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ARAŞTIRMA / RESEARCH

Efficacy of adrenomedullin on cisplatin ototoxicity in rats

Ratlarda cisplatin ototoksisitesinde adrenomedüllinin etkinliği

Nagihan Bilal[1], Ergül Belge Kurutaş[2], Abdulkadir Yasir Bahar[3], Bora Bilal[4], Selman Sarıca[1], İsrafil Orhan[1], Adem Doğaner[5], Meltem Güngör[6]

[1]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Otorhinolaryngology, [2]Department of Biochemistry, Faculty of Medicine, [3]Department of Pathology, [4]Department of Anesthesia and reanimation, [5]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey,
[6]Toros University, School of health services, Mersin, Turkey

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Abstract

Purpose: The aim of this study was to evaluate the efficacy of adrenomedullin on damage associated with cisplatin ototoxicity created in an experimental animal model.

Materials and Methods: A total of 32 Wistar Albino rats were separated into 4 groups of 8. To the rats in Group 1 (control), 1% acetic acid was administered intraperitoneally and intratympanically, to Group 2, cisplatin (10mg/kg/2 days) + intratympanic acetic acid, to Group 3, cisplatin + intraperitoneal adrenomedullin (12 µg/kg) and to Group 4, cisplatin +0.1- 0.3ml intratympanic adrenomedullin (12 µg/kg). The drugs were administered on the first 5 days and signal-to-noise ratio (SNR) measurements of the auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were made on days 0, 5 and 15. The rats were sacrificed and serum and ear tissue samples were taken for histopathological examination and examination of oxidative stress levels.

Results: A statistically significant difference was determined in the cisplatin+intratympanic adrenomedullin group in the Auditory brain-stem response (ABR) measurements on days 0, 5 and 15 . In the Distortion product-evoked otoacoustic emission (DPOAE) measurements, the between-day differences at 16000 frequency in the cisplatin + intraperitoneal adrenomedullin group were accepted as statistically significant

Conclusion: Adrenomedullin, which is a strong vasodilator and antioxidant, was found to have a protective effect against cisplatin ototoxicity in the cochlea.

Keywords: Adrenomedullin, cisplatin, cochlea, intratympanic, ototoxicity

Öz

Amaç: Bu çalışmanın amacı daha önce hiç çalışılmamış olan adrenomedüllinin deneysel hayvan modelinde oluşturulan cisplatin ototoksisitesine bağlı hasarı üzerindeki etkinliğini değerlendirmektir.

Gereç ve Yöntem: 32 wistar albino rat 4 gruba ayrıldı. Grup 1 kontrol grubu intraperitoneal ve intratimpanik %1 asedik asit verilen, grup 2 cisplatin (10 mg/kg/ 2 gün)+ intratimpanik asedik asit verilen, grup 3 cisplatin + intraperitoneal adrenomedüllin (12mikrogr/kg) verilen, grup 4 cisplatin + 0,1-0,3 ml intratimpanik (12mikrogr/kg) adrenomedüllin verilen grup olarak her bir grupta rastgele 8 rat olacak şekilde düzenlendi. İlk 5 gün ilaç uygulamaları yapıldı. ABR ve DPOAE deki sinyal gürültü oranları (SNR) 0. 5. ve 15. gün ölçümleri yapıldı. Ratlar sakrifiye edildi, histopatolojik inceleme, serum ve kulak doku örneklerinde oksidatif stres düzeyleri çalışıldı.

Bulgular: İşitsel uyarılmış beyinsapı potansiyelleri (ABR) ölçümlerinde C+IT ADM grubunun 0. gün 5. gün ve 15. gün değerleri arasındaki farklılık istatistiksel olarak anlamlı olarak değerlendirilmiştir. Distorsiyon ürünü otoakustik emisyon (DPOAE) ölçümlerine göre 16000 hz frekanstaki cisplatin + intraperitoneal adrenomedüllin grubundaki günler arasındaki farklılık istatistiksel olarak anlamlı olarak kabul edilmiştir

Sonuç: Güçlü bir vazodilatattör ve antioksidan olan adrenomedüllinin cisplatin ototoksisistesinde kokleayı koruyucu etkisinin olduğu bulundu.

Anahtar kelimeler: Adrenomedüllin, cisplatin, kohlea, intratimpanik, ototoksisite

Yazışma Adresi/Address for Correspondence: Dr. Nagihan Bilal, Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Otorhinolaryngology, Kahramanmaras, Turkey E-mail: nagihanyazan@gmail.com
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INTRODUCTION

Cisplatin is an anti-neoplastic agent widely used as an inhibitor of the growth of certain types of malignant neoplasm. However, in addition to the antineoplastic effect of cisplatin, as there are side effects such as nephrotoxicity, neurotoxicity and ototoxicity, clinical use is limited[1].

The ototoxic effect of cisplatin is characterised by tinnitus and irreversible, bilateral, progressive sensorineural hearing loss at high frequencies. The incidence of this ototoxicity is affected by factors such as the route of administration, age, diet factors, genetic factors, serum protein levels and cranial radiotherapy history[2]. Cisplatin ototoxicity is dose-related and cumulative. Generally, ototoxicity occurs less in low-dose, long-term treatments. Cisplatin affects the outer hair cells in the organ of Corti, spiral ganglion cells and stria vascularis cells in the basal cochlea[3].

Adrenomedullin (ADM) is a long-term effective vasoactive peptide, originating from human pheochromocytomas[4]. Tyrosine amino acid, which is found in the carboxy terminal of human adrenomedullin is a molecule in the peptide structure formed from the 52 amino acids contained in the disulphide connections between the 16 th and the 21st carbons. As ADM has shown homology with peptide (CGRP) related to the calcitonin gene, the calcitonin/CGRP/amylin peptide family is affected[5]. ADM, which is produced extremely quickly, is not stored and is expressed as soon as it is synthesised. However, it has been suggested that it may be stored in the pancreas endocrine cells and in adrenal medulla cell granules[6].

ADM is a member of the CGRP family, but is not only synthesised in neural tissue like CGRP, but is widely found in several tissues. ADM immunoreactivity has been determined in cardiac myocytes, vascular smooth muscle cells, endothelial cells, renal distal and collecting tubercles, in the epithelium of the respiratory and reproductive system with mucosal and glandular epethelium in the digestive system, in the supraoptic nucleus in the hypothalamus and in the magnocellular area of the paraventricular nucleus, in the blood, in the urine, in the cerebrospinal fluid and in amniotic fluid [7].

The aim of this study in which cisplatin ototoxicity was created in an experimental animal model, was to evaluate the efficacy of adrenomedullin, which is known to be a strong vasodilator and has recently been shown to have antioxidant activity in ischaemic reperfusion injury in some tissues.

MATERIALS AND METHODS

Approval for the study was granted by the Animal Care and Use Committee. The animal care in this study was based on the criteria of the Ethics Review Committee for Animal Experimentation and NIH Guidelines for the Care and Use of Laboratory Animals (approval number: 08 dated: 01.12.2015).

The study included 32 adult rats, aged 3-3.5 weeks. The rats were randomly separated into 4 groups of 8. Before the treatment, the basal hearing thresholds were determined in all the rats with auditory brainstem response (ABR) and any animals with hearing loss were excluded from the study. The animals were kept in an evironment of 20°-22°C temperature, a 12-hour light/dark cycle and humidity of 55%-65%. The rats were kept in cages in groups of 4 and given free access to drinking water and prepared pellet food. The cages were cleaned once a day. Due to fluid loss in the groups administered with cisplatin, additional intraperitoneal saline was given.

Group 1 (n:8): for 4 days, only 1 ml 1% acetic acid (glacial = HOAc) and intratympanic 0.1-0.3 1% acetic acid solution was given (because adrenomedullin is dissolved in 1% acetic acid) and this group was defined as the control group.

Group 2 (IP C) (n:8): to be able to create ototoxicity, intraperitoneal (ip) cisplatin (Onko, Kocsel 50mg/100 ml flacon) was administered for 2 days at doses of 10 mg/kg to reach a cumulative dose of 20 mg/kg + intratympanic 0.1-0.3 1% acetic acid administered for 4 days.

Group 3 (CP+IP ADM) (n.8): to be able to create ototoxicity, ip cisplatin was administered for 2 days at doses of 10 mg/kg to reach a cumulative dose of 20 mg/kg and for 4 days, an infusion was administered for 30 minutes of 12 μgr/kg adrenomedullin (adrenomedullin 11-50 rat 0.5mg Sigma-Aldrich Chemistry Ltd. co. USA).

Group 4 (CP+IT ADM) (n:8): to be able to create ototoxicity, ip cisplatin was administered for 2 days at doses of 10 mg/kg to reach a cumulative dose of 20 mg/kg and for 4 days, intratympanic 0.1-0.3 adrenomedullin (12 μgr/kg) was administered.

The intratympanic injection was made with a 28

gauge dental injector into the tympanic membrane anterosuperior quadrant in a manner to completely fill the middle ear with solution. Intratympanic injections were made to both ears in all the groups. Both the intratympanic and intraperitoneal applications of adrenomedullin were made 30 minutes before the administration of cisplatin.

Anaesthesia

Before the Auditory brain-stem response (ABR) and Distortion product-evoked otoacoustic emission (DPOAE) measurements on days 0, 5 and 15, the rats were applied with an intramuscular cocktail of 40 mg/kg ketamine hydrochloride (Ketalar® 50mg/ml flacon Pfizer Drugs Ltd.co., Istanbul, Turkey) and 5 mg/kg xylazine (Alfazyne® 2%, 20 mg/ml, Alfasan). The depth of anaesthesia was determined with the pedal reflex. To maintain anesthesia, a half-dose of the initial cocktail was administered when necessary. To prevent dryness of the eyes during anaesthesia, artificial eyedrops were applied to the eyes of the rats.

Auditory brain-stem response (ABR) and Distortion product-evoked otoacoustic emission (DPOAE) testing

Before the treatment, the basal hearing thresholds were determined in all the rats with auditory brainstem response (ABR) and any animals with hearing loss were excluded from the study. On the 5th day of the study, 1 day after having waited for the final dose of intratympanic adrenomedullin to have disappeared, anaesthesia was applied and the ABR and DPOAE measurements were taken. To keep the body temperature of the animals at 37.5°C, the measurements were taken on a hypothermic blanket. The ABR responses were recorded with a 3 platin-irridium needle electrode placed on the sub-dermal vertex (positive), the mastoid (negative) and the dorsum (reference/base) regions.

In a double-walled room, sound stimuli were applied comprising a spectral peak of 3-4 kHz click sounds (rate 21.1/s, duration 100 µsec) made through an evoked potential system (Universal Smart Box, Intelligent Hearing System, Miami, FL, USA). The stimuli were delivered through a tube which connected an earphone (ER-2 Insert Earphone) to the external auditory canal, and the average of 1000 stimulus presentations was used to develop the response curve. ABRs were first recorded at 70 dB sound pressure level, and stimulus intensity was reduced in 10 dB steps until there was no longer any identifiable response. The stimulus was then increased in 5 dB steps until a reliable and replicable peak 5 in ABR waveforms was present. (Figure 1).

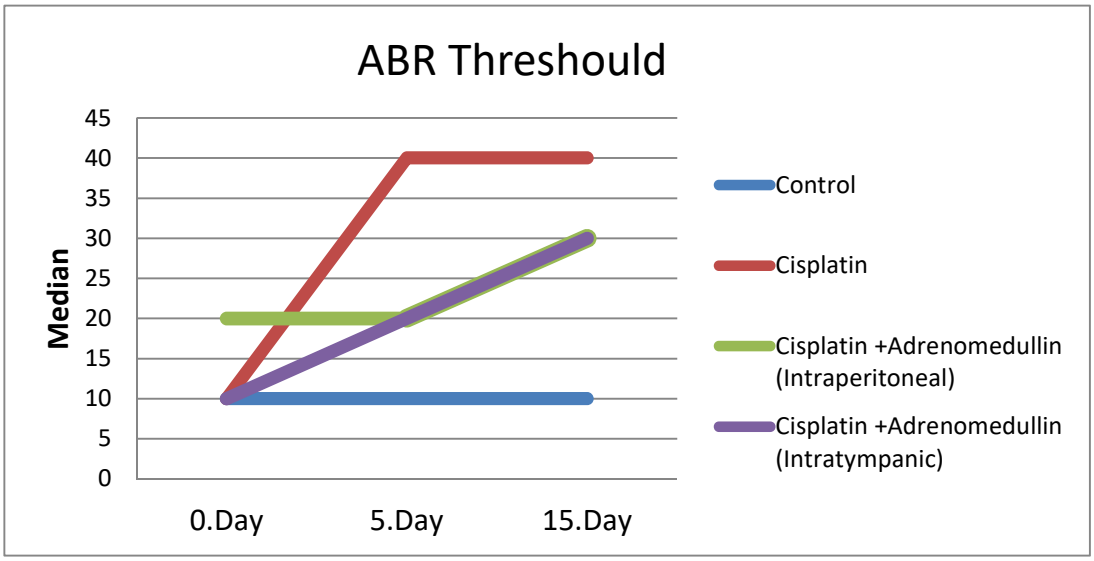

Figure 1. ABR measurements of the rats on days 0, 5 and 15

The DPOAE measurements were taken in a silent room with sound presssure level <45 dB. Using a plastic adapter to keep the probe in the external ear canal, the primary tones were introduced into the animal's external ear canal through an insert earphone (high frequency transducers smart DPOAE). These stimulating 2 pure tones (F1 and F2; F1/F2 ratio = 1.22) were at 65 decibel sound pressure level (dB SPL). The otoacoustic emission results were evaluated as 4000, 8000, 12000, 16000, 24000, and 32000 Hz. The signal/noise ratio (SNR) was measured at these frequencies.

On the 15th day, the same anaesthesia protocol was applied and the final DPOAE and ABR measurements were taken. Blood samples were taken to examine the oxidative stress levels (malondialdehyde [MDA], superoxide dismutase [SOD] and catalase [CAT]). The animals were then euthanised with 60mg/kg phenobarbitol applied intraperitoneally and after decapitation, the cochlea were dissected.

Histopathological examination

The right cochlea of all the animals in all groups were evaluated under light microscope. After dissection of the cochlea, using a 1ml injector, a window was opened 13mm in length and 4.5mm in width in the cochlear apex. Using the same injector, fixation was made with 10% formaldehyde via oval and round windows. The dissected cochlea were left in 10% formaldehyde at room temperature for 24 hours. After fixation, decalcification was applied by treating with 10% EDTA for 7 days at room temperature. The specimens were left in water for 24 hours and dried in increasing concentrations of alcohol (70%, 80%, 90% and 100%), then embedded in paraffin blocks. A series of slices 5µm in thickness were cut longitudinally passing parallel to the modiolus. The slices were stained with eosin and examined under a light microscope with a x40 lens (Olympus BX53F, Japan) at x400 magnification and digital images were taken. Cisplatin-origin ototoxicity was evaluated with the 4-point scoring system defined by Freitas et al [8].

The organ of Corti was evaluated according to the number of outer hair cells (OHC). The number of OHC expected for each section was compared with the control animals. Two basal, one mid and one apical turn midmodiolar sections were evaluated; 15 OHCs were counted (basal turn: 6 cells, middle turn: 6 cells, apical turn: 3 cells). According to the subjective evaluation of stria vascularis, marginal cell blebbing, cytoplasmc vascularisation, intermediate cell atrophy (shrinkage), categorisation was as follows: normal thickness of the stria vascularis and no marginal cell blebbing, cytoplasmic vacuolization, or shrinkage was evaluated as 0, slight as 1, mild, as 2, moderate as 3, and severe as 4. Subjective evaluation was also made in the categories of no change = 0, mild =1, moderate = 2 and severe = 3 for the degree of change seen in spiral ganglion cells, vacuolization, and nuclear degeneration.

Preparation of homogenate

Ear tissues were removed, weighed, blotted on filter paper, and homogenized with three volumes of ice-cold 1.15 % KCI. Activities of antioxidant enzymes (catalase and superoxide dismutase) and the levels of mda as an indicator of oxidative stress were measured in the supernatant obtained following centrifugation at 14.000 rpm.

Biochemical analysis

Using the Fridovich method, Superoxide Dismutase (SOD) activity was measured in the tissue samples [9]. In this method, xanthine and xanthineoxidase are applied to generate superoxide radicals which react with p-iodonitrotetrazlium violet (INT) to form a red formazan dye which was measured at 505 nm. The assay medium was formed of 0.01 M phosphate buffer, CAPS (3-cyclohexilamino-1-propanesulfonicacid) buffersolution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH 10.2, a solution of substrate (0.05 m Mxanthine, 0.025 mM INT) and 80 UL xanthineoxidase. SOD activity was evaluated and stated as U/mg protein.

To determine Catalase (CAT) activity, the decrease in hydrogen peroxide concentration at 230 nm was measured using the Beutler method [10]. The assay medium was formed of 1 M Tris HCI, 5 mM Na_2 EDTA buffer solution (pH 8.0), 10 mM H_2O_2 and the tissue sample in a final volume of 1.0 ml. CAT activity was determined and stated as U/mg protein.

The TBA test was applied to measure the Malondialdehyde (MDA) level in the tissue samples [11]. The reaction mixture contained 0.1 ml sample, 0.2 ml of 8.1 % sodiumdodecylsulphate (SDS), 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % aqueous solution of TBA. The pH of the mixture was adjusted to 3.5 and the volume was finally brought up to 4.0 ml with distilled water and 5.0 ml of the mixture of nbutanolandpyridine (15:1, v/v) wasthen added.

After vigorous shaking of the mixture, centrifugation was applied at 4000 rpm for 10 min, and the absorbance of the organic layer was measured at 532 nm.

Statistical analysis

Statistical analyses were made using SPSS vn 22 software (IBM SPSS for Windows version 22). The values were examined in the Control, Cisplatin, Cisplatin +Intraperitoneal Adrenomedullin and Cisplatin +Intratympanic Adrenomedullin groups. Conformity to normal distribution of the data was examined with the Shapiro Wilk test. The homogeneity of the data as assessed with the Levene test. In the comparison of variables with normal distribution, Univariate, One Way ANOVA and the Tukey and Dunnett paired comparison tests were used. In the comparison of groups not showing normal distribution, the Kruskal Wallis test and the MannWhitney U-test were used. Statistical paramters were stated as mean±standard deviation (SD), and data not showing normal distribution as median (Q1-Q3). A value of $p<0.05$ was accepted as statistically significant.

RESULTS

The mean weight of the rats in the study was calculated as 225.3±19.8 gr (range, 193-263 gr).

ABR measurement results

The evaluation of the ABR measurements of the groups is given in Table 1. In the C+IT ADM group, the difference between the values on days 0, 5 and 15 was determined to be statistically significant ($p=0.001$) (Figure 2). The difference between the groups on Day 5 and on Day 15 was determined to be statistically significant ($p<0.001$, $p<0.001$).

DPOAE measurement results

The DPOAE results at 4000, 8000, 16000, 24000 and 32 000 Hz frequencies are shown in Table 2. According to the results at 16000 Hz frequency, the difference between the days was statistically significant in the C+IP ADM group ($p=0.003$). No statistical significance was determined in any of the other results.

Table 1. Evaluation of the ABR measurements according to the groups

Group	ABR Threshould			
	0.Day	5.Day	15.Day	
	Median (Q1-Q3)	Median(Q1-Q3)	Median(Q1-Q3)	p
Control	10.00(10.00-10.00)	10.00(10.00-20.00)	10.00(10.00-20.00)	0.240
Cisplatin	10.00(10.00-20.00)	40.00(30.00-45.00)	40.00(35.00-55.00)	0.001*
Cisplatin +Adrenomedullin (intraperitoneal)	20.00(10.00-20.00)	20.00(20.00-30.00)	30.00(20.00-40.00)	0.002*
Cisplatin +Adrenomedullin (İntratympanic)	10.00(10.00-20.00)	20.00(20.00-50.00)	30.00(20.00-50.00)	0.001*
p	0.198	p<0.001*	p<0.001*	

Kruskal Wallis H test;Mann-Whitney U test;α:0,05;*Difference is statisticallysignificant

Histopathological results

According to the Freitas 4-point scoring system used in the study, there was a statistically significant difference between the 4 groups in respect of the evaluation of the stria vascularis ($p<0.001$).The C+IT ADM group was evaluated as mean grade 1 and this was observed to be close to the value of the control group (Table 3) (Image 1).

In the evaluation of OHCs, a statistically significant difference was determined between the 4 groups (p:0.002). The C+IP ADM and C+IT ADM groups were evaluated as mean grade 1 and this was observed to be close to the value of the control group (Table 3).

In the evaluation of spiral ganglion, a statistically significant difference was determined between the 4 groups ($p=0.001$). The C+IT ADM group was evaluated as mean grade 1 and this was observed to be close to the value of the control group (Table 3) (Figure 3).

Table 2. DPOAE results at 4000, 8000, 16000, 24000 and 32 000 Hz frequencies

		0.Day	5.Day	15.Day	
		Mean±SD	Mean±SD	Mean±SD	p
4000	Control	5.75±5.94	3.56±6.00	3.60±6.21	0.512
	Cisplatin	5.00±7.39	-0.17±8.31	2.08±7.14	0.265
	Cisplatin +Adrenomedullin (intraperitoneal)	1.42±5.35	4.83±3.95	2.42±5.95	0.262
	Cisplatin +Adrenomedullin (İntratympanic)	-0.57±5.89	4.71±6.96	3.71±7.32	0.102
	p	**0.027***	0.201	0.896	
8000	Control	21.37±10.35	9.19±12.44	8.07±12.01	**0.004***
	Cisplatin	19.42±9.20	6.17±9.26	11.75±6.92	**0.002***
	Cisplatin +Adrenomedullin (intraperitoneal)	17.00±11.39	10.50±7.72	15.42±9.57	0.244
	Cisplatin +Adrenomedullin (İntratympanic)	13.71±10.67	7.43±12.23	12.43±5.96	0.225
	p	0.238	0.764	0.224	
16000	Control	4.06±14.80	.75±9.48	-.53±8.25	0.505
	Cisplatin	12.33±19.75	-0.58±6.50	2.67±3.39	**0.037***
	Cisplatin +Adrenomedullin (intraperitoneal)	8.50±14.74	.17±7.76	-0.50±9.15	0.097
	Cisplatin +Adrenomedullin (İntratympanic)	6.64±7.32	0.14±4.94	-1.93±6.68	**0.003***
	p	0.517	0.974	0.445	
24000	Control	5.56±7.54	9.31±7.12	9.07±7.29	0.282
	Cisplatin	11.08±4.48	6.92±3.78	6.25±6.61	0.055
	Cisplatin +Adrenomedullin (intraperitoneal)	5.17±10.41	4.83±6.60	2.67±9.06	0.755
	Cisplatin +Adrenomedullin (İntratympanic)	5.36±6.39	5.21±6.02	3.79±5.16	0.738
	p	0.156	0.194	0.099	
32000	Control	5.31±13.78	0.44±8.51	-0.27±8.31	0.281
	Cisplatin	4.50±7.75	-0.17±5.86	-1.17±6.41	0.104
	Cisplatin +Adrenomedullin (intraperitoneal)	2.58±7.29	1.08±4.87	2.50±6.39	0.806
	Cisplatin +Adrenomedullin (İntratympanic)	0.14±6.36	-1.86±4.72	1.71±7.41	0.329
	p	0.490	0.662	0.562	

Univariate;Post-hoc: Tukey test and Dunnett test; α:0,05;*Difference is statisticallysignificant

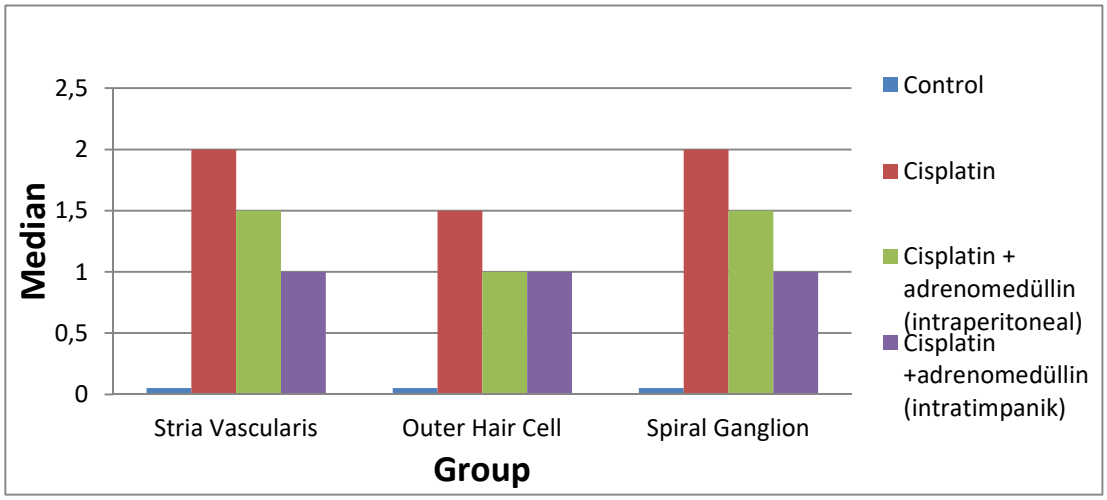

Figure 2. According to the Freitas 4 point scoring system, comparison of the histopathological results.

Biochemical results

The CAT and SOD values examined in erythrocytes and the MDA values examined in plasma are shown in Table 4.

The CAT values in the C+IP ADM and C+IT ADM groups were determined to be statistically

significantly higher than those of IP C (Cisplatin). The difference between the control group and the C+IP ADM and C+IT ADM groups was determined to be statistically significant. The difference between the control group and IP C was not statistically significant (p=0.007).

In the SOD values, a statistically significant difference was determined between the 4 groups (p<0.001). The mean SOD value was determined at the lowest level in IP C at 1086.83±376.35 U/g Hb and in the control group it was 2049.25±446.63 U/g Hb. The SOD values of the C+IT ADM group were found to be close to those of the control group.In the MDA values, a statistically significant difference was determined between the 4 groups (p<0.001). The mean MDA value was determined as 4.09±6.6 in Group 2 and 1.94±5.4 in the control group. The MDA values of the C+IT ADM group were found to be close to those of the control group (Table 4).The CAT, SOD and MDA values examined in the ear tissue are shown in Table 5.

Table 3. According to the Freitas 4-point scoring system, Comparison of Histopathological results

		Median(Q1-Q3)	p
StriaVascularis	Control	0.00(0.00-1.00)	**p<0.001***
	Cisplatin	2.00(2.00-3.00)	
	Cisplatin + Adrenomedullin (intraperitoneal)	1.50(1.00-2.00)	
	Cisplatin +Adrenomedullin (intratympanic)	1.00(1.00-2.00)	
Outer Hair Cell	Control	0.00(0.00-0.00)	**0.002***
	Cisplatin	1.50(1.00-2.00)	
	Cisplatin + Adrenomedullin (intraperitoneal)	1.0(1.00-2.00)	
	Cisplatin +Adrenomedullin (intratympanic)	1.00(1.00-1.00)	
Spiral Ganglion	Control	0.00(0.00-1.00)	**0.001***
	Cisplatin	2.00(2.00-2.00)	
	Cisplatin + Adrenomedullin (intraperitoneal)	1.50(1.00-2.00)	
	Cisplatin +Adrenomedullin (intratympanic)	1.00(1.00-2.00)	

Kruskal Wallis H test;α:0,05;* Groups differences are statistically significant.

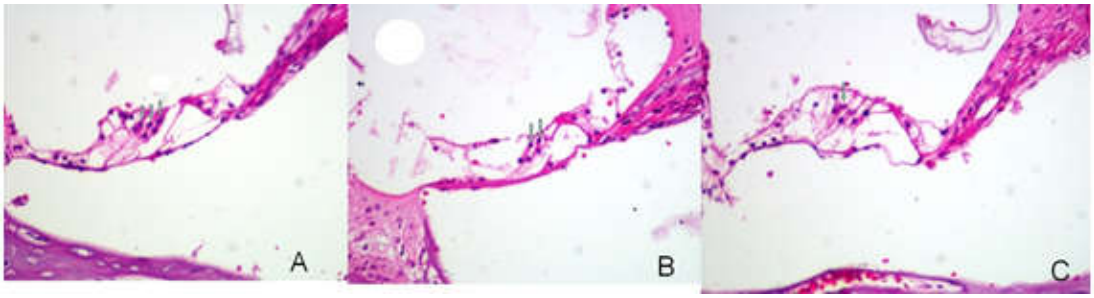

Image 1. Light microscopy image of outer hair cells (OHCs) A:(Score 0) group 1: Control group. B: (Score 1) group 4: Cisplatin +intratympanic adrenomedullin C: (Score 2) group 2: Cisplatin.

Table 4. MDA results in the plasma and CAT and SOD results in the erythrocytes

		Mean±SD	P
Catalase (U/g Hb)	Control	6.17±2.65	**0,007***
	Cisplatin	2.30±1.15	
	Cisplatin + adrenomedullin (intraperitoneal)	6.25±2.15	
	Cisplatin +adrenomedullin (intratympanic)	6.34±2.18	
SOD (U/g Hb)	Control	2049.25±446.63	**p<0,001***
	Cisplatin	1086.83±376.35	
	Cisplatin + adrenomedullin (intraperitoneal)	1276.67±298.53	
	Cisplatin +adrenomedullin (intratympanic)	1354.29±249.88	
Malondialdehyde (nmol/mL)	Control	1.94±0.54	**p<0,001***
	Cisplatin	4.09±0.66	
	Cisplatin + adrenomedullin (intraperitoneal)	3.09±0.63	
	Cisplatin +adrenomedullin (intratympanic)	2.31±0.29	

One-WayAnova; Post-Hoc: Tukey test: Dunnett test;α:0,05; *Groupsdifferencesarestatisticallysignificant

A statistically significant difference was determined between the 4 groups in respect of the CAT values ($p<0.001$). The CAT values of the C+IP ADM and C+IT ADM groups were determined to be close to those of the control group. In the SOD values in the ear tissues, a statistically significant difference was determined between the 4 groups ($p<0.001$). The values of the Cisplatin group were determined to be statistically significant compared to those of the C+IP ADM, C+IT ADM and control groups. The values of the C+IP ADM and C+IT ADM groups were observed to be close to those of the control group.

In the MDA values in the ear tissues, a statistically significant difference was determined between the 4 groups ($p<0.001$). The difference between the C+IP ADM and C+IT ADM groups and the control group was determined to be statistically significant. The MDA values of the C+IP ADM and C+IT ADM groups were observed to be close to those of the control group (Figure 4).

Table 5. Comparison of CAT, SOD and MDA values in the cochlea.

		Mean±SD	p
Catalase (U/g Hb)	Control	13.81±2.00	**p<0.001***
	Cisplatin	6.83±1.18	
	Cisplatin + adrenomedüllin (intraperitoneal)	12.17±2.54	
	Cisplatin +adrenomedüllin (intratimpanik)	13.12±2.29	
SOD (U/g Hb)	Control	11.57±2.63	**p<0.001***
	Cisplatin	7.34±1.19	
	Cisplatin + adrenomedüllin (intraperitoneal)	11.54±1.49	
	Cisplatin +adrenomedüllin (intratimpanik)	12.80±1.95	
Malondialdehyde(nmol/mL)	Control	0.13±0.03	**p<0.001***
	Cisplatin	0.38±0.10	
	Cisplatin + adrenomedüllin (intraperitoneal)	0.11±0.03	
	Cisplatin +adrenomedüllin (intratimpanik)	0.09±0.02	

One-WayAnova; Post-Hoc: Tukey test: Dunnett test;α:0,05;*Groupsdifferencesarestatisticallysignificant

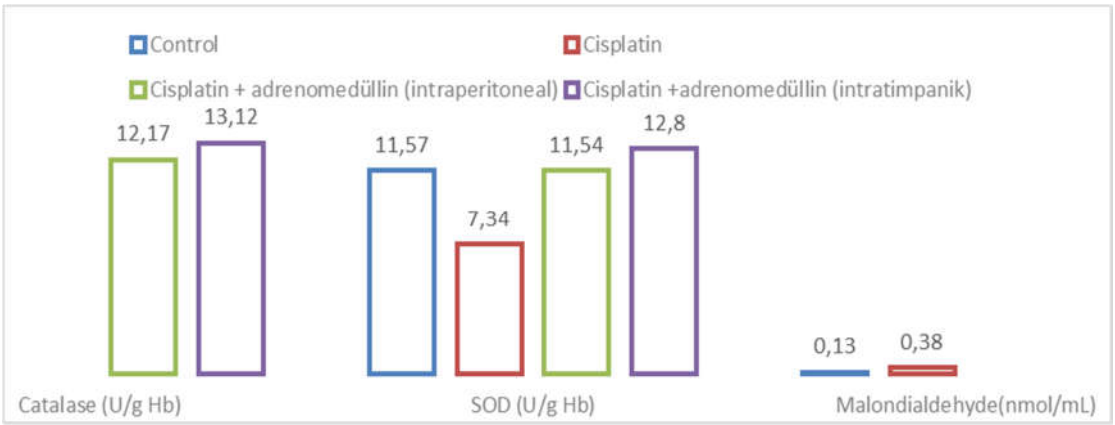

Figure 3. CAT, SOD and MDA values in the cochlea

DISCUSSION

Adrenomedullin has a role in physiological and pathological processes such as vasodilation, angiogenesis, apoptosis, and cell growth regulation and differentiation. ADM has been reported to have a protective effect against oxidative stress caused by stress factors such as hypoxia of various cells, ischaemia reperfusion, and hydrogen peroxide [12, 13]. As the effect created by the use of cisplatin does not correct endogenous antioxidants, there are concerns about the ototoxicity effect [14]. Therefore, trials have been made of antioxidant agents. In this study, the efficacy of the use of both intratympanic and intraperitoneal adrenomedullin was investigated in an experimental animal model of the ototoxic effect of cisplatin.

The results of this study showed that the ABR measurements on days 0, 5 and 15 were significant in the C+IT ADM group. In addition the differences between the groups were evaluated as statistically significant. Previous in vitro studies have shown that cisplatin causes hearing loss by affecting various areas of the cochlea. The most common symptom of this ototoxicity is the destruction of outer hair cells (OHC). The stereo cilia of the OHC are initially damaged and this is followed by loss of OHC in the apex. This damage includes atrophy of the stria vasculars and cells supporting the cochlear organ and collapse of the Reissner membrane [15, 16]. In the current study, the intratympanic application of adrenomedullin was found to have a protective effect on the cochlea. The intratympnic administration of drugs has been recently proposed and is a safe and applicable method. In practice, an increased concentration of steroids and aminoglycocytes is provided in the perilymph with intratympanic application with this method via a round window [14, 17]. In the current study, in the intratympanic application in general the results obtained were significant.

In recent studies, hearing has generally been evaluated up to 12000 Hz and significant changes have been observed in SNR and DPOAE. In the current study, measurements taken from 4000 to 32000 Hz were evaluated and a statistically significant difference was determined between the days in the SNR measurements at 16000 Hz of the C+IP ADM group. When lycopene, hesperetin and melatonin, which have antioxidant properties, have been evaluated in cisplatin ototoxicity, the SNR of DPOAE at 6000 to 10000 frequencies has been determined to be statistically significant [18, 19, 20]. In a study conducted using tetramethylpyrazine, SNR was evaluated up to 35000 Hz and it was seen to have a protective effect on the cochlea.

It has been previously determined that antioxidant enzymes increase in cisplatin-induced ototoxicity [21]. The reason for this is thought to be the production of free oxygen species in the cochlea following exposure to cisplatin [22]. The activation of caspase-3 which causes oxidative stress apoptosis causes cochlear cell death [23]. The reaction of free oxygen species with cell membrane lipids creates toxic aldehydes, which then trigger apoptotic cell death in the spiral ganglion and the organ of Corti, leading to inner ear hair cell degeneration [24]. SOD and CAT contain antioxidant enzymes which play a role in the defence system against oxygen toxicity [25]. SOD is an enzyme which forms hydrogen peroxide found in the cytoplasm, but CAT eliminates hydrogen peroxide, transforming it to water [26, 27]. In the current study, CAT was observed to be increased in both the C+IP ADM and C+IT ADM groups in both the ear tissue and erythrocytes when compared with the control group. The SOD level of the C+IP ADM and C+IT ADM groups was observed to be lower than that of the control group and higher than the value of the cisplatin group. It is thought that the lower SOD level compared to the control group could be a compensatory response to protect the cell against the damage created by oxidative stress.

MDA is an indicator of oxidative stress within the cell and is the end product of lipid peroxidase. There are studies which have shown an increase in lipid peroxidase in inflammatory diseases [28]. In the current study, an increase was observed in the MDA levels in erythrocytes in the C+IP ADM and C+IT ADM groups compared to the control group, but it was seen to be lower than that of the cisplatin group. This can be attributed to the intratympanic and intraperitoneal application of adrenomedullin having reduced the antioxidant effects of the MDA level. That the MDA levels in the ear tissue were determined to be lowest in the intratympanic adminstered group can be considered to be evidence of the protective effect of adrenomedullin against oxidative stress on the cochlea.

According to the histopathological examinations, although the results of the intratympanic administered adrenomedullin were close to those of the control group and this difference was statistically significant, the intratympanic administration of adrenomedullin showed a greater protective effect on the cochlea.

In the last 10 years, there have been more than 1200 publications related to ADM. Although primarily assumed to only be a vasodilator and natriuretic peptide, it is currently accepted as a multifunctional mediator, with various effects in embryogenesis, normal and tumoural growth, inflammation and immunity [29]. In a study of ischaemia reperfusion injury in rats, Kirişci et al. demonstrated that the use of adrenomedullin formed a protective effect to a certain degree on oxidative stress [4].

To the best of our knowledge, there has been no previous study in literature on the use of adrenomedullin on cisplatin ototoxicity. Studies are

rare which have examined high frequency DPOAE, ABR, histopathology and oxidative stress levels together.

Adrenomedullin, which is a strong vasodilator and antioxidant, was found to have a protective effect on the cochlea in cisplatin ototoxicity. That the intratympanic application of adrenomedullin was more effective was supported by the ABR and histopathological findings and the antioxidant enzyme levels.

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Bilgilendirilmiş Onam: Katılımcılardan yazılı onam alınmıştır.
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