The effects of hyperbaric oxygen therapy on an experimental cisplatin-induced ototoxicity model

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ABSTRACT

Objectives: This study aims to investigate the protective effect of hyperbaric oxygen therapy (HBOT) as an antioxidant agent on cisplatin ototoxicity.

Materials and Methods: Between June 4th, 2014 and June 13th, 2014, a total of 15 adult female Wistar albino rats were used in this study. The rats were divided into the following groups: Group A comprised of five rats and had prophylactic one course of HBOT before intraperitoneal (IP) cisplatin injection, followed by two more courses of HBOT. Group B comprised of five rats receiving three cycles of HBOT after 10 mg/kg of IP cisplatin administration. Group C served as the control group, including five rats, and received 10 mg/kg IP cisplatin without any additional treatment. The distortion product otoacoustic emission amplitudes of the rats were obtained before and after the study.

Results: Group A and Group C showed a significant decrease in all measurements (4,004 Hz, 4,761 Hz, 5,188 Hz, 5,652 Hz, 6,726 Hz, and 7,996 Hz) after cisplatin administration compared to that of baseline measurement. In Group B, a significant decrease was observed in the majority of the measurements, although no significant difference was found in 4,761 Hz.

Conclusion: Our study results suggest that HBOT neither provides a protective nor therapeutic effect in a rat cisplatin-ototoxicity model.

Keywords: Cisplatin ototoxicity, distortion product otoacoustic emission, hearing loss, hyperbaric oxygen.

Ototoxicity is a generic name given to the damage to the cochlear and vestibular organs caused by exposure to various therapeutic agents and chemicals.[¹] The sensitivity of the inner ear to multiple chemicals has been well known and is still an essential cause of ototoxicity, hearing loss, and balance disorder.[¹] It is known that certain antibiotics, diuretics, anti-inflammatory, and antineoplastic agents, antimalarial drugs, and some other drugs on the market cause ototoxicity.[¹]

Cisplatin, an antineoplastic agent, is widely used in the treatment of many malignant diseases such as squamous cell carcinoma of the head and neck, testes, ovaries, bladder, prostate, cervical tumors, and non-small cell lung carcinomas.[²] Although there are tolerable side effects such as nausea and vomiting, dose limitation is mostly required in the presence of nephrotoxicity and ototoxicity.[²] Adequate fluid intake and diuretics have been attempted to manage nephrotoxicity; however, an effective
treatment option for ototoxicity still remains unknown.[2]

Recently, many researches have been conducted to prevent cisplatin-related ototoxicity.[3] For that purpose, the otoprotective effects of a variety of antioxidant agents, administered through intratympanic (IT) or intraperitoneal (IP) route, have been evaluated, so far.[3] Although there are many experimental studies on this subject and some positive results have been reported, an ideal agent or treatment method has not been suggested, yet.[4]

In this study, we aimed to investigate the protective effect of hyperbaric oxygen therapy (HBOT), as an antioxidant agent, on cisplatin ototoxicity.

**MATERIALS AND METHODS**

This study was conducted with the approval of the Ethics Committee of Istanbul University Animal Experiments (2014/33) in the animal laboratory of Istanbul University Experimental Medicine Research Institute between June 4th, 2014 and June 13th, 2014. During the study, the principles of the Helsinki Declaration (2004-Tokyo) on experimental animals were followed.

**Selection and housing of experimental animals**

The female Wistar albino rats were obtained from the animal laboratory of Istanbul University Experimental Medical Research Institute. The study was conducted with 18 adult female Wistar albino rats with normal outer eardrums after the otoscopic examination. The weights of the rats varied between 200 and 220 g. The control and experimental groups were kept at room temperature at 21 to 22°C in 12 h of light and 12 h of dark cycles, and the baseline background noise levels were less than 45 dB.

**Preliminary study**

A preliminary study was conducted to determine the toxic symptoms of the drug, the appropriate dose of ototoxic effect, and the time when animal welfare began to deteriorate against the given cisplatin. For that purpose, three of 18 rats were randomly selected, and distortion product otoacoustic emission (DPOAE) measurements were performed under general anesthesia. The IP cisplatin (cisplatin 25 mg/50 mL 1 vial, Kocak Farma, Istanbul, Turkey) at the doses of 10 mg/kg, 16 mg/kg, and 20 mg/kg were administered. At the end of the preliminary study, emission measurements were repeated in all rats, and ototoxicity was observed at the end of follow-up. According to the preliminary study results, a dose of 10 mg/kg was found to be sufficient for the ototoxicity model without toxic symptoms.

**Experimental model**

All rats underwent DPOAE measurements under general anesthesia before any drug administration. Before the start of HBOT sessions, the HBOT chamber was ventilated with oxygen for 10 min to maintain 100% oxygen concentration. The rats were, then, exposed to HBOT at 2.5 atmospheres absolute, 60 min/day, including applications of both decompression and compression cycles and were divided into the following groups: Group A comprised of five rats and had prophylactic one course of HBOT in a small HBOT chamber. To achieve a rat ototoxicity model, 10 mg/kg of IP cisplatin was injected. Then, two more courses of (three sessions in total) HBOT were administered on the following third and fifth days. Group B included five rats receiving three cycles of HBOT on the following first, third, and fifth days after 10 mg/kg of IP cisplatin administration. Group C served as the control group, including five rats, and received 10 mg/kg IP cisplatin, without any additional treatment.

**DPOAE test**

All rats were anesthetized with intramuscular ketamine hydrochloride (Ketalar, Eczacibasi Ilac San. Tic., Istanbul, Turkey) 50 mg/kg and xylazine (Rompun, Bayer Turk Kim. San. Tic. Ltd. Şti., Istanbul, Turkey) 10 mg/kg. The same investigator performed all measurements in a soundproof room with Diagnostic OAE System device ILOv6 software, (Otodynamics, Hatfield Herts, UK) using a neonatal probe. Before the testing procedure, calibration and placement of the probe were performed automatically. The measurements were performed in 30 ears of 15 rats. The rats with normal DPOAE measurements before cisplatin
administration were included in the study. During the procedure, DPOAE measurements could not be taken from 4 ears in the A and B groups and from 5 ears in the control group. Ears for which DPOAE measurement could not be taken were not included in the statistical analysis. The DPOAE was measured as 2f1-f2 cubic distortion product components. The ratio between f2 and f1 frequencies (f2/f1) was maintained at 1.22. The stimulus intensity was taken as L1 for f1 and L2 for f2 and adjusted at L1 = L2 (L1 = 80, L2 = 80 dB). The results were shown in the geometric mean of the primary tones (f1 and f2).

Otoacoustic emissions were stimulated using two different loudspeakers for two stimuli (f1 and f2) placed in the outer ear canal. The DPOAE levels were measured at the 2f1-f2 frequency with a microphone located in the outer ear canal and recorded at 4,004 Hz, 4,761 Hz, 5,188 Hz, 5,652 Hz, 6,726 Hz, and 7,996 Hz frequencies in the geometric mean of f1 and f2. The test time lasted approximately 45 sec. The DPOAE amplitudes above 3 dB of noise threshold were considered significant.[5]

The signal-to-noise ratio (SNR) defined as the geometric average of 2f1-f2 cubic distortion products were used for the interpretation of the DPOAE results. The mean ratio of each subject was calculated. The DPOAE measurements after drug administration were obtained seven days after the first test, and the findings were compared with baseline DPOAE measurements.

**Statistical analysis**

Statistical analysis was performed using the IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency. The Kolmogorov-Smirnov test was used to determine the distribution of the continuous variables. The Kruskal-Wallis test was used for the analysis of the quantitative data between the study groups. The Wilcoxon signed-rank test was used for the comparison of repeated measurements. A p value of <0.05 was considered statistically significant.

**RESULTS**

Group A and Group C showed a significant decrease in all measurements (4,004 Hz,

| Table 1. Comparison of the DPOAE measurements of the study groups |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                | Group A (n=6)    | Group B (n=6)    | Group C (n=5)    |                                |                                |                                |                                |
|                                | Mean±SD Median Min-Max | Mean±SD Median Min-Max | Mean±SD Median Min-Max |                                |                                |                                |                                |
| 4,004 Hz before cisplatin      | 15.0±14.3 10.4 2.3-32.5 | 12.8±11.8 8.6 1.4-31.2 | 4.2±4.6 5.8 3.3-8.5 | 0.509                                 |
| 4,004 Hz after cisplatin       | 10.2±13.3 5.3 1.8-27.2 | 8.7±9.6 7.1 0.6-25.3 | 0.6±3.8 1.2 4.3-5.8 | 0.07 |
| 4,761 Hz before cisplatin      | 19.9±14.6 21.9 2.1-33.6 | 18.3±17.0 10.7 1.5-41.4 | 8.3±6.1 10.6 1.1-14.6 | 0.55 |
| 4,761 Hz after cisplatin       | 10.3±16.3 4.9 4.2-32.6 | 13.8±17.7 9.2 2.8-41.1 | 4.4±4.6 4.3 0.6-10.5 | 0.77 |
| 5,188 Hz before cisplatin      | 25.0±16.4 25.7 2.5-41.9 | 23.0±16.3 17.7 4.4-45.7 | 11.8±8.4 13.4 2.1-19.8 | 0.38 |
| 5,188 Hz after cisplatin       | 13.3±18.3 7.3 2.1-44.9 | 17.9±17.0 13.5 5.5-43.8 | 7.1±8.9 6.6 5.3-17.7 | 0.73 |
| 5,652 Hz before cisplatin      | 28.6±17.9 27.1 10.3-49.3 | 26.9±12.9 24.3 8.7-45.4 | 13.0±13.5 16.5 10.2-24.6 | 0.38 |
| 5,652 Hz after cisplatin       | 16.0±18.2 15.2 4.3-44.6 | 19.5±10.7 20.9 4.4-35.6 | 9.4±8.2 12.0 4.0-16.0 | 0.74 |
| 6,726 Hz before cisplatin      | 23.5±10.2 23.0 10.5-35.8 | 24.5±15.4 25.9 0.4-40.9 | 18.1±7.9 20.0 4.5-25.3 | 0.68 |
| 6,726 Hz after cisplatin       | 17.5±16.2 24.1 4.2-32.3 | 15.0±14.3 21.9 9.9-38.7 | 15.0±14.3 16.0 4.8-19.2 | 0.76 |
| 7,996 Hz before cisplatin      | 31.4±11.7 31.1 17.1-44.6 | 23.7±16.8 23.8 4.4-47.6 | 21.5±14.8 25.0 3.3-34.0 | 0.66 |
| 7,996 Hz after cisplatin       | 19.9±18.5 24.5 3.1-42.6 | 15.2±23.2 15.9 13.4-44.2 | 17.2±15.7 20.0 9.9-29.0 | 0.82 |

DPOAE: Distortion product otoacoustic emission; SD: Standard deviation; Min: Minimum; Max: Maximum; n: Number of ears.

Wilcoxon test

- 4,004 Hz before/after cisplatin; Group A p=0.04; Group B p=0.02; Group C p=0.04
- 4,761 Hz before/after cisplatin; Group A p=0.02; Group B p=0.11; Group C p=0.03
- 5,188 Hz before/after cisplatin; Group A p=0.04; Group B p=0.04; Group C p=0.04
- 5,652 Hz before/after cisplatin; Group A p=0.04; Group B p=0.02; Group C p=0.03
- 6,726 Hz before/after cisplatin; Group A p=0.02; Group B p=0.02; Group C p=0.04
- 7,996 Hz before/after cisplatin; Group A p=0.02; Group B p=0.04; Group C p=0.04
4,761 Hz, 5,188 Hz, 5,652 Hz, 6,726 Hz, and 7,996 Hz) after cisplatin administration, compared to baseline measurement. In Group B, a significant decrease was observed in the majority of the measurements, although no significant difference was observed in 4,761 Hz. In addition, there was no significant difference among the three groups in terms of baseline and post-cisplatin treatment 4,004 Hz, 4,761 Hz, 5,188 Hz, 5,652 Hz, 6,726 Hz, and 7,996 Hz measurements (Table 1).

The amount of change between baseline and post-cisplatin administration measurements at 4,004 Hz, 4,761 Hz, 5,188 Hz, 5,652 Hz, 6,726 Hz, and 7,996 Hz did not significantly differ (Table 2).

**DISCUSSION**

The mechanism of ototoxicity formation due to cisplatin is still unclear. The most emphasized ototoxicity theory is the toxic effects of reactive oxygen species (ROS) and the insufficiency of the antioxidant system in outer hair cells (OHC).[6,7] Cisplatin causes progressive damage moving from the basal to the apical layer of the OHSs in the cochlea and has an effect on the Reissner’s membrane. On the other hand, cisplatin ototoxicity is not limited to hair cells: it leads to atrophy of striae vascularis, the collapse of the Meissner membrane, and damage to supporting cells in the Corti organ.[8]

A variety of protective agents have been evaluated in conjunction with cisplatin therapy to reduce ototoxicity without altering cisplatin’s antitumoral activity.[9,10] Over the last decade, majority of the studies have focused on the effects of antioxidant drugs, preventing cisplatin or amikacin-induced ototoxicity in OHCs. For that purpose, antioxidant agents such as phosphomycin, sulfur compounds, vitamin C and E, sodium thiosulfate, diethyl carbamate, adrenocorticotropic hormone, lipoic acid, melatonin, Ginkgo Biloba, and D-methionine have been investigated.[11-14] Giordano et al.[15] reported protective antioxidant effects of systemic application of D-methionine in 12 rats to treat cisplatin-induced ototoxicity, at 4.0 to 12 kHz bands with otoacoustic emission and auditory brainstem response (ABR) tests. Similarly, Kalkanis et al.[9] found a significant protective effect of a single vitamin E dose administered 30 min before cisplatin injection at 8-, 16-, and 32-kHz ABR thresholds in a rat ototoxicity model. Vitamin E is a potentially otoprotective agent by terminating the lipid peroxidation chain reactions. These studies differed regarding the administration routes of antioxidant substances. Systemic or IT applications of antioxidant agents have advantages and disadvantages.

Systemic antioxidant agents may interact with cisplatin and transform it into its inactive compounds. This process may reduce the effectiveness of cisplatin in cancer treatment. Saito et al.[16] reported reduced antitumor effect of cisplatin when combined with systemic sodium thiosulfate, which is known as ototoxicity, preventing antioxidants. Similarly, Rybak et al.[17] reported the adverse effect of the systematic administration of sodium thiosulfate...
and N-acetylcysteine on cisplatin anticancer treatment.

Considering this unintended effect due to systemic drug administration, many researchers have focused on transferring antioxidant agents through the inner ear. However, IT administration raised some concerns such as the accumulation of insufficient drug and damage to the eardrum. The other limiting factor in rats is the permeability of the circular window membrane, which is known as decreasing the permeability of substances with molecular weights larger than 45 kD.\cite{18,19} Besides, IT applications require a delicate intervention in experimental studies. The injury or perforation risk of the eardrum may hinder the results of otoacoustic emissions. Therefore, experimental rat ototoxicity models may not be suitable in studies requiring dose repetitions.

The HBOT is known to have antioxidant effects. It has been reported that HBOT prevents the ROS-related toxicity caused by cisplatin via increasing superoxide dismutase (SOD) and glutathione peroxidase.\cite{20} In a study, Yassuda et al.\cite{21} investigated the effect of three courses of HBOT on cisplatin ototoxicity in eight guinea pigs. They evaluated the cochlear function and morphological structures and reported that hearing functions and external shaky hair cell morphology were significantly preserved in the HBOT group. In another study, Cobanoglu et al.\cite{5} evaluated the antioxidant effect of HBOT in 30 adult Wistar rats in a cisplatin-induced ototoxicity model. They administered HBOT on the same day or 72 h after 15 mg/kg of cisplatin administration. The effect of ototoxicity was measured with DPOAE on the following first, third, and seventh days. They revealed that HBOT groups had better SNR values compared to the cisplatin only group. They also concluded that same day HBOT had better SNR values compared to after 72-h HBOT group. In our study, due to the disadvantages of IT treatment discussed above, we preferred non-invasive systemic HBOT administration to avoid interaction with the molecular structure of cisplatin. We found a significant decrease in all mean DPOAE frequencies in the control group, indicating that a single dose of 10 mg/kg was sufficient to induce cisplatin-induced ototoxicity.

We administered HBOT prior to cisplatin in Group A to evaluate the preventive effect of HBOT. However, no significant improvement was observed. Besides, the SNR values significantly decreased at all frequencies, except for 6,726 Hz in Group A, which was served as a prophylactic treatment group, and except for 4,761 Hz in Group B.

Although at 6,726 Hz in Group A and at 4,761 Hz in Group B provided an insight into the protective efficacy of HBOT, there was no statistically significant difference among the groups. Therefore, our results cannot support the protective effect of HBOT on cisplatin ototoxicity. Similarly, in a placebo-controlled amikacin-induced ototoxicity study conducted by Amora Lde et al.\cite{22} the authors could not observe the otoprotective effect of HBOT based on DPOAE measurements and morphological structures.

The lack of biochemical antioxidant enzyme measurements after HBOT is the main limitation of the present study. In addition, the evaluation of OHC morphology by electron microscopy could provide more information about the structure of drug-induced ototoxicity.

In conclusion, there is still no consensus on appropriate antioxidant agents, effective doses, and treatment regimens. Based on our study results, HBOT seems to have no protective effect on cisplatin-induced ototoxicity. However, there is still a need for further studies investigating laboratory and histological results of HBOT protocols or different antioxidant agents to confirm their reliability.

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