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The protective effect of dexamethasone and lactate against cisplatin-induced ototoxicity*

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Aim: To compare the protective effect of dexamethasone and lactate against cisplatin-induced ototoxicity.

Materials and methods: Thirty Wistar rats were randomly divided into 4 groups. After the rats were sedated with intraperitoneal (IP) ketamine hydrochloride (50 mg/kg) and xylazine (7.5 mg/kg), baseline ABRs (auditory brainstem evoked responses) were measured in response to clicks and tone pips of 4, 8, 12, and 16 kHz. After auditory thresholds were determined, the animals received drug administration as follows: Group 1 (n: 6) received intratympanic (IT) saline (0.9% NaCl) solution, Group 2 (n: 8) IP cisplatin (20 mg/kg) alone, Group 3 (n: 8) IT dexamethasone (0.1-0.3 mL), and Group 4 (n: 8) IT lactated Ringer's (LR) solution (0.1-0.3 mL) followed after 30 min by 20 mg/kg cisplatin. Dexamethasone, LR solution, and saline application were continued for 3 days. At the end of the study, ABR testing was performed and threshold changes were recorded.

Results: Group 2 animals showed marked hearing loss with average threshold shifts of 39,6 dB for clicks, 7.2 dB at 4 kHz, 8.4 dB at 8 kHz, 71.1 dB at 12 kHz and 71.8 dB at 16 kHz. No significant loss was observed in Group 3 with average threshold shifts of 1.6 dB, 4.7 dB, 8.7 dB, and 4.2 dB for clicks and tone pips at 4, 8, 12, and 16 kHz, respectively. Similar findings were observed in Group 4 with shifts of 3.5 dB, 6.8 dB, 11.3 dB, and 15.2 dB for clicks and tone pips at 4, 8, 12, and 16 kHz, respectively. Significant protection was seen in Group 3 and 4 animals compared with Group 2 animals. There was no side effect in IT administration of LR solution and dexamethasone for hearing functions. Both of these appear to be easier and safer to apply and have a usable protective effect against cisplatin ototoxicity.

Conclusion: IT administration of LR solution and dexamethasone appear to be easy and safe to apply and have a useful protective effect. Clinical applications including these agents could be considered for use in order to reduce the side effects of ototoxic chemotherapy protocols.

Key words: Cisplatin, ototoxicity, dexamethasone, lactated ringer's solution, otoprotection, rat, intratympanic route

Sisplatin ototoksisitesinde dekzametazon ve laktat'ın koruyucu etkinliği

Amaç: Sisplatin ototoksisitesinde intratimpanik deksametazon ve laktatın koruyucu etkinlikleri karşılaştırıldı.

Yöntem ve gereç: Otuz Wistar cinsi sıçan rastgele dört guruba ayrıldı. Sıçanlar intraperitonal ketamine hydrochloride (50 mg/kg) ve xylazine (7.5 mg/kg) ile sedatize edildikten sonra, Bütün hayvanların uygulamalar öncesinde ABR (Auditory brainstem response) ile 4, 8, 12 ve 16 kHz'de klik ve "tone-pips" uyaranlarla bazal işitme eşikleri saptandı. Oditör eşikler saptandıktan sonra, sıçanlara aşağıdaki sırayla ilaçlar uygulandı: 1. Grup (n: 6) intratimpanik % 0,9 salin

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(NaCl) solusyonu; 2. Grup (n: 8), yalnızca intraperitoneal sisplatin (20 mg/kg); 3. Grup (n: 8) intraperitoneal sisplatin ile birlikte intratimpanik dekzametazon (0.1-0.3 mL) ve 4. Grup (n: 8) 20 mg/kg sisplatin uygulamasından 30 dakika sonra intratimpanik Ringer laktat (RL) solusyonu (0.1-0.3 mL) uygulandı Dekzametazon, RL solusyonu ve % 0,9 salin uygulamasına üç gün boyunca devam edildi. Çalışmanın sonunda, ABR testi uygulandı ve eşik değişimleri ölçüldü.

Bulgular: 2. Grup taki sıçanlar uygulama öncesi ve sonrası işitme eşikleri arası ortalama fark 39,6 dB olup 4 kHz de 7,2 dB, 8 kHz de 8,4 dB,12 kHz de 71,1 dB ve 16 kHz de 71,8 dB lik eşik farkları saptandı. 3. grupta belirgin işitme kaybı gözlenmedi. Klik ve "tone-pips" uyaranlara karşı işitme eşikleri arası ortalama fark 4, 8, 12 ve 16 kHz frekansları için sırasıyla 1,60 dB, 4,75 dB, 8,70 dB, ve 4,26 dB olarak ölçüldü. Benzer bulgular 4. gruptada saptandı. Bu grupta klik ve "tone-pips" uyaranlara karşı işitme eşikleri arası ortalama fark 4, 8, 12 ve 16 kHz frekansları için sırasıyla 3,56 dB, 6,87 dB, 11,34 dB, ve 15,29 dB olarak ölçüldü. 2. gruba kıyasla 3. ve 4. grup sıçanlarda belirgin olarak işitmenin korunduğu saptandı. Ayrıca işitme fonksiyonları üzerine intratimpanik RL ve dekzametazonun herhangi bir yan etkisinin olmadığı ve bu iki ajanın sisplatin ototoksisitesinde kolay uygulanabilir, güvenli ve koruyucu olduklarını söyleyebiliriz.

Sonuç: İntratimpanik RL solusyonu ve dekzametazon kolay uygulanabilen ve güvenli ajanlardır. kemoterapi protokollerinin ototoksik yan etkilerini azaltmak için klinik uygulamalara bu ajanların dahil edilmesi uygun olacaktır.

Anahtar sözcükler: Cisplatin, ototoksisite, dekzametazon, ringer laktat solusyonu, sıçan, intratimpanik yöntem

Introduction

Cisplatin is an effective antineoplastic agent widely used in medullablastoma, neuroblastoma, osteosarcoma, and various cancer treatments (testicular, ovarian, cervix, bladder, lung, and brain) (1). However, there are various side effects related to non-specific cytotoxic influences. A common dose side effect is ototoxicity (2). Cisplatin ototoxicity in adults and children may lead to hearing loss starting as sensorineural tinnitus. Hearing loss begins at high frequencies and then progresses towards lower frequencies, which are crucial for hearing speech (2-4). The resultant hearing loss depends on the dose. Hearing loss may be cumulative, bilateral, and permanent. Some 60% to 80% of patients experience some hearing loss and approximately 15% experience permanent hearing loss (5,6). Cisplatin provides development reactive oxygen species (ROT) as superoxide anions at a molecular level. Glutathione and antioxidant enzymes are released as ROT rise. In this case, superoxide hydrogen peroxide and toxic lipids cause apoptosis with calcium entrance to cochlear cells (7-9). Many experimental studies have been done to find the most suitable otoprotective agent mostly as an antioxidant supplement against ROT at early stages of ototoxicity (10). Unfortunately, most of these agents inhibit antitumoral effects of cisplatin (1). As a result, there are no clinical agents that prevent cisplatin ototoxicity at present.

Glucocorticoids (GCs) are used in hearing loss treatment for various cochlear diseases when the etiology is not clear as in otoimmune inner ear disorder, endolymphatic hydrops, Méniere's disease, tinnitus, and sudden or idiopathic rapidly progressing hearing loss (11). It has been shown that the existence of GCs receptors in the rat's inner ear structure is effective in limiting steroid ROT development (12-14). The protective effect of lactated Ringer's (LR) solution is not known yet. However, it is thought that LR solution is effective via nicotinamide adenine dinucleotide (NADH). Lactate dehydrogenase H isoenzyme (LDH-H), which is the originator of the endogenic antioxidant NADH converts lactate into pyruvate.

It has been shown that cisplatin doses do not cause LDH inhibition in clinical or experimental studies. At the same time, perilymph concentrations of LDH and lactate are found 3 times more than those of blood or cerebrospinal fluid (15,16).

One of the aims of the experiment described herein was to identify a laboratory animal that would be ototoxically susceptible and consequently would have the potential to be used as a model in the study of early effects of cisplatin on auditory function. For this purpose, we used Wistar rats as an animal model to determine the ototoxicity of cisplatin. Cisplatin was systemically administered with 2 high doses to rats, and the ototoxic effects were evaluated. If ototoxicity occurs, it can be prevented by IT administration of dexamethasone or LR solution. In addition, this study investigated the role of auditory brainstem evoked response (ABR) as an indicator of cisplatin-induced ototoxicity and ABR thresholds were used to compare the ototoxicity in these animals.

Materials and methods

This study was approved by the Committee for Ethics in Animal Experiments of the Current Haydarpaşa Numune Training and Research Hospital with protocol number (13/2009). Thirty healthy Wistar rats (190-300 g) were housed in temperature controlled rooms with 12-h light/dark cycles. These animals were provided with free access to food and water. They were allowed to acclimatize to their cages for at least 48 h after shipment. They were sedated using an intraperitoneal (IP) solution of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 7.5 mg/kg xylazine (Rompun, Bayer, Germany). Rectal temperatures were continuously monitored while the animals were under anesthesia. The animals were also placed on a warming blanket calibrated to maintain body temperature at 35 °C. The rats were divided into 4 study groups after baseline ABR testing was performed (Table 1). All tympanic membranes were examined with an operating microscope (Zeiss, S1, Germany) before earphone placement to ensure normal middle ear appearance.

Group 1: (6 rats, 12 ears) Saline (0.9% NaCl) solution was selected as the control agent because it is the solvent in which dexamethasone was stored and administered for 3 days.

Group 2: (8 rats, 16 ears) cisplatin 20 mg/kg was given as an IP infusion (Cisplatin-teva 10 mg 1 flakon, Med-ilac, İstanbul, Turkey) for 2 days.

Group 3: (8 rats, 16 ears) cisplatin 20 mg/kg was given IP as a slow infusion and rats received 4 mg/kg IT dexamethasone (Onadron flakon, I.E. Ulagay, İstanbul, Turkey), followed after 30 min and 24 h by an IP infusion of cisplatin. This was administered under an operating microscope, slowly through a myringotomy in the anterosuperior quadrant, 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 min, the procedure was performed in the other ear.

Group 4: (8 rats, 16 ears) received intratympanic (IT) LR solution (lactate, 28 mEq/L) (approximately 0.1 to 0.3 mL) (Eczacıbaşı-Baxter, İstanbul, Turkey), followed after 30 min and 24 h by IP cisplatin.

After an observation period of 3 days, the animals were again sedated and follow-up ABR testing was performed to determine the degree of threshold change compared with baseline measures. No tympanic membrane perforations or complications were observed as a result of these procedures.

Auditory brainstem evoked responses testing

A total of 30 rats were used for ABR recording. Rats were sedated with xylazine and ketamine. Baseline ABRs were measured using the Smart EP evoked potential system (Intelligent Hearing Systems, Miami, FL, USA). Responses to 100 μs clicks and tone pips with an 8-ms plateau and a 1-ms rise fall time at 4, 8, 12, and 16 kHz were averaged using this instruments signal generating averaging system. The stimuli were presented inside a double-walled radio frequency-shielded sound booth using an Etymotic ER-2 earphone placed directly into the ear canal. Clicks and tone pips were presented at a rate of 5 times per second. Animals were presented with a stimulus intensity series, which began at 10 dB sound pressure level (SPL) and reached a maximum of 90 dB SPL. Stimulus intensity was progressively increased in 10 dB increments. Resulting ABRs were observed on a

Table 1. Distribution of rats and application numbers according to study groups.

Groups	Used procedure	Description	The rats (n)	Applications (n)
1	IT. saline solution	Negative control with IT injection	6	3
2	IP. cisplatin	Ototoxicity group	8	2
3	IP. cisplatin with IT.dexamethasone	Treatment group	8	5
4	IP. cisplatin with IT. lactate	Treatment group	8	5

video monitor. Intensities that appeared to be near threshold were repeated. Threshold was defined as the lowest intensity capable of producing a visually detectable, reproducible response. The voltage associated with threshold was 0.5 μV. Subdermal electrodes were used to record brain potentials differentially. The active electrode was positioned at the vertex and the reference electrode at the mastoid tip contralaterally. The ground electrode was located over the mastoid tip ipsilaterally. Potentials were amplified 1000 times inside the sound attenuation booth (bandwith 0.1-10 kHz), and signals were further amplified to produce an overall gain of nearly 100,000 and viewed on an oscilloscope. The ABR were sampled for 20.5 ms after stimulus onset. Stimuli were repeated 5 times per second, and a total of 512 trials were averaged using an analog to digital converting system. After an observation period of 3 days, the animals were again sedated, and underwent follow-up ABR testing to determine the degree of threshold change from baseline.

Statistical analysis

NCSS 2007 & PASS 2008 Statistical Software (Utah, USA) was used. This software suggested that a sample size of 30 animals would be sufficient for statistical significance. Considering possible unforeseen events resulting in the loss of animals during the study, we used 30 animals in 4 groups of 6, 8, 8, 8, i.e. 30 animals were randomly assigned to 4 groups, each group including 16 ears, except the control group. The data were analyzed using the Wilcoxon paired 2-sample test, Mann-Whitney U test, and Kruskal-Wallis variance analysis in SPSS 11.0 for Windows. A P value of less than or equal to 0.05 was considered significant.

Results

ABR threshold shifts for clicks were compared following drug administration; the results are shown in Table 2. No significant change in hearing was seen in animals receiving saline (Group 1) with shifts of 1.1 dB, 1.4 dB, 2.5 dB, and 3.3 dB for clicks at 4, 8, 12, and 16 kHz, respectively. There were no significant differences in the ABR thresholds ($P > 0.05$) after administration of saline. The injections had no toxic effect on cochlear emissions in any of the rats. Marked hearing loss was noted in animals receiving cisplatin (Group 2) with average threshold shifts of 39.6 dB for all frequencies, 7 dB at 4 kHz, 8 dB at 8 kHz, 7 dB at 12 kHz, and 7 dB at 16 kHz. Differences in ABR thresholds at frequencies 4 to 8 kHz were not statistically significant (4 kHz $P = 0.17$, 8 kHz $P = 0.08$). However, ABR thresholds decreased significantly at frequencies 12 to 16 kHz 3 days after IP cisplatin injection (12 kHz $P = 0.003$, 16 kHz $P = 0.003$) (Figure). A significant degree of otoprotection was observed in Group 3 animals with average threshold

Table 2. Mean hearing levels (dB) before (pre) and after (post) drug administration.

Hearing frequencies		Group 1 dB	Group 2 dB	Group 3 dB	Group 4 dB
4 kHz	Pre	12	16	23	23
	Post	13	23	24	26
8 kHz	Pre	10	13	12	21
	Post	11	22	17	28
12 kHz	Pre	31	15	34	37
	Post	33	86	43	48
16 kHz	Pre	28	16	32	32
	Post	31	88	36	48

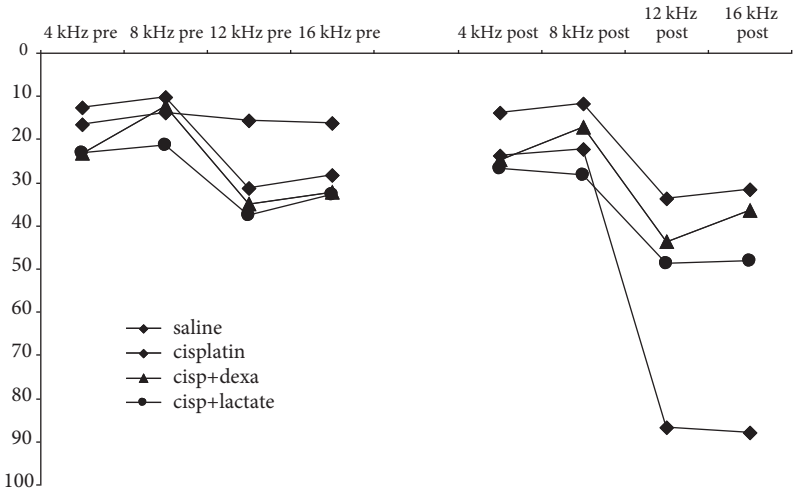

Figure. Mean hearing levels (dB) before (pre) and after (post) drug administration.

shifts of 4.8 dB for all frequencies, 1 dB for 4 kHz, 4 dB for 8 kHz, 8 dB for 12 kHz, and 4 dB for 16 kHz. Moreover, a significant degree of otoprotection_was observed in Group 4 animals with average threshold shifts of 9.2 dB for all frequencies, 3 dB for 4 kHz, 6 dB for 8 kHz, 11 dB for 12 kHz, and 15 dB for 16 kHz (Table 3). In Group 3 as well as in Group 4, there were no significant differences in ABR thresholds before and after administration of IT dexamethasone or RL solution ($P > 0.05$) suggesting that IT dexamethasone or RL solution had an otoprotective effect in subjects given 2 high doses of cisplatin ($P < 0.01$).

Discussion

Cisplatin is now the most widely used anticancer drug for a variety of human neoplasms especially for squamous cell carcinoma of the head and neck. However, it is an antineoplastic agent with an ototoxic side effect. Factors affecting the incidence of ototoxicity are application route, cumulative dose, age, dietary factors, plasma protein level, genetic factors, and cranial radiotherapy history. The ototoxic effect usually appears on the 2nd day of treatment and may continue up to 7 days after treatment (1-4).

The ototoxicity of cisplatin first appears histopathologically on the first row of cells on the curve of the cochlea then moves upwards towards the outer hair cells and damages inner hair cells together with the organ of corti, spiral ganglion, and stria vascularis (10,17-19). Nitric oxide (NO) occurrence is blamed for the ototoxicity of cisplatin, which is a result of excessive production of ROT and nitric oxide synthase (NOS) (20).

Table 3. The hearing loss average shift levels (dB) before and after drug application.

Frequencies	**Group 1**	**Group 2**	**Group 3**	**Group 4**
	dB	**dB**	**dB**	**dB**
4 kHz	1.10	7.25	1.60	3.56
8 kHz	1.45	8.44	4.75	6.87
12 kHz	2.55	71.12	8.70	11.34
16 kHz	3.35	71.83	4.26	15.29

Many studies have been conducted concerning the ototoxicity of cisplatin. Clinical studies done in the last 8-10 years have shown that L- and D-methionine, sodium thiosulfate, ebselen, and 4-methyl thiobenzoic acid are significant effective agents (21-27). These agents are used locally or systemically. Reduction of cisplatin anti-tumor activity is observed in animal studies during the course of systemic antioxidant application except for ebselen (27). Animal studies have shown that GCs reduce NO related harm on cochlear cells in ototoxicity of cisplatin and aminoglycoside ototoxicity, which are estimated to have similar pathogenesis and protective effects as well as inhibiting release of reactive nitrogen mediator (14,28). In this respect, GCs are used as IT as a current method in local inner ear treatment. Diffusion to the inner ear spreads through the round window membrane. In this manner, GCs can provide a higher concentration in the inner ear compared to other oral or parenteral routes. It was found that an IT injection of methylprednisolone produced perilymph concentrations that were 33-fold higher and plasma concentrations that were 136-fold lower than the respective concentrations from parenteral dosing (29). Separately local administration prevents systemic absorption, avoiding the common systemic side effects of steroids including hyperglycemia, peptic ulcers, hypertension, osteoporosis, and more problematic reduced efficacy of chemotherapeutic agents (11,14).

GCs have been used to safely and widely treat other inner ear disorders such as sudden sensorineural hearing loss and Méniere's disease for several years (11). Daldal (28) and Hill et al. (30) demonstrated the protective effect of IT dexamethasone against cisplatin-induced ototoxicity in guinea pigs and IT dexamethasone had no ototoxic or systemic side effects on DPOAE measurements. The present study suggests that dexamethasone does not have a side effect on cochlear function, and the findings concur with those of recent studies.

We hypothesize that IT dexamethasone may also have a place for preventing cisplatin ototoxicity. We used local application to exclude possible adverse effects of systemic application and to achieve a higher concentration of dexamethasone in the cochlear fluid more rapidly. Only 0.1 to 0.3 mL was enough to cover the round window, although sampling from the inner ear for the measurement of the diffused drug concentration was not done. In the present study, there were no significant differences in the pre- and post-drug injection measurements of ABR threshold shifts to the click in Group 3.

Even though lactate's protective action is not clearly explained yet, current evidence shows that cisplatin and lactate (in the form of LR solution) may have important effects on outer hair cell metabolism, both revolving around the depletion and repletion of intracellular NADH, which is an endogenous antioxidant. Of the other components of LR solution, lactate is the most likely to provide the protective role. All of the other components have been found to have either equivocal or potentiating effects on cisplatin ototoxicity (15). In a study of protectivity of LR solution and N-acetlysystein, these 2 substances are given by IT method to guinea pigs simultaneously after creating cisplatin ototoxicity. The N-acetlysystein applied group demonstrated mid-grade improvement in their hearing level but the LR solution applied group demonstrated nearly full improvement. This study explains that the reason for lactate's otoprotective effect being higher is the lower molecular weight of lactate compared with N-acetlysystein, allowing it to pass through the round window membrane more easily (15). We also found that there were no statistically significant differences in hearing threshold shifts after administration of cisplatin in the LR solution applied group. In the present study, there were no significant differences in the pre- and post-drug injection measurements of ABR threshold shifts for the click in Group 3, but we observed that the dexamethasone applied group demonstrated more improvement in their hearing level compared to the LR solution applied group ($P > 0.05$).

In the present study no differences were found in the average ABR thresholds for Groups 1, 3, and 4 before and after application. However, significant differences were observed especially at 12 and 16 kHz for Group 2, which indicates the side effect of cisplatin's ototoxicity. The present study demonstrated a smaller hearing loss after application of cisplatin for Groups 3 and 4 related to dexamethasone and LR solution having a protective effect over cisplatin's ototoxicity. Our findings support a few articles in the

literature written about this issue. Moreover, we used ABR testing, which measures threshold, as opposed to using otoacoustic emissions.

In our study we used dexamethasone and LR solution because there was no research comparing the effectiveness of these 2 agents in relation to cisplatin's ototoxicity. There have been concerns about IT treatment in recent years. Knowledge gained from this kind of experimental application with several agents could be readily transferred into clinical practice to increase the safety of cancer treatments in the future.

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