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Effects of L-carnitine on oxidative stress parameters in oophorectomized rats

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KEYWORDS

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Abstract *Aims:* The aim of this study was to investigate the antioxidant effects of L-carnitine on the oxidative stress parameters in oophorectomized rats.
Methods: Twenty-four female albino Wistar rats were used. Rats were divided into four groups: laparotomy-only (LO_{SALINE}) group, oophorectomy plus L-carnitine 100 mg/kg/day (OX_{L100}) group, oophorectomy plus L-carnitine 500 mg/kg/day (OX_{L500}) group, and oophorectomy-only (OX_{SALINE}) group. Experimental protocol was started on day 21 post-castration. Various dosage forms of L-carnitine or isotonic saline were administered intraperitoneally for 14 consecutive days. Nitric oxide (NO), malondialdehyde (MDA), total antioxidant status (TAS), total oxidative stress (TOS) and oxidative stress index (OSI) were evaluated in the tissues including kidney, liver and heart, and sera.
Result(s): In the heart tissue samples, there was no difference in the levels of NO, OSI and TOS between the groups. However, MDA levels in OX_{SALINE} group were significantly higher than OX_{L500} group. On the other hand, there was no statistically significant differences between the groups in terms of levels of NO, MDA, TAS, TOS and OSI in liver, kidney and sera samples.
Conclusion(s): Levels of MDA in the heart tissue were significantly higher in OX_{SALINE} group compared to OX_{L500} group. Thus, it may be suggested that L-carnitine reduces oxidative stress at least in the heart of oophorectomized rats.

1. Introduction

Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. A variety of physiological changes occur in the body; some of

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them are related to cessation of ovarian functions, and others are because of the aging process.[1] It is also known that level of lipid peroxide, a marker of free-radical production and cell membrane lipid peroxidation (LPO), increases after menopause.[2] In various studies, it has been shown that the menopause impairs oxidative systems and causes increase in LPO.[2,3] LPO is a process mediated by free oxygen radicals. During this process, the polyunsaturated fatty acids that are the structure of phospholipid, glycolipid, glyceride or sterol present in cell membrane are converted into various noxious products such as peroxides, alcohols, aldehydes, and hydroxy fatty acids. One of the major end products of lipid peroxidation, is malondialdehyde (MDA).[4]

Oxygen-free radicals more generally are known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS), and one of them is also nitric oxide.[4] Harmful effects of oxidative stress are counteracted by the action of antioxidants. It is well known that various estrogens scavenge reactive oxygen species (ROS) efficiently in both aqueous and lipophilic cellular components.[5]

Furthermore, many in vitro and animal studies have reported that L-carnitine is a free radical scavenger, which protects antioxidant enzymes from oxidative damage.[6] L-carnitine is a quaternary amine containing seven carbon atoms with a molecular mass of 161.2 g/mol, water-soluble, small, highly polar compound which is widely distributed in nature.[7] Carnitine exists in two isomeric forms: L-carnitine and D-carnitine. Only the L-isomer of carnitine is metabolically active and synthesized primarily in liver also in the kidney. Tissues containing high levels of L-carnitine are unable to synthesize it. Tissues, other than liver and kidney, are dependent on the active uptake of carnitine from blood into tissues.[8,9] It has been reported that L-carnitine is a powerful antioxidant, and L-carnitine exerts its antioxidant effect by preventing LPO.[10–12]

In this study, we aimed at investigating the protective effects of L-carnitine against oxidative stress in various tissues at the surgically menopausal rats.

2. Material and method

This study was conducted in the Experimental Medical Research Unit (DETAB, Kırıkkale University Medical Faculty, Kırıkkale, Turkey). The study protocol was approved by Kırıkkale University Animal Research Ethical Committee on 22.08.2011 (Approval Number 11/8-11-210).

2.1. Animals

A total of 24 adult female Wistar albino rats weighing 200–250 g were used in the study. The rats were given ad libitum access to a standard rat chow and tap water, and were housed as a group per cage under standard controlled temperature (20–22 °C), humidity (40–60%), and light (12 h light/12 h dark) in a facility for each group. All animal procedures compiled with the items of Helsinki Final Act (1986) related to experimental animals.

2.2. Study protocol

The rats were randomly allocated to 4 groups: laparotomy-only group (LO_{SALINE} group; $n = 6$), oophorectomized-only group (OX_{SALINE} group; $n = 6$), oophorectomy plus 100 mg/kg/day L-carnitine group (OX_{L100} group; $n = 6$) and oophorectomy plus 500 mg/kg/day L-carnitine group (OX_{L500} group; $n = 6$). The rats were anaesthetized with intraperitoneal injections of ketamine 80 mg/kg (Ketalar®, 50 mg/ml vial; Pfizer Pharmaceuticals, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun®, 100 mg/ml vial; Bayer, Leverkusen, Germany) cocktail. All the interventions were performed under aseptic conditions and the rats were spontaneously breathing during surgical procedure. Surgery on each animal was performed through a ventral midline incision. No intervention other than incision was done in the SL group. The ovaries were exposed and bilateral oophorectomy was performed in the other three groups. Abdominal layers were closed anatomically, using 4/0 atravmatic Vicryl® and the animals were allowed to recover from anesthesia.

Experimental protocol was started on day 21 post-castration. In rats, antioxidant dosage recommendations for L-carnitine were 100 mg/kg[13,14] and 500 mg/kg[15,16] in the literature.

LO_{SALINE} group was administered 0.12 ml/day of isotonic saline solution, OX_{L100} group was administered 100 mg/kg/-day of L-carnitine, OX_{L500} group was administered 500 mg/kg/day of L-carnitine and OX_{SALINE} group was administered 0.12 ml/day of isotonic saline solution, intraperitoneally, for 14 consecutive days. On day 35 post-castration, all the animals were anesthetized again and blood samples were obtained via intra-cardiac puncture before the animals were sacrificed. The blood samples were centrifuged at 10.000 rotations per minute (rpm), at +4 °C for 10 min (Eppendorf; 5804/R, Germany). Sera obtained from blood samples were transferred to polyethylene tubes. After removal the liver, heart and kidney tissue samples were immediately washed with cooled isotonic saline and placed on ice-cold plate. Sera and tissue samples (liver, heart and kidney) were stored at −80 °C until they were used for analysis of nitric oxide (NO), malondialdehyde (MDA), total antioxidant status (TAS), and total oxidative stress (TOS).

Flowchart of the study is presented in Fig. 1.

2.2.1. MDA measurement

The evaluation of MDA was based on the modified version of Yagi's fluorometric method.[17] MDA levels were calculated using a standard curve and values are expressed as nmol/g protein.

2.2.2. NO measurement

Nitrates/nitrites were assayed according to the method of Miranda et al.[18] NO (nitrates/nitrites) levels were calculated using a standard curve and multiplying by the dilution factor. Values are expressed as μmol/g protein.

2.2.3. TOS measurement

Erel's method was used for measuring TOS.[19] TOS was assayed with TOS commercial kit (Rel Assay Diagnostics®, Gaziantep, Turkey, 2010) adapted to an Olympus® AU 480 autoanalyzer (Fully Automatic Chemistry Analyser [AU480] Olympus Diagnostics, Japan). The results are expressed as micromolar hydrogen peroxide equivalents per g protein (μmol H_2O_2 equiv/g protein).

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