The Journal of International Advanced Otology

Int Adv Otol 2014; 10(3): 217-21 •DOI: 10.5152/iao.2014.369

Original Article

The Protective Effect of Intratympanic Alpha Lipoic Acid on Cisplatin-Induced Ototoxicity on Rats

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OBJECTIVE: The objective of this study is to investigate the effect of intratympanic alpha lipoic acid (α-LA) on cisplatin-induced ototoxicity by using distortion product otoacoustic emission (DPOAE).

MATERIALS and METHODS: A total of 24 Wistar albino rats were randomly divided into three groups: group 1 received intraperitoneal (IP) saline + intratympanic (IT) saline as a control group for 4 days (n=8), group 2 received IP cisplatin (20 mg/kg) + IT saline (0.1-0.3 mL) for 4 days (n=8), and group 3 received IP cisplatin (20 mg/kg) + IT α-LA (25 mg/mL) for 4 days (n=8). DPOAEs were performed prior to the procedure and at the end of the experiment on day 5.

RESULTS: Statistically significant DPOAE amplitude reductions were found in the group 2 at all frequencies (2–9 kHz) that demonstrate severe cisplatin ototoxicity ($p<0.01$). IT α-LA injection provided a protective effect against cisplatin-induced ototoxicity at 3.2, 3.8, 4.5, 5.4, 6.3, and 7.6 kHz frequencies ($p<0.05$).

CONCLUSION: In our study IT α-LA showed a protective effect against cisplatin-induced ototoxicity in a large frequency range.

KEY WORDS: Ototoxicity, cisplatin, alpha lipoic acid, otoacoustic emission, intratympanic

INTRODUCTION

Cisplatin is a potent antineoplastic agent with a very wide area of use, especially in head and neck squamous cell carcinoma. However, it has serious side effects, such as nephrotoxicity, ototoxicity, myelotoxicity, gastrointestinal toxicity, and peripheral neuropathy[1]. The ototoxic effect of cisplatin is characterized by high-frequency-affecting, irreversible, bilateral, and progressive sensorineural hearing loss and tinnitus. Cisplatin both is capable of producing reactive oxygen species (ROS), such as superoxide ions and hydroxyl radicals, and may inhibit antioxidant enzymes in the normal tissues. Therefore, to reduce the damage caused by cisplatin, many protective agents have been tested in cisplatin ototoxicity. Of these, many protective agents such as amifostine, lycopene, erdosteine, vitamin C and E, dexamethasone, sodium salicylate, aminoguanidine, resveratrol, diethyldithiocarbamate, and methionine, were previously used for this purpose[2-8]. These protective agents could be administered both by systemic and intratympanic (IT) route. Referring to previous studies, IT-administered drug was observed at higher concentrations in the cochlea and perilymph regarding systemic administration[9].

Alpha lipoic acid (α-LA) is known to be a powerful antioxidant and anti-inflammatory agent. It has a dithiolane ring. Dihydrolipoic acid (DHLA) is a more active metabolite of α-LA. Besides being a potent antioxidant, α-LA is a cofactor for mitochondrial enzymes, metal chelator, and free radical scavenger[10]. It regenerates ascorbate, coenzyme Q10, glutathione, and vitamin E directly and indirectly[11]. It is both lipid- and water-soluble. Free radicals can also pass into intercellular fluid and can damage the cell membrane, mitochondria, or DNA. In this regard, unlike vitamin E and C, α-LA is able to fight free radicals in any part of the cell. It also improves cellular metabolism, energy capacity, and the healing process.

The aim of our study is to investigate the protective effect of IT administration of α-LA[12], which was previously shown to reduce cisplatin ototoxicity with systemic administration, by using distortion product otoacoustic emission (DPOAE).

MATERIALS AND METHODS

This study was carried out at the Marmara University Experimental Medical Research Institute animal laboratory and was approved by Marmara University Local Ethics Committee for Animal Experiments (77.2012.mar). The study included 24 healthy adult female and male albino rats with a normal ear canal and tympanic membrane. The animals weighed 190-300 g (mean weight: 240 g), and the rats were housed in an environment with a light cycle (12 h light, 12 h dark) at 25°C temperature. The animals were fed standard

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Submitted: 31.08.2014 **Accepted:** 22.09.2014

Table 1. DPOAE amplitudes in control group

	Median (mean)							
Control group (n: 8)	**2.7 kHz**	**3.2 kHz**	**3.8 kHz**	**4.5 kHz**	**5.4 kHz**	**6.3 kHz**	**7.6 kHz**	**9 kHz**
Pre-treatment (dBSPL)	11.90 (7.68)	13.00 (16.25)	19.90 (18.87)	21.10 (23.87)	21.00 (22.11)	25.60 (28.36)	33.10 (35.68)	33.40 (36.53)
Post-treatment (dBSPL)	8.35 (6.35)	8.70 (6.27)	9.7 (10.94)	11.20 (14.33)	10.60 (13.21)	19.10 (19.32)	22.10 (23.70)	29.10 (29.38)
P	0.483	0.008	0.012	0.008	0.008	0.008	0.008	0.008

Wilcoxon test 95% confidence interval
dBSPL: decibel sound pressure level
kHz: kilohertz

Table 2. DPOAE amplitudes in cisplatin group

	Median (mean)							
Cisplatin group (n: 8)	**2.7 kHz**	**3.2 kHz**	**3.8 kHz**	**4.5 kHz**	**5.4 kHz**	**6.3 kHz**	**7.6 kHz**	**9 kHz**
Pre-treatment (dBSPL)	13.35 (14.76)	24.30 (23.75)	29.70 (27.25)	33.80 (30.38)	32.85 (34.85)	38.15 (36.84)	40.35 (41.55)	38.30 (37.24)
Post-treatment (dBSPL)	1.90 (1.62)	-0.10 (2.35)	4.90 (-0.54)	3.40 (3.02)	4.00 (5.08)	-2.00 (-0.93)	5.60 (5.50)	2.20 (2.59)
P	0.01	0.003	0.003	0.003	0.003	0.003	0.003	0.003

Wilcoxon test 95% confidence interval
dBSPL: decibel sound pressure level
kHz: kilohertz

diet and water. Following induction of anesthesia with 90 mg/kg of intramuscular ketamine hydrochloride (Ketalar; Pfizer, New York, USA and 10 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany), the external ear canal and eardrum were examined by an operating microscope[13,14].

Study Design

The animals were divided into three groups, including a control group, cisplatin-saline group, and cisplatin-α-LA group. Then, 0.9% saline was administered to the control group intratympanically and intraperitoneally for 4 days (8 animals, 16 ears). The cisplatin group received a 20-mg/kg cumulative dose (10 mg dose for 2 days) of intraperitoneal cisplatin (Cisplatin; Koçak Pharma, İstanbul, Turkey) and intratympanic saline solution for 4 days (8 animals, 16 ears). The α-LA group received intraperitoneal cisplatin with the same protocol and 25 mg/mL of intratympanic α-LA (Thioctacid; Meda Pharma, Bad Homburg, Germany)solution, as an otoprotective agent, 30 min before cisplatin administration for 4 days (8 animals, 16 ears).

Transtympanic injections were done with a 28-gauge needle through the antero-superior quadrant of the tympanic membrane. The injection was continued for about 15 s until the liquid was observed filling the middle ear and coming back to the outer ear (approximately 0.2 mL). Transtympanic injections were done 30 minutes before cisplatin injections to presumably allow a reasonably high concentration of α-LA in the inner ear. At the end of the fifth day, the same anesthesia protocol was applied, and DPOAE measurement was performed to the animals, and the experiment was terminated. Before the DPOAE measurement, animals were submitted to manual otoscopy to assess the external auditory canal and tympanic membrane, and those with signs of otitis media or with a difficult-to-remove cerumen were excluded. DPOAE was measured at the beginning of treatment and after 4 days of drug injections with a Bio-logic Navigator PRO Scout Diagnostic OAE (Natus Medical, California, USA).

The primary tones were introduced into the animals' external ear canal through an insert earphone, using a plastic adapter that sealed the probe in the external ear canal. DPOAEs were recorded at the 2f1-f2 frequency with a constant frequency ratio (f2/f1=1.22). The intensities of primary stimuli were held constant and set as equilevel (L1=L2) at 65 dBSPL (decibel sound pressure level). DPOAE data were recorded for different frequency regions, ranging from 2.7 to 9 kHz (2.7, 3.2, 3.8, 4.5, 5.4, 6.3, 7.6, 9), and plotted as a function of f2. An emitted response was accepted if DPOAE amplitude was 3 dB above the magnitude of the noise floor level at each of the 8 test frequencies.

The data were expressed as mean and medians. The Kruskal-Wallis test was used for the between-group comparison, and the Mann-Whitney U-test was used for sub-analyses. The Wilcoxon test was used in the analyses of intra-group repeated measurements. Statistical Package for the Social Sciences (SPSS) 15.0 software (IBM SPSS Statistics, IBM Corporation; Chicago, IL, USA) program was used for analyses.

RESULTS

Four rats, three from the cisplatin group and one from the α-LA group, died due to toxicity associated with cisplatin. When amplitude values of the control group before and after drug administration were compared, although the decrease in the amplitude of 2.7 kHz frequency was not statistically significant, the amplitude reductions in all other frequencies were statistically significant (Table 1). Statistically significant amplitude reductions were found in the cisplatin group at all frequencies. The maximum amplitude reductions were at the 4.5, 6.3, 7.6, and 9 kHz frequencies (Table 2). The amplitude decreases in the α-LA group, regarding before and after drug administration, were found to be statistically significant at all frequencies. The maximum reductions were at the 6.3, 7.6, and 9 kHz frequencies (Table 3).

However, when amplitude reductions among groups were examined according to median values, the maximum amplitude reduction was

Table 3. DPOAE amplitudes in α-LA group

	Median (mean)							
α-LA group (n: 8)	**2.7 kHz**	**3.2 kHz**	**3.8 kHz**	**4.5 kHz**	**5.4 kHz**	**6.3 kHz**	**7.6 kHz**	**9 kHz**
Pre-treatment (dBSPL)	12.10 (11.93)	17.50 (17.90)	21.70 (21.66)	24.10 (22.46)	26.70 (26.00)	35.20 (32.65)	36.90 (34.82)	38.70 (35.86)
Post-treatment (dBSPL)	5.25 (3.92)	8.40 (8.06)	6.60 (4.94)	10.10 (9.73)	7.70 (8.83)	7.00 (6.40)	9.20 (8.29)	6.50 (7.32)
P	0.012	0.003	0.001	0.001	0.007	0.001	0.001	0.001

Wilcoxon test 95% confidence interval
dBSPL: decibel sound pressure level
kHz: kilohertz
α-LA: alpha lipoic acid

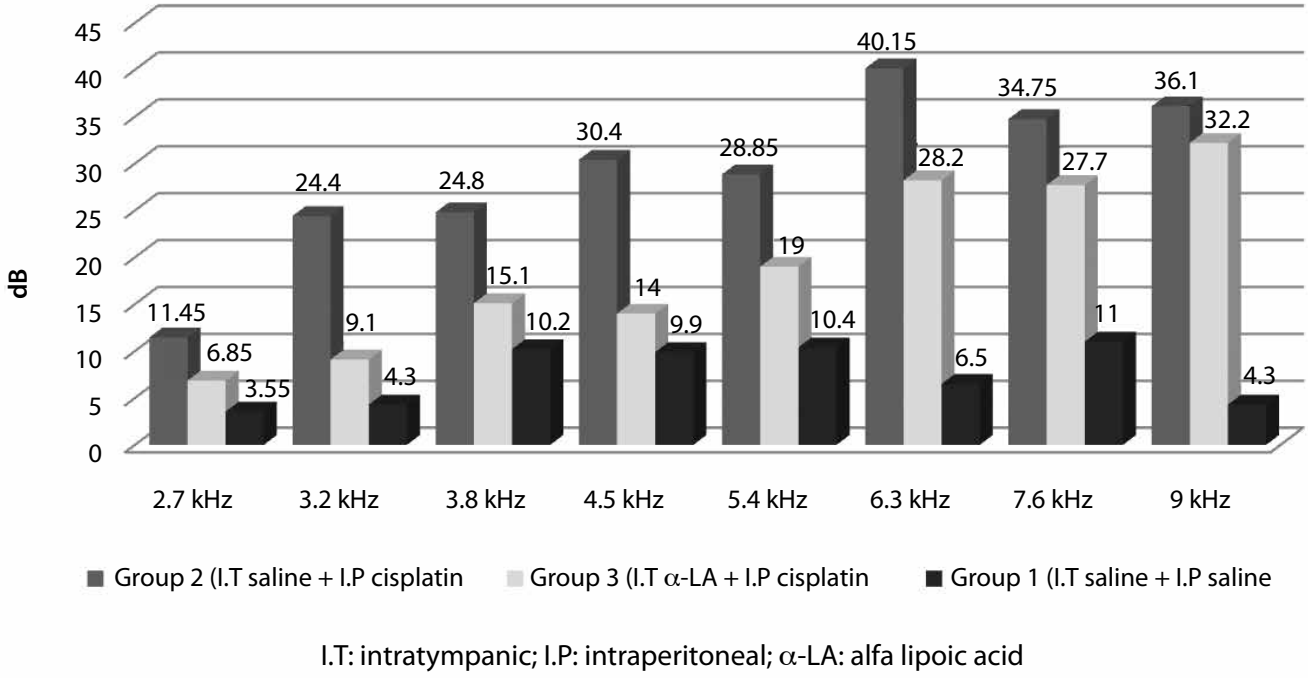

Figure 1. DPOAE amplitude reductions according to median values (dB)
dB: decibel; I.T: intratympanic; I.P: intraperitoneal

found to be in cisplatin group, then in the α-LA group, and naturally least in the control group. No statistically significant difference was observed at the 3.2, 3.8, 4.5, 5.4, 6.3, and 7.6 kHz frequencies in the α-LA group compared to the cisplatin group ($p<0.05$). This difference was maximum, especially at the 3.2, 3.8, and 4.5 khz frequencies ($p <0.01$); on the other hand, no significant difference was observed between the two groups at a frequency of 9 kHz ($p>0.05$) (Figure 1).

DISCUSSION

Cisplatin is a potent alkylating antineoplastic agent, used for the treatment of a variety of neoplastic diseases, with serious side effects, such as ototoxicity, nephrotoxicity, neurotoxicity, and myelotoxicity[8, 15]. Ototoxicity is seen at a high ratio of 36% in patients receiving cisplatin[16]. In animal studies, the effect of cisplatin on cochlear function was shown as a decrease in endocochlear potentials and increase in cochlear microphonic and compound action potential thresholds[17]. Histomorphologically, cisplatin affects the organ of Corti, spiral ganglion, spiral ligament on the lateral wall, and stria vascularis. The experimental studies on rats showed that cisplatin has effects, such as compression, strial edema, bulging, rupture, and reduction in cytoplasmic organelles of marginal cells in the basal fold of the stria vascularis[18]. At the molecular level, cisplatin induces the formation of ROS, such as superoxide anion[19, 20]. Glutathione and antioxidant enzymes are depleted with increased ROS[21]. With the depletion of antioxidant enzymes, toxic products, such as superoxide, hydrogen peroxide, and 4-Hydroxynonenal, lead to calcium entry into cochlear cells and trigger apoptosis. Van Ruijv et al. have immunohistochemically localized this apoptosis and platinum-coated DNA in nuclei of cochlear outer hair cells, supporting cells of the organ of Corti, stria vascularis of marginal cells, and spiral ligament cells at the basal fold[22]. As a result, degeneration in the basal toward the apex and losses are seen in inner ear hair cells due to oxidative stress in the ototoxicity of cisplatin.

In recent years, many chemoprotective and antioxidant agents have been studied experimentally in order to antagonize the toxic effects of cisplatin on the cochlea, and their otoprotective effects have been investigated [6, 7, 18, 23-28]. Intratympanic administration of such agents, including α-LA, is applied today more as a rational method[6, 8, 26, 29].

The importance of antioxidants found in our daily diet in the prevention of ototoxicity is increasing with the recent studies. Resveratrol (grapes), vitamin C (citrus fruits, kiwi, melon, etc.), and lycopene (tomatoes, grapefruit), which are present in foods that we consume daily, are antioxidants used for this purpose[2, 3, 26]. α-LA, which we used in our study, is a natural antioxidant found in broccoli, spinach, tomatoes, and kidney, liver, and heart tissue from animal products[11]. It has a dithiolane ring. In its oxidized form, it is a disulfide derivative of octanoic acid forming an intramolecular disulfide bond. Dihydrolipoic acid (DHLA), its reduced form, is a more active metabolite. Besides being a potent antioxidant, α-LA is a cofactor for mitochondrial enzymes, metal chelator, and free radical scavenger [10]. It directly and indirectly regenerates ascorbate, coenzyme Q10, glutathione, and vitamin E [11].

α-LA is both lipid- and water-soluble, in contrast to lipid-soluble vitamin E and water-soluble vitamin C. With all of these features, α-LA is an antioxidant agent that is close to ideal. Rybak et al.[30] have demonstrated that systemic α-LA provides protection of cochlear glutathione levels and prevents increases in malondialdehyde levels in cases of cisplatin-induced ototoxicity and that α-LA has otoprotective effects by cleaning ROS and chelating cisplatin before causing toxic effects on the cochlea in their next studies [10, 12]. As systemic administration of α-LA has the concern of antitumor effects, besides protective efficacy, it was used intratympanically. In a study by Zhou CH et al., α-LA was shown to be at concentrations about 5 times higher in perilymph with intratympanic administration compared to IV administration [9].

The protective effect of intratympanic vitamin C, an antioxidant used in cisplatin-induced ototoxicity, was evaluated at 20 mg/kg administration by DPOAE in the range of 2-8 kHz. In this study, a higher protective effect was demonstrated at 2 kHz than at other frequencies[26]. In another study in rats, the otoprotective effect of intratympanic vitamin E in cisplatin ototoxicity was evaluated in the range of 4-16 kHz by ABR. An otoprotective effect was shown at the 12 and 16 kHz frequencies in this study[6]. In our study, the maximum otoprotective effect of intratympanic α-LA in cisplatin ototoxicity was observed in the 3-4.5-kHz range.

Rybak et al. have investigated the protective effects of 100 mg/kg intraperitoneal α-LA in cisplatin and carboplatin ototoxicity in two separate studies. An otoprotective effect was observed in cisplatin-administered rats at all frequencies, and otoprotective effects were observed at 16 and 32 kHz frequencies in the carboplatin-receiving group, although this effect could not be detected at the 2.4 and 8 kHz frequencies. Also, no otoprotective effect was observed with 25 and 50 mg/kg of α-LA versus 100 mg/kg cisplatin. According to this study, the protective effect of α-LA has been shown to increase with higher administered doses in cisplatin ototoxicity[10,12]. In our study, 25 mg/mL of α-LA was shown to have a statistically significant otoprotective effect in the 3.2, 3.8, 4.5, 5.4, 6.3, and 7.6 kHz frequency range. This otoprotective effect reached the maximum level at the 3.2, 3.8, and 4.5 kHz frequencies ($p<0.01$). Also, with intratympanic administration of this thiol group antioxidant, the possible tumoricidal activity, lowering the effect of systemically administered cisplatin, was eschewed.

Although ABR was carried out to evaluate the protective efficacy in cisplatin ototoxicity in some studies, we chose to use DPOAE in our study, as in other studies, [3, 26] due to it being a cochlea-selective non-invasive method and being a sensitive method in the functional status assessment of OHC and identifying hearing processes at an early stage[31, 32].

Many studies have been carried out in order to find a way to prevent cisplatin ototoxicity, a common problem. But, no specific agent has been found and transferred into routine clinical practice as a result of these studies. In addition to there not being a consensus about agents that can be used for otoprotective purposes, there is also no consensus available regarding the way of application of these agents. In this regard, although there are studies showing that systemic use of α-LA is effective in ototoxicity of cisplatin, there is no study about the use of intratympanic administration in the literature. Defined as an easy and reliable way in recent years and being popular in the treatment of inner ear diseases, by using intratympanic administration, we believe that α-LA has protective effects in cisplatin ototoxicity. However, to show this effect at the maximum level, studies on the optimal dose of intratympanic α-LA are needed.

Ethics Committee Approval: Ethics committee approval was received for this study from Marmara University Local Ethics Committee for Animal Experiments (77.2012.mar).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.A., S.A.; Design - N.A., Z.E.B.; Supervision - A.S., S.A.; Funding - N.A., Z.E.B.; Materials - N.A., Z.E.B. Data Collection and/or Processing - N.A., S.A.; Analysis and/or Interpretation - S.A., A.S.; Literature Review - S.A., N.A.; Writing - N.A., S.A.; Critical Review -S.A., N.A.; Other - A.S., E.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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