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ORIGINAL ARTICLE

Protective Effect of Methylprednisolone Against Cisplatin-Induced Ototoxicity: Comparison of Route of Administration

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Objective: We investigated the otoprotective effect of methylprednisolone against cisplatin-induced ototoxicity by comparing the effectiveness of route of administration.

Materials and Methods: Thirty-five adult, albino guinea pigs were randomly divided into 5 groups and were treated as follows: Group (1) received cisplatin alone 13 mg/kg, (2) intratympanic methylprednisolone alone, (3) cisplatin and intratympanic methylprednisolone, (4) intravenous methylprednisolone alone, (5) cisplatin and intravenous methylprednisolone. Baseline and 72 hours post-treatment distortion product otoacustic emissions were measured. Cochleae were harvested and processed for electron microscopic examination after completion of auditory testing.

Results: Mean post-treatment distortion product otoacustic emission amplitudes significantly decreased in group 1 at 6 to 8 kHz ($p=0.000$). There were no significant differences between pretreatment and posttreatment DPOAE amplitudes in group 3 animals suggesting that intratympanic methylprednisolone had an otoprotective effect ($p>0.05$). Although significant otoprotection was not observed in group 5 animals, the measurements were close to significance ($p<0.05$). There were no significant differences in emission amplitudes between before and after methylprednisolone injections in group 2 and 4 animals ($p>0.05$). The electron microscopy observations demonstrated an increased intracytoplasmic myeline figures in the outer hair cells in group 1 animals whereas normal morphology of the outer hair cells was preserved in group 3 animals.

Conclusion: These results demonstrate that intratympanic methylprednisolone protects against cisplatin ototoxicity.

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Introduction

Cisplatin(cis-diamminedichloroplatinium II) is a potent antineoplastic drug and is widely used in the treatment of various soft tissue malignant neoplasms, including testicular, overian, bladder, cervical, head and neck and nonsmall cell lung cancers. However, its clinical use is limited by severe side effects such as nephrotoxicity, myelotoxicity, gastrointestinal toxicity, ototoxicity, and peripheral neuropathy[1]. In particular, nephrotoxicity and ototoxicity are dose-limiting side effects. The ototoxic effect of cisplatin is characterized by irreversible, progressive, bilateral, high-frequency, sensorineural hearing loss with associated with tinnitus. Factors that affect the incidence of ototoxicity include the administration route, cumulative dose, age, dietary factors, serum protein level, genetic factors, and cranial radiotherapy history. The hearing loss appears to result from the death of outer hair cells (OHCs) in the organ of Corti by apoptosis[2]. The ototoxicity of cisplatin is not limited to hair cells but also includes atrophy of the stria vascularis, collapse of Reissner's membrane, and damage to the cells supporting the organ of Corti[3].

The mechanism of cisplatin-induced ototoxicity has been intensively investigated. A large interindividual variability in ototoxicity has been well documented in experimental models and in humans. It has been show that the accumulation of reactive oxygen species mediates cisplatin ototoxicity. Cisplatin-induced ototoxicity results in depletion of the cochlear antioxidant system and increased lipid peroxidation within cochlear tissues. Reactive nitrogen species have

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also been implicated in cisplatin-induced ototoxicity[4].

Several agents have been reported to ameliorate cisplatin ototoxicity, including Ginkgo Biloba Extract(EGb 761), glutathione ester, erdosteine, α-tocopherol, vitamine B and E[5-10].

Corticosteroids are widely used to treat inner ear disorders such as sudden idiopathic sensorineural hearing loss, autoimmune hearing loss and Meniere's disease[11]. Both short-term and long-term complications from systemic steroids are well known to otolaryngologists. More recently, otolaryngologists have begun to instill steroids directly into the middle ear space, instead of oral steroids. Methylprednisolone and dexamethasone are the most widely used agents for the intratympanic administration protocols. The intratympanic administration has two theoretical advantages: the potential for steroid uptake through the round window membrane, which results in higher perilymph levels, and the possible reduction of systemic steroid absorption and toxicity. Furthermore, IT steroids have been shown to increase cochlear blood flow in an experimental study by Shirwany et al[12].

The aim of this study was to investigate the protective effect of methylprednisolone against cisplatin-induced ototoxicity by comparing the effectiveness of the route of administration.

Materials and Methods

Animals

Healthy 35 adult, female, albino guinea pigs (weight 400 to 650 g.) were used in this study. All animals had free access to commercial food and water, and were housed in temperature-controlled rooms with 12-hour light/dark cycles. They were kept in the animal laboratory at the university hospital. This study was approved by the Institutional Laboratory Animal Care and Use Committee.

Anesthesia

The animals were anesthetized with 30 mg/kg ketamine and 4 mg/kg xylazine administered intraperitoneally before cisplatin administration and testing. The depth of anesthesia was determined with the pedal reflex and to maintain anesthesia during testing, half-doses of the xylazine/ketamine were administered as needed.

Experimental Design

The animals were randomly divided into five groups of seven guinea pigs each. Auditory function was assessed with distortion product otoacustic emissions (DPOAEs). In all groups, baseline DPOAE testing preceded the administration of the drugs. Body weights, clinical signs were recorded regularly. The animals were tested before and 3 days after cisplatin treatment with or without chemoprotection.

Group 1 *(cisplatin only)* received cisplatin 13 mg/kg IP (Cisplatin DBL, Faulding Pharmaceuticals, Warwickshire, UK) as a slow infusion over 30 minute period.

Group 2 *(intratympanic methylprednisolone only).* Under an operating microscope, an intratympanic injection of methylprednisolone at 4 mg/mL was given slowly through a myringotomy in the anterosuperior quadrant of the tympanic membrane, with a 28-gauge dental needle to fill the middle ear cavity (approximately 0.1 to 0.3 mL). After keeping the animal in the same position for 30 minutes, the procedure was performed in the other ear.

Group 3 *(cisplatin and intratympanic methylprednisolone)* received intratympanic methylprednisolone followed after 30 minutes by IP slow infusion of cisplatin (13 mg/kg).

Group 4 *(intravenous methylprednisolone)* received the standard dose of methylprednisolone (4mg/kg) was administered through the external jugular vein.

Group 5 *(cisplatin and intravenous methylprednisolone)* received the standard dose of I.V. methylprednisolone (4mg/kg) followed after 30 minutes by IP slow infusion of cisplatin (13 mg/kg).

There was no interaural differences in distortion product (DP) amplitudes for each animal. The DP amplitudes from the both ears were compared for each frequency. The status of the middle ear was noted before each injection and DPOAE measurement. Ears that developed persistant fluid, blood, or purulence

were excluded from the study. The animals were killed under anesthesia following the last testing using an IP injection of 26% sodium pentobarbital and 10% isopropyl alcohol.

DPOAE Measurements

The animals were anesthetized with ketamine hydrochloride and xylazine before testing. Before the DPOAEs were measured, the ear was examined under an operating microscope to assess the external auditory canal, tympanic membrane, and signs of otitis media. The DPOAEs was measured at the beginning of treatment and after 3 days of drug treatment using the Vivosonic 600R computer-based system. A probe consisting of a microphone assembly and two transducer tubes was acoustically coupled to the external ear canal of the test animal. The DPOAEs were measured as the sound pressure level (SPL) with stimuli at constant intensity and different frequencies. The stimulus intensity of f1 and f2 was 65dB SPL. The F_2/F_1 ratio was set at 1.22. The upper frequency limit of the distortion product otoacoustic emissions is considered to be 8 kHz. Distortion product functions were obtained at frequencies (F_2) of 1 to 8 kHz in quarter-octave steps at an intensity (L_2) of 65 dB sound pressure level. Input/output(I/O) functions were obtained at frequencies (F_2) ranging from 1 to 8 kHz. Seperate threshold and I/O functions were calculated for each group of animals. Specimen Preparation for

Transmission Electron Microscopy

The animals were killed immediately after completion of DPOAEs recordings using an IP injection of 26% sodium pentobarbital. After decapitation, the cocleae were harvested. The round and oval windows and the apex of the cochlea were perforated with a small pick. The perilymphatic space was perfused with 2.5% glutaraldehyde at 4°C in 0.1 mol/L cacodylate (Cac) buffer. The specimens were then placed in the glutaraldehyde solution and refrigerated overnight. The specimens were perfused with 1% osmium tetroxide and placed on a tissue rotator for 15 minutes. The samples were then rinsed in 1.0 mol/L of Cac three times. The bony capsule of the cochlea was carefully removed and the lateral wall was cut away to reveal the organ of Corti. The specimens were dehydrated in a graded series of alcohol and then embedded in Araldite(r) CY212. The thin (60-90 nm) sections were obtained with ultramicrotome (Leica). The tissue was then viewed, and photographs were taken from basal turn of the cochlea using a transmission electron microscope (Carl Zeiss Libra 120).

Statistical Analysis

The DPOAEs levels for the left and right ears of each subject were averaged to provide the final DPOAE results. If one of the two ears was excluded for the above reasons, the remaining ear represented that animal's DPOAE result. Comparisons between pre-treatment and post-treatment results were analyzed using the Wilcoxon paired 2-sample test, SPSS 15.0 for Windows. Statistical significance was accepted at a p value of less than 0.05.

Results

DPOAE Amplitudes Changes

Baseline DPOAE amplitudes were similar in groups 1, 3, and 5 animals ($p>0.05$, Figure 1). Group 2 and 4 baseline DPOAE amplitudes were also similar with group 1, 3 and 5 animals. The post-treatment DPOAE amplitudes decreased significantly in group 1 at 6 to 8 kHz ($p=0.000$). Significant otoprotection was observed in DPOAE amplitudes in group 3 animals ($p>0.05$). Although significant otoprotection was not observed in group 5 animals, the differences of the measurements were close to significance in this group ($p<0.05$). Comparison of the post-treatment DPOAE amplitudes of the group 1,3 and 5 animals are shown in Figure 2. The post-treatment DPOAE amplitudes were significantly higher in group 3 animals at 6 to 8 kHz when compared with group 1 and 5 animals at those frequencies ($p=0.004$). There were no significant differences in DPOAE amplitudes between before and after intratympanic methylprednisolone injection in group 2 and 4 ($p> 0.05$) suggesting that I.T. and I.V. methylprednisolone injection alone had no toxic effect on cochlear emissions.

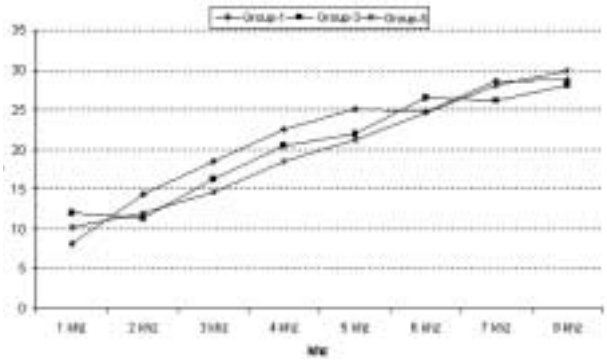

Figure 1. Mean baseline DPOAE amplitudes in groups 1, 3 and 5. There was no significant amplitude difference between those groups (p>0.05).

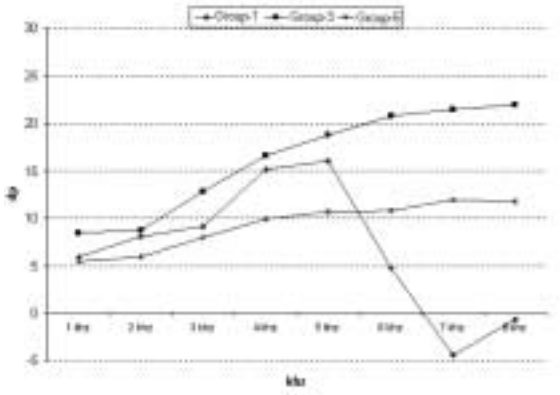

Figure 2. Mean post-treatment amplitudes in groups 1,3 and 5. DPOAE amplitudes decreased significantly in group 1 at 6 to 8 kHz (p=0.000), whereas significant otoprotection was observed in group 3 animals at those frequencies (p>0.05). Otoprotection was close to significance in group 5 (p<0.05).

Cochlear Morphology

Morphologic photographs were taken from basal turn of the cochlea. There were no morphological changes in mitochondria, endoplasmic reticulum and nucleus of the OHCs in group 2 and 4 animals receiving only IT methylprednisolone or I.V. methylprednisolone (Figure 3A). Increased intracytoplasmic myelin figures were observed in group 1 animals receiving only cisplatin (Figure 3B). Normal morphological integrity of the OHCs was observed in group 3 animals (Figure 3C). Normal morphological integrity of the OHCs was also observed in group 5 animals (Figure 3D).

Two animals (group 1) died due to the systemic toxicity of cisplatin. No tympanic membrane injury, such as perforation, was observed in any test animal.

Discussion

The mechanism of cisplatin ototoxicity is not completely understood. There is compelling evidence that the formation of free radicals, particularly superoxide anion, occurs, leading to depletion of intracellular antioxidants[13]. Glutathione, a major substrate in the antioxidant pathway, has been shown to undergo significant depletion in cisplatin-treated rats. A significant increase in the level of malondialdehyde, a product of cellular free radical oxidation, was also observed within cochlea [14]. Some authors suggest that the principle mechanism of ototoxicity is related to the production of NO, which is induced by the production of reactive oxygen species and the over-induction of inducible NOS (NOS II) synthesis. The major toxicity of NO is modest but is greatly potentiated by its reaction with superoxide to form peroxynitrite. Peroxynitrite has direct negative effects on proteins, lipids, and DNA, and the resulting increase in protein oxidation and lipid peroxidation may damage DNA and cause apoptotic changes [15].

Ototoxic agents primarily affect the OHCs before affecting other cochlear elements. OAE measurements are ideal for monitoring cochlear functions in drug-induced ototoxicity. Evoked OAEs, especially DPOAEs due to frequency specificity, were shown to be more sensitive for evaluating OHCs than conventional audiometry, ultra high frequency audiometry, and auditory brainstem response [16]. Sockalingam et al. reported that the recording of DPOAEs is a sensitive method for the evaluation of the functional state of OHCs and albino guinea pigs are the most sensitive animals in term of cisplatin ototoxicity, with alteration in DPOAE and damage to OHCs [17]. So, we used DPOAEs to assess cochlear function in this experimental study.

The present study sought to determine the effectiveness of IT methyprednisolone as an otoprotectant against cisplatin ototoxicity in guinea pigs. Daldal et al. reported that IT dexamethasone had a significant protective affect against cisplatin-induced

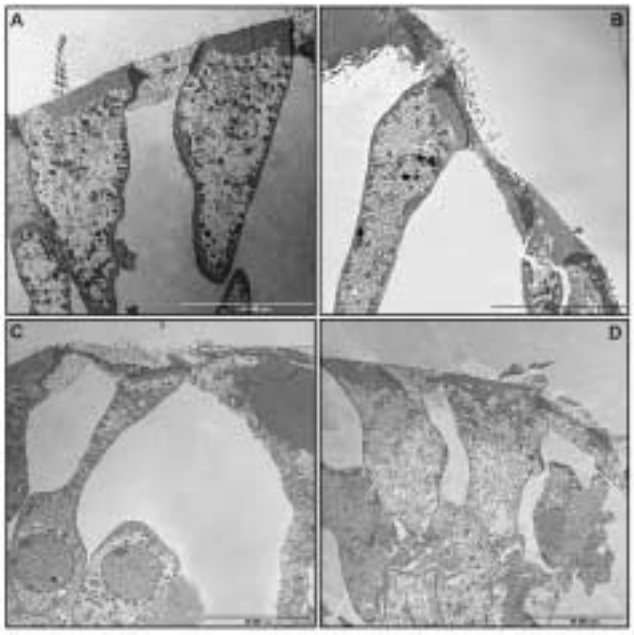

Figure 3. Photomicrographs from basal turn of the cochlea using transmission electron microscopy. (A) Normal morphology of the outer hair cells. (B) Increased in intracytoplasmic myelin figures and marked intercellular adhesion complex were seen in group 2 animals. Black arrows indicates intracytoplasmic myelin figures. (C) Preservation of normal morphology of the outer hair cell was observed in group 3 animals. (D) Normal morphology of the outer hair cell was also observed in group 5 animals.

ototoxicity [18]. IT dexamethasone showed significant otoprotection without interfering with the chemotherapeutic actions of cisplatin by Hill et al.[19] However, these two experimental studies had lack of the electron microscopic and histologic examinations. Cisplatin ototoxicity is manifested by progressive high frequency hearing loss and frequently effects basal turn of cochlea. Because the steroid from intratympanic injection penetrates through the round window to perilymph in the cochlea, steroids may influence the basal turn more than the apex. So we had actually expected that hearing protection might be in high frequencies (basal turn) than low frequencies (apical turn) with IT methylprednisolone injection in this study. Significant otoprotection was demonstrated in group 3 animals receiving IT methylprednisolone and cisplatin by pre-treatment and post-treatment DPOAE measurements. These findings were supported by transmission electron microscopy that normal morphological integrity of the OHCs was demonstrated in group 3 animals whereas increased intracytoplasmic myelin figures and marked intercellular adhesion complex were seen in the OHCs in group 1 animals (Figure 3C). Scanning electron microscopy demonstrates outer hair cell loss in cisplatin ototoxicity. The current study demonstrated and emphasized the morphological changes in the OHCs using transmission electron microscopy.

In animal models, intratympanic steroid administration results in significantly higher perilymph concentrations of steroids than intravenous or oral administration [20]. Chandrasekhar has also demonstrated significantly higher perilymphatic drug concentrations when steroids are instilled intratympanically compared with systemic administration [21]. In addition to higher perilymphatic concentrations, IT steroids avoid the risk of systemic side effects and minimize the risk of drug interactions. Parnes et al. also reported that methylprednisolone showed more effective absorption and reached higher perilymphatic concentration than dexamethasone [20]. We also used IT methylprednisolone instead of dexamethasone for otoprotection in this experimental study.

Recent studies have proved that the instillation of steroids in inner ear disease is safe and has no adverse effects on hearing [11,22]. Shirwany et al. showed that IT injection of steroid had no side effect on auditory sensitivity or cochlear histology in guinea pig[12]. Yilmaz et al. also demonstrated that intratympanic steroid injection had no significant negative effect on the accuracy of transient evoked otoacoustic emissions [23]. Our study also suggest that methylprednisolone does not affect cochlear function, and our findings concur with those of recent studies.

Conclusion

We demonstrated that IT methylprednisolone had a significant protective effect against cisplatin ototoxicity. In addition, no ototoxicity in DPOAE results was observed with IT and I.V. methylprednisolone administration. IT administration of methylprednisolone resulted in a significantly higher protection against cisplatin ototoxicity compared with I.V. administration. These results demonstrate that IT methylprednisolone can be safely used for otoprotection against cisplatin ototoxicity.

References

1. Boulikas T, Vougiouka M. Recent clinical trail using cisplatin, carboplatin and their combination chemotherapy drugs. Oncol Rep 2004; 11:559-95.

2. Rybak LP. Mechanisms of cisplatin of cisplatin ototoxicity and progress in otoprotection. Curr Opin Otolaryngol Head Neck Surg 2007; 15:364-9.

3. Tange RA,Vuzevski VD. Changes of the stria vascularis of the guinea pig due to cis- platinum. Arch Otorhinolaryngol 1984; 239:41-7.

4. Lee JE, Nakagawa T, Kim TS, Endo T, Shiga A, Iguchi F, et al. Role of reactive radicals in degeneration of the auditory system of mice following cisplatin treatment. Acta Otolaryngol 2004; 124:1131-5.

5. Huang X, Whitworth CA, Rybak LP. Ginkgo Biloba Extract (EGb 761) protects against cisplatin-induced ototoxicity in rats. Otol Neurotol 2007; 28:828-33.

6. Campbell KCM, Larsen DL, Meech RP, Rybak LP, Hughes LF. Glutathione ester but not glutathione protects against cisplatin-induced ototoxicity in a rat model. J Am Acad Audiol 2003; 14:124-33.

7. Kalcioglu MT, Kizilay A, Gulec M, Karatas E, Iraz M, Akyol O, et al. The protective effect of erdosteine against ototoxicity induced by cisplatin in rats. Eur Arch Otorhinolaryngol 2005; 262:856-63.

8. Fetoni AR, Sergi B, Ferraresi A, Paludetti G, Troiani D. Protective effects of alpha-tocopherol and tiopronin against cisplatin-induced ototoxicity. Acta Otolaryngol 2004; 124:421-6.

9. Guneri EA, Serbetcioglu B, Ikiz AO, Guneri A, Ceryan K. TEOAE monitoring of Cisplatin induced ototoxicity in guinea pigs: the protective effect of vitamin B treatment. Auris Nasus Larynx. 2001; 28(1):9-14.

10. Kalkanis JG, Whitworth C, Rybak LP. Vitamin E reduces cisplatin ototoxicity. Laryngoscope 2004; 114:538-42.

11. Doyle KJ, Bauch C, Battista R, Beatty C, Hughes GB, Mason J, et al. Intratympanic Steroid Treatment: A review. Otol Neurotol 2004; 25(6):1034-39.

12. Shirwany Na, Seidman MD, Tang W. Effect of transtympanic injection of steroids on cochlear blood flow,auditory sensitivity, and histology in the guinea pig. Am J Otol 1998; 19:230-5.

13. Dehne N, Lautermann J, Petrat F, Rauen U, de Groot H. Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. Toxicol Appl Pharmacol 2001; 174:27-34.

14. Ravi R, Somani S, Rybak LP. Mechanism of cisplatin ototoxicity: antioxidant system. Pharmacol Toxicol 1995; 76:386-94.

15. Takumida M, Anniko M, Popa R, Zhang DM. Pharmacological models for inner ear therapy with emphasis on nitric oxide. Acta Otolaryngol 2001; 121:16-20.

16. Ress BD, Sridhar KS, Balkany TJ, Waxman GM, Stagner BB, Lonsbury-Martin BL. Effects of cis-platinum chemotherapy on otoacoustic emissions: the development of an objective screening protocol. Otolaryngol Head Neck Surg 1999; 121:693-701.

17. Sockalingam R, Freeman S, Cherny TL, Sohmer H. Effect of high-dose cisplatin on auditory brainstem responses and otoacoustic emissions in laboratory animals. Am J Otol 2000; 21:521-7.

18. Daldal A, Odabasi O, Serbetcioglu B. The protective effect of intratympanic dexamethasone on cisplatin-induced ototoxicity in guinea pigs. Otolaryngol Head Neck Surg 2007; 137:747-52.

19. Hill GW, Morest DK, Parham K. Cisplatin-induced ototoxicity: effect of intratympanic dexamethasone injections. Otol Neurotol 2008; 29:1005-11.

20. Parnes LS, Sun AH, Freeman DJ. Corticosteroid pharmacokinetics in the inner ear fluids: an animal study followed by clinical application. Laryngoscope 1999; 109:1-17.

21. Chandrasekhar SS, Rubinstein RY, Kwartler JA, Gatz M, Connelly PE, Huang H, et al. Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents. Otolaryngol Head Neck Surg 2000; 122:521-8.

22. Silverstein H, Isaacson JE, Olds MJ, Rowan PT, Rosenberg S. Dexamethasone inner ear perfusion for the treatment of Meniere's disease: a prospective randomized, double-blind, cross trial. Am J Otol 1998; 19:196-201.

23. Yilmaz I, Yilmazer C, Erkan AN, Aslan SG, Ozluoglu LN. Intratympanic dexamethasone injection effects on transient-evoked otoacoustic emission. Am J Otol 2005; 26:113-7.