cage, a window of 10.5 cm diameter was made in the 70 cm part of the cage in which to place the rats, and a similar window was made in the 30 cm part of the cage to release FA. Formaldehyde being heavier than air precipitates into the bottom of the cage, and a ball of diameter 1 cm was formed by wrapping 0.5 g a thin cotton rope in 4.5 g of cotton using thin string to hang in the 30 cm division.

One cotton ball was hung for LDFA level, two for MDFA and 3 for HDFA in the 30 cm division. To adjust the amount of FA in the cage to the desired level, 40 ml 37% FA was put into Eppendorf tubes which were used as stores that could be controlled by a drop-adjusting apparatus used in serum kits that was connected to an air pump. The apparatus was placed in the holes opened on the cage. The pumps were adjusted so that they would continuously supplement 0.002 ppm FA per minute to the cage for 24 h. The main function of the air pumps was not to supplement FA, but to prevent precipitation of FA so that a more homogeneous distribution was achieved in the cage. The cotton balls ensured that FA was slowly released to the environment, since they contained at their center 1 ml 37% FA for steady release over 4 days. FA concentrations in the glass cages were constantly measured by a calibrated monitor (Environmental Sensors Co. Boca Raton FL33432 USA–Catalog No: ZDL-300) recommended by OSHA (Occupational Safety and Health Administration). The targeted concentrations were adjusted according to an 8 h weighted mean (Dose = (concentration × time)/8 h). When the FA level dropped, 1 mL 37% FA was injected into the center of the cotton ball. In the FA inhalation room (except for control and sham groups kept in a different room where no FA was present), FA levels were varied from 0.004 to 0.009 ppm FA, coming from a slow release from the cotton balls injected with 1 mL 37% FA.

Certain people, e.g. pathologists and morticians, generally get exposed to FA for 8 h a day through inhalation because of their occupation. To make the animal experiment closer to the real life situation, all groups except the control and sham groups were exposed to FA through inhalation for 8 h. After exposure, the animals were kept in the normal animal house and fed with rat pellet and water ad libitum, as were the control and sham-treated rats. The animals to be exposed to FA treated from 08.00 until 16.00 daily (week days only) over 4 weeks. All animals were single housed during the experiment period of 4 weeks (except for control and sham groups kept in a different room where no FA was present). Before experiments all cages were also sanitized in a commercial cage washer with a soap wash and a high temperature (100 °C) rinse. Throughout the animals had a 12 h light/12 h dark cycle. Animals' rooms were kept clean, quiet, and uncluttered. All rats were kept at 22 ± 1 °C. Also, animal health status, room conditions, food and water levels were checked daily, including weekends and holidays, no exceptions. At the end of the experiment, the rats were killed by an overdose of anesthetic. Half of the blood samples taken were sent for analysis of lipid parameters and glucose, whereas the other half was reserved to measure irisin