N-Acetyl Cysteine Reduces Cisplatin Ototoxicity

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ORIGINAL ARTICLE

ABSTRACT

Objective: To investigate the changes in otoacoustic emissions (OAEs) for detecting the ototoxicity of cisplatin and to study the possible preventive effect of N-acetylcysteine (NAC) in cisplatin ototoxicity.

Materials and Methods: This study was conducted on 21 Wistar albino rats in four groups. Cisplatin, cisplatin plus NAC, NAC alone, and saline were intraperitoneally administered. The rats were anesthetized to study the otoacoustic emissions before and after the experiment.

Results: The OAEs were attenuated in the cisplatin group: (−1.31/−4.90), (6.28/4.58), (4.00/0.93), (2.73/−3.74), (2.66/−4.53).

The group that received NAC in addition to cisplatin had better OAEs.

Conclusion: Cisplatin ototoxicity can be detected by OAE testing in rats, and NAC may reduce the ototoxic effect.

Keywords: Ototoxicity, cisplatin, NAC, N-acetylcysteine, hearing

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INTRODUCTION

Ototoxicity is a health problem that emerged after the powerful treatments in serious health conditions (1-3). Ototoxicity is sometimes inevitable when treatment of the underlying disease is obligatory; such as cancer. For cancer patients, cisplatin is a common antineoplastic agent, and was investigated previously to reveal increased nitrogen and reactive oxygen radicals that can damage hair cells resulting in ototoxicity (3-8).

There are a few agents, including sodium thiosulfate, amifostine, D-methionine, vitamin E, dexamethasone, salicylates, neurotropins, flunarizine, lipoic acid, ebselen, diethyldithiocarbamate, and 4-methylthiobensoic acid, that are reported to prevent ototoxicity (9-15). N-acetylcysteine (NAC), previously shown to decrease ototoxicity caused by different agents, is known to be a powerful *in vitro* antioxidant (16-18). The current study aimed to determine the effect of NAC on the reduction of cisplatin ototoxicity.

Permanent ototoxicity is a disabling condition that could further remove the patient from the environment, additional to the devastating effects of the primary disease. Detection by otoacoustic emissions (OAE) and prevention by NAC would be very beneficial for a patient who is already dealing with the primary disease.

MATERIALS and METHODS

The study was approved by Erciyes University Local Ethics Committee for Animal Experiments. The experiments were performed in Erciyes University Experimental Research and Application Center Laboratory.

A total of 21 male Wistar albino 5-month-old rats with an average weight of 300–350 g, which were reared under the same environmental conditions and given a standard laboratory diet were used in the experiments. All the rats were kept in cages in the same room and under the same environmental conditions, namely in a room that was illuminated and darkened for 12/12 hour cycles at a temperature of 22°C±3°C with a background noise level of under 50 dB, and the rats having free access to food and water. All the procedures were performed in compliance with the Helsinki Declaration and International Guiding Principles for Biomedical Research Involving Animals.

Initially, each rat was anesthetized with intraperitoneal (i.p.) 40 mg/kg ketamine (Ketalar flacon, Pfizer; New York, USA) and 5 mg/kg xylazine (Rompun, Bayer; Leverkusen, Germany). Following the anesthesia, the ear canals

and tympanic membranes of each rat were examined by otomicroscopic inspection (Opmi 1, Zeiss; Oberkochen, Germany), which revealed no pathological finding. Distortion product otoacoustic emission (DPOAE) tests were performed for both ears of each animal for baseline hearing threshold evaluation, and 42 functionally normal ears of 21 rats were included in the study. During the experiments, three rats belonging to the control group, the cisplatin group, and the NAC group and two rats belonging to the cisplatin + NAC group did not recover from anesthesia and were excluded from the study.

Preliminary trial
First, a preliminary trial for ototoxicity on 20 rats was conducted. Cisplatin (Cisplatin 100 ml, 100 mg flacon, Orna; İstanbul, Turkey) was administered to 15 Wistar albino rats, and ototoxicity was determined with decrement in OAEs. Wistar albino rats were administered cisplatin, first 5 mg/kg, followed by 10 mg/kg, and then 15 mg/kg until ototoxicity resulted (Table 1).

Formation of the groups and experimental procedures
The subjects were randomized into four distinct groups, as follows (Table 2):

Group 1 Control Group (n=5): Each of the five rats included in this group was given saline (1 cc, i.p.).

Group 2 NAC Group (n=5): Intraperitoneal 500 mg/kg NAC (Asist, Hüsnü Arsan; İstanbul, Turkey) was administered to each of the five rats in this group.

Group 3 Cisplatin Group (n=5): Intraperitoneal 15 mg/kg cisplatin (Cisplatin 100 mL, 100 mg flacon, Orna, İstanbul, Turkey) was administered to each of the five rats in this group.

Group 4 Cisplatin + NAC Group (n=6): Initially, the rats were given 15 mg/kg cisplatin (Cisplatin 100 mL, 100 mg flacon, Orna, İstanbul, Turkey). Four hours later, intraperitoneal 500 mg/kg NAC (Asist, Hüsnü Arsan; İstanbul, Turkey) was administered to each of the six rats in this group.

The rats in the third and fourth groups were administered cisplatin only once during the study. To the fourth group, NAC was administered a total of three times: on the first day 4 hours after cisplatin, and then on the second day and third days. At days 0 and 7, the subjects underwent anesthesia followed by DPOAE evaluation for hearing functions, and the results were recorded.

Otoacoustic emission measurement
Signal-to-noise ratio (SNR) values, which were calculated by subtracting the background noise level from the DPOAE measurements in decibels (dBs), were used for interpretation of the test results. The Madsen (Capella, Denmark) OAE system and neonatal probes were used for DPOAE screening. The f2/f1 ratio was fixed at 1.22, and the L1-L2 difference was adjusted to a 10 dB sound pressure level (SPL) (L1=70 dB SPL; L2=60 dB SPL). DPOAEs were measured at the tones equal to 2f1-f2, and generated at the frequencies corresponding to the geometric mean of f1 and f2. SNR values were recorded from both ears on days 0 and 7, and at 2000, 3000, 4000, 6000, and 8000 Hz.

Table 1. Preliminary study of cisplatin ototoxicity on 20 rats

Group	Treatment protocol	Result
Group 1 (n=5)	Saline	Ototoxicity (-)
Group 2 (n=5)	Cisplatin 5 mg/kg	Ototoxicity (-)
Group 3 (n=5)	Cisplatin 10 mg/kg	Ototoxicity (-)
Group 4 (n=5)	Cisplatin 15 mg/kg	Ototoxicity (+)

Table 2. N-acetylcysteine (NAC) against cisplatin ototoxicity on 21 rats

Group	Treatment Protocol
Group 1 (n=5)	1 cc Saline
Group 2 (n=5)	NAC 500 mg/kg
Group 3 (n=5)	Cisplatin 15 mg/kg
Group 4 (n=6)	Cisplatin 15 mg/kg+NAC 500 mg/kg

Statistical analysis
SPSS for Windows 16.0 (SPSS Inc.; Chicago, IL, USA) was used for analyzing the findings of this study. For statistical analysis, the variables were expressed and used as the number (n), percentage (%), and mean ± standard deviation. The Shapiro–Wilk test, Q-Q, and histograms were used for assessment of the normality of the data. Comparisons were made by using two-way repeated measures analysis of variance. The one-way Kruskal–Wallis test was used for comparing DPOAE results between groups before and after drug administration The Bonferroni test was performed for multiple comparisons. Intra-group comparisons were utilized from Wilcoxon analysis. Values of p<0.05 were considered as statistically significant.

Evaluation of the DPOAE results
Among all the groups and intra-group, baseline DPOAE values were not markedly different. After cisplatin application, DPOAE values were attenuated. There were statistically significant differences of the DPOAE values before and after cisplatin application (p<0.05) (Table 3). Both other groups receiving cisplatin plus NAC, saline, or NAC alone, exhibited no significant change in hearing thresholds after treatment (Table 3). The cisplatin treatment markedly decreased DPOAE responses in all frequencies (Table 3). Changes in DPOAE responses in the other three groups were not significant.

DISCUSSION

Although there are other methods to detect ototoxicity, including postmortem histopathology, surveillance with otoacoustic emissions is a reliable way. A decrease in OAE produced by outer hair cells provides evidence of ototoxicity, which has been previously studied (5, 7, 17).

N-acetylcysteine decreases hydrogen peroxide and increases cellular glutathione, thus it is known to reduce cisplatin ototoxicity (19, 20). However, there is a debate about whether NAC can also decrease the antitumor effect since it is known to interact with the cisplatin molecule. To prevent this attenuation in the antitumor effect, NAC was introduced via a totally different route, i.e., transtympanic, which ensured that the two molecules did not

Table 3. Pretreatment and posttreatment Distortion Product Otoacoustic Emission (DPOAE) responses in all frequencies (Mean SNR and SD value)

	Saline		NAC		Cisplatin		Cisplatin+NAC			
	SNR	SD	SNR	SD	SNR	SD	SNR	SD	P (CN)	P (C)
Pre2000	1.56	5.09	-0.76	5.46	-1.31	4.25	6.85	3.89		
Post2000	2.13	6.82	-2.77	5.88	-4.90	5.29	6.33	4.33	1.00	0.03*
Pre3000	7.92	1.91	5.10	5.50	6.28	0.86	12.55	5.32		
Post3000	5.32	3.14	5.69	3.86	4.58	2.36	11.71	5.86	0.75	0.00*
Pre4000	6.63	5.25	6.65	5.23	4.00	5.52	18.28	9.00		
Post4000	5.66	4.94	3.87	8.64	0.93	4.02	15.96	6.75	0.58	0.04*
Pre6000	10.53	8.78	6.14	12.08	2.73	7.23	21.91	11.67		
Post6000	5.36	2.23	7.23	9.60	-3.74	7.16	18.16	11.36	0.15	0.01*
Pre8000	11.37	10.30	14.06	14.69	2.66	5.20	25.69	16.22		
Post8000	8.91	7.74	4.56	11.56	-4.53	8.38	17.95	19.32	0.05	0.01*

Cisplatin treatment markedly decreased distortion product otoacoustic emission (DPOAE) responses in all frequencies. DPOAE changes in other three groups were not marked.
NAC: n-acetylcysteine; C: cisplatin; CN: cisplatin + NAC; SNR: signal-to-noise ratio; SD: satandard deviation
*Posttreatment statistically significant.

interact (21). The transtympanic introduction of different protective molecules, *e.g.*, sodium thiosulfate, was also tried by others (22). To solve the interaction problem, Muldoon et al. (23) introduced NAC 4 h after chemotherapy, and claimed that NAC chemoprotection did not alter cisplatin therapy if delayed until 4 h after chemotherapy. They also claimed that this kind of protocol prevents ototoxicity. To test this in our current study, we also introduced the protective NAC 4h after cisplatin injection, which turned out to reduce ototoxic effect cisplatin. While otoacoustic emissions were decreased in the cisplatin group; NAC given 4 h after cisplatin did not alter OAE. We were able to clearly detect ototoxicity with these measurements *in vivo.* The cisplatin group revealed the worst results in the OAE tests. OAE were not altered in the group that received NAC, meaning NAC did not affect hearing by itself. Low et al. (18) evaluated NAC administered 72 h after radiation and claimed to see less oxygen radicals in the inner ear, which resulted in less apoptosis cochlea. This is similar to the mentality that we tested in the current study. This paper reveals the preliminary OAE results of a broader study investigating evoke response audiometry and histopathologic findings, which are being processed at the moment.

CONCLUSION

N-acetylcysteine prevented the attenuation of OAE caused by cisplatin. Other similar studies are also necessary to support the delayed introduction of the protective agent.

Ethics Committee Approval: Ethics committee approval was received for this study from Erciyes University Ethics Committee on Animal Research.

Informed Consent: N/A.

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Authors' Contributions: Conceived and designed the experiments or case: MAS, CY, IG, MG. Performed the experiments or case: MAS, CY, IG. Analyzed the data: MAS, DA, SÜ. Wrote the paper: MAS, DA. All authors have read and approved the final manuscript.

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