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ORIGINAL ARTICLE

The Effect of L-Carnitine on the Prevention of Experimentally Induced Cisplatin Ototoxicity in Rats

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Objective: To investigate the preventative effect of L-Carnitine (LC) on cisplatin induced ototoxicity in rats by evaluating distortion product otoacoustic emissions (DPOAEs) levels and electron microscopic sections of cochlea.

Materials and Methods: Forty Wistar rats were divided into four groups of ten rats; group 1) cisplatin (12mg/kg single dose, i.p.); group 2) cisplatin (12mg/kg single dose, i.p.) plus LC) (100 mg/kg/day, orally); group 3) cisplatin (12mg/kg single dose, i.p.) plus physiological saline (5 ml, i.p.), and group 4 as control group, physiological saline (5 ml, i.p.). All animals were tested by DPOAEs three days before and after cisplatin administration. Additionally electron microscopic evaluation of cochlea was performed.

Results: At all tested frequencies DPOAEs were significantly decreased in animals receiving cisplatin compared to animals receiving cisplatin plus LC. Otherwise, when cisplatin plus LC group was compared to the control group, statistically significant decrease in DPOAEs at 2, 3, 4, 6 and 8 kHz was not observed. Additionally degenerative changes in OHCs and outer phalangeal correct?, please check throughtout the text cells were encountered in the cisplatin group as evidenced by electron microscopy. In the cisplatin plus LC group, normal morphological appearance in OHCs and outer phalangeal cells were observed. Stereocilia at the apical surface of OHCs were minimally shortened compared to the control group.

Conclusions: Our results suggested that LC administration may reduce the severity of cisplatin induced hearing loss.

Key words: Cisplatin, distortion product otoacoustic emissions, ototoxicity, rat

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Introduction

Cisplatin is a platinum-containing compound which is widely used in the treatment of epithelial tumors. However this cytotoxic drug produces several adverse effects such as renal insufficiency, peripheral neuropathies and ototoxicity. Cisplatin induced ototoxicity is generally manifested as sensorineural hearing loss, which may be accompanied by tinnitus. The hearing loss is dose related, usually permanent, and typically bilateral and begins at higher frequencies, progressing to lower frequencies with continued treatment [1,2]. The histological lesions in the cochlea after cisplatin administration are most prominent in the organ of Corti, and consist of degeneration and loss of the sensory cells. This loss typically starts at the first row of outer hair cells (OHCs) in the basal turn of the cochlea followed by the other OHC rows and, with increasing doses or prolonged administration, progresses towards the more apically located cochlear turns and eventually to the inner hair cells (IHCs). Free radical production with resulting glutation depletion and subsequent lipid peroxidation is a proposed mechanism for cisplatin ototoxicity. Several biochemical changes are linked to cochlear injury and OHC loss including an increase in free radicals or reactive oxygen species (ROS) [3,4]. A change in the level of reduced glutathione and the activity of glutathione utilizing enzymes has been correlated with OHC loss due to cisplatin exposure. Endogenous antioxidant enzymes are important to the health and survival of OHCs such as superoxide dismutase and glutathione peroxidase [4,5].

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The L-carnitine (LC) is synthesized in the body from lysine and methionine aminoacids. It has been found to be a co-factor in the oxidation of long chain fatty acids in the mitochondria. It also has an important role in carbohydrate metabolism as well as in the maintenance of cell viability [6]. LC has been reported to be a powerful antioxidant and free radical scavenger. [7] Many reports have shown that LC could protect against the toxicities of several anticancer and toxic agents [8,9]. It could protect against magnetic field immunotoxicity,[10] testicular toxicity [11]. Also our previos studies demonstrated that LC could be a preventive agent for laryngotracheal stenosis and myringosclerosis in rats [12, 13].

These previous studies stimulated our interest to investigate the possible protective effects of LC against cisplatin induced ototoxicity. For systemic cisplatin administration, the rats are a well established model of ototoxicity. In this study, we aimed to investigate the preventative effect of LC on cisplatin induced ototoxicity in rats by evaluating distortion product otoacoustic emissions (DPOAEs) levels and electron microscopic sections of cochlea. To our knowledge, this is the first study investigating the effect of LC on the prevention of cisplatin induced ototoxicity in the English literature.

Materials and Methods

In this study, Wistar, forty male adult albino rats (200-250 gr) were obtained from the Animal Care Center, Mersin University, Turkey Mersin University Animal Research Committee approved this study. Rats were housed in controlled environmental conditions (25 ˚C and a 12 hours light/dark cycle). The animals had free access to pulverized standard pellet food and tap water. The electrophysiological recordings were made with the animal placed in a soundproof room in Animal Care Center. All animals were submitted to the exam of DPOAEs, under anesthesia with intramuscular ketamine hydrochloride (50mg/kg) and xylazin (5mg/kg) . The device used was the Madsen Capella DPOAE System (GN Otometrics A/S Taastrup, Denmark), with the frequency relation 2F1-F2 and the ratio F1/F2=1.22, and the primary tones were both 65 dB sound pressure level (SPL). DPOAEs were defined as positive response if it is amplitude was ≥6 dB above the magnitude of the noise floor around that frequency. The inclusion criterion in this study was an initial DPOAEs exam showing normal responses, which revealed outer hair cells integrity.

Intraperitoneal (i.p.) administration of cisplatin was accompanied by hydration with 10ml physiological saline. LC was used orally (100 mg/kg/day) five days before cisplatin administration. Three days before and after cisplatin administration all animals were tested by DPOAE. The animals were randomly divided into four groups and were treated as follows: group 1; cisplatin (12mg/kg single dose, i.p., n=10), group 2; cisplatin (12mg/kg single dose, i.p.) plus LC (100 mg/kg/day, orally) (n=10), group 3; cisplatin (12mg/kg single dose, i.p.) plus physiological saline (5 ml, i.p.), (n=10), and group 4 as control group, physiological saline (5 ml, i.p. n=10).

Data were statistically evaluated by using paired samples t-test and ANOVA test (SPSS Inc, Chicago, IL). Normality assumption was checked for continues variables. There wasn't any deviation from normal distribution. Therefore parametric statistical tests were performed. P values less than 0.05 were considered statistically significant.

Electron Microscopic Evaluation

Two rats were randomly selected from each group and the electron microscopic evaluation was performed. Under xylazin + ketamine anesthesia, chest wall of the rats were opened and through the left ventricle the circulatory system was flushed with %0.9 cold izotonic salt solutions. Following the flushing, animals were fixed by perfusion with %4 paraformaldehyde through the same way. The inner ear and the surrounding tissues were removed by using a surgical microscope (Leica M651TM, Leica Microsystems GmbH, Wien, Austria) after the perfusion protocol. Then tissue samples were decalcified in 10% EDTA solution. The inner ear tissue was dissected under a stereo dissection microscope (Olympus SZ-61TM, Olympus GmbH, Germany) after decalcification. For electron microscopic investigations, tissue samples were fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol series, cleared with propylene oxide and embedded in epoxy resin. Thin sections (50-70 nm) were cut by ultramicrotome (Leica UCT-125TM, Leica Microsystems GmbH, Wien, Austria) and contrasted with uranyl acetate and lead citrate. Sections were examined by an electron microscope (JEOL JEM-

1011TM, Jeol Ltd. Tokyo, Japan) and photographed by a digital camera attached to the electron microscope.

Results

In the experimental process, neither infection nor mortality was observed in any animals.

DPOAEs levels

There was a significant decrease at DPOAEs signals especially at 6 and 8 kHz in animals receiving cisplatin compared to control group (p=0.000 and p=0.01). All tested frequencies at DPOAEs were significantly decreased in animals receiving cisplatin compared to animals receiving cisplatin plus LC (p values for 2, 3, 4, 6 and 8 kHz; p=0.012, p=0.010, p=0.020, p=0.024 and p=0.042, respectively). Otherwise, when group 2 animals receiving cisplatin plus LC compared to control group, statistically significant decreasing in DPAOE at 2, 3, 4, 6 and 8 kHz was not observed (p=0.160, p=0.428, p=0.817, p=0.61 and p=0.219, respectively) (figure 1).

Electron microscopic findings

Normal morphological appearance in the hair cells was encountered in group 4 when observed by electron microscopy (figure 2A, B). However degenerative changes in OHCs and outer phalengeal cells were seen in group 1 animals receiving cisplatin. In these animals marked shortening observed in stereocilia at apical surface of OHCs (figure 3A, B). In group 2 animals receiving cisplatin plus LC, normal morphological appearance in OHCs and outer phalengeal cells were observed. Stereocilia at apical surface of OHCs were minimally shortened compared to the control group (figure 4A, B).

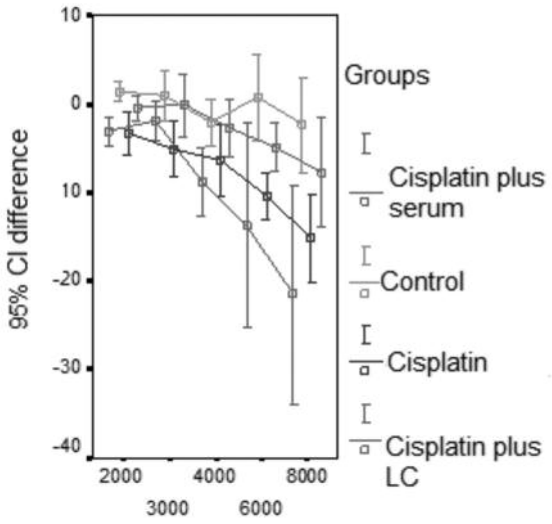

Figure 1. The changes of distortion product otoacoustic emissions (DPOAEs) all tested frequencies among all groups.

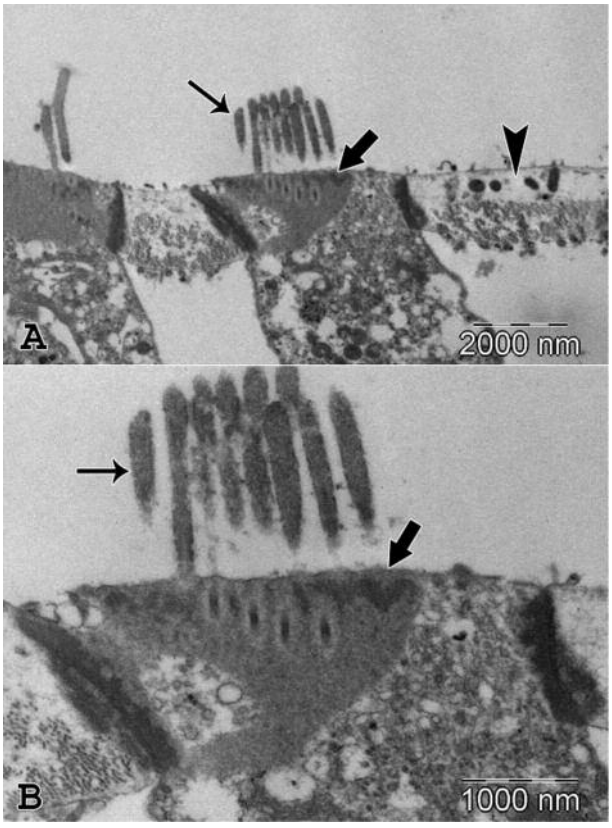

Figure 2. (A.B) Normal morphological appearance in OHCs (large arrow), outer phalengeal cells (arrow head) and stereocilia at apical surface of OHCs (small arrow) were encountered in group 4 when observed by electron microscopy.

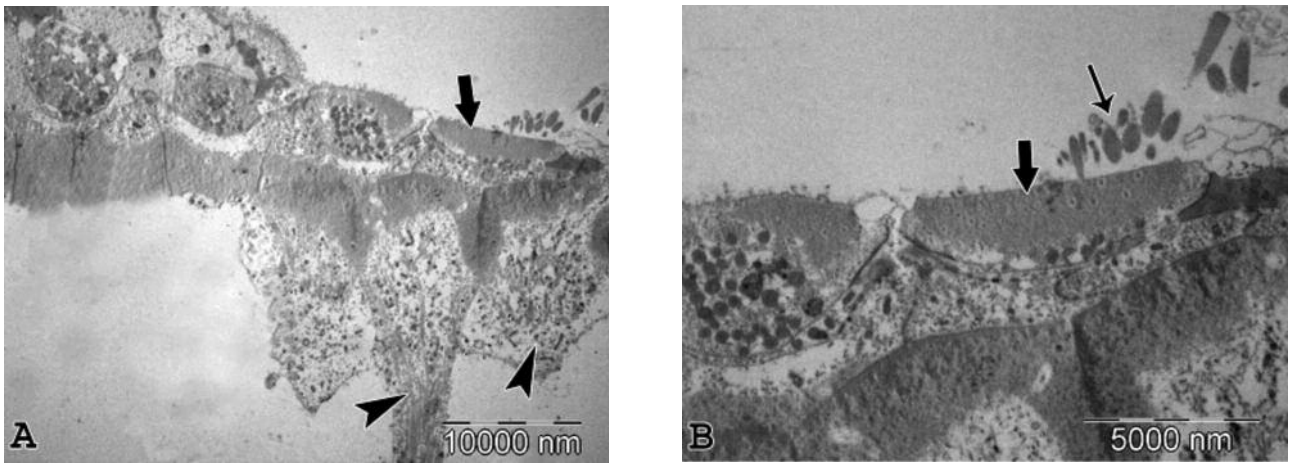

Figure 3. (A.B) In group 2 animals receiving cisplatin plus LC, normal morphological appearance in OHCs (large arrow) and outer phalengeal cells (arrow head) were observed. Stereocilia at apical surface of OHCs (small arrow) were minimal shortened compared to the control group.

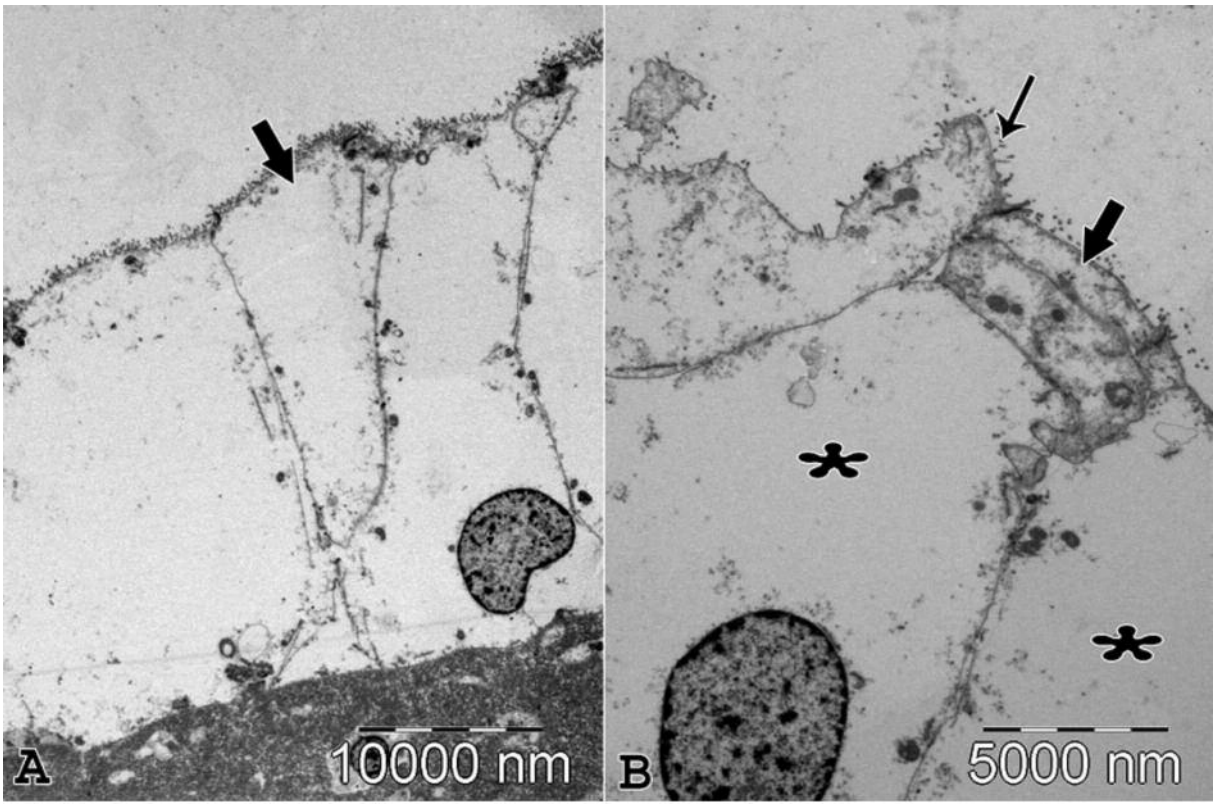

Figure 4. (A.B) In group 2 animals receiving cisplatin, degenerative changes were seen in OHCs (large arrow) and outher phalengeal cells (star) and marked shortening was observed in stereocilia at the apical surface of OHCs (small arrow).

Discussion

The pathogenesis of cisplatin ototoxicity is multifactorial. Animal research indicates that the presence of ROS leads to damage in the organ of Corti, stria vascularis and spiral ganglion cells. In the organ of Corti, cisplatin causes damage primarly to the hair cell stereocilia. Hair cell destruction is most pronounced in the first row and least in the third row of OHCs. IHCs shows damage and degeneration only after all three rows of OHCs in the same region have degenerated [1,4]. Several studies on cisplatin ototoxicity point to alterations in cell anti-oxidant potentials. It has been shown that levels of glutathione and antioxidant enzymes as superoxide dismutase, catalase, glutation peroxidase and glutation reductase are reduced in these tissues leading to lipid peroxidation, thus, to cellular toxicity. Cisplatin-induced ototoxicity results in depletion of the cochlear antioxidant system and increased lipid peroxidation within cochlear tissues [4,14]. Several compounds tested for protection against cisplatin ototoxicity, such as: 4-methylthiobenzoic acid,[15] sodium thiosulfate,[16] ebselen, [17] D-methionine,[18] N-acetylcysteine,[19] vitamin E,[20] aminoguanidine [21].

The neuroprotective action of LC was described in the literature [8,10,22]. It has been demonstrated that LC is able to attenuate the rate of neuronal mortality, to enhance the response to nerve growth factor, and to decrease the neurotoxicity evoked by mitochondrial uncoupling factors or inhibitors. Protective effect of LC was initially presumed to be the result of an increase in the regenerative capacity of neurons, secondary to increased mitochondrial oxidative capacity and nerve growth factor (NGF) responsiveness. Hart et al. reported that LC treatment eliminates sensory neuronal death after peripheral axotomy and resulted in an increased number of regenerating neurons [22,23].

The present study evaluated the efficacy of LC as an otoprotectant agent against cisplatin-induced ototoxicity in rats. LC plays an essential role in intermediary metabolism. Some of the properties exhibited by LC include neuroprotective and neurotrophic actions, antioxidant activity, positive actions on mitochondrial metabolism, and stabilisation of intracellular membranes [12,13]. In several experimental settings, the prophylactic administration of LC prevented the occurrence of peripheral neurotoxicity commonly induced by chemotherapeutic agents [24]. Pisano et al. reported that LC is a protective agent for cisplatin induced neuropathy without showing any interference with antitumor activity of cisplatin [25]. In the literature several compounds have been used for protection against cisplatin ototoxicity. Some otoprotectant agents neutralized the antitumor effect of cisplatin by using systemic administration. To avoid this problem some studies were conducted using intratympanic administration of otoprotectatn agents [26,27]. But systemic administration of LC not diminish the antitumor efficiency of cisplatin [25]. Additionally, treatment with cisplatin is associated with an increase in renal carnitine excretion, most likely due to inhibition of carnitine reabsorption by the proximal tubule of the nephron [28]. Therefore LC supplementation may be beneficial for improving the carnitine deficiency during cisplatin therapy.

In our study, there was significant decrease at DPOAEs signals especially at 6 and 8 kHz in animals receiving cisplatin compared to the control group. Also our study clearly demonstrated that all tested frequencies at DPOAEs were significantly decreased in animals receiving cisplatin compared to animals receiving cisplatin plus LC. However we could not observe any statistically significant change in DPOAEs levels between the cisplatin plus LC and the control groups. These results suggested that additional LC administration could reduce the severity of cisplatin induced hearing loss. Our electrophysiological findings were supported by electron microscopic evaluations of the cochlea. Normal morphological appearance in the hair cells was encountered in the control group, however degenerative changes in OHCs and the outer phalengeal cells were encountered in the cisplatin group. In these animals marked shortening was observed in the stereocilia at apical surface of OHCs. In the cisplatin plus LC group, normal morphological appearance in OHCs and outer phalengeal cells were observed. Stereocilia at the apical surface of OHCs were minimally shortened compared to the control group. These morphological findings supported that LC administration may reduce the ototoxic effects of cisplatin therapy.

Conclusion

LC can inhibit mitochondrial injury induced by oxidative stress and mitochondria-dependent apoptosis of various types of cells. We hypothesized that oxidative injury of mitochondria and cell death induced by cisplatin in the cochlear tissues might be reduced by LC. Our study demonstrated this protective effect electrophysiologically and morphologically, however further biochemical studies are needed for the explanation of the exact protective mechanism of LC in inner ear.

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