Original Article / Çalışma - Araştırma

Preventing cisplatin induced ototoxicity by N-acetylcysteine and salicylate

Cisplatine bağlı gelişen ototoksisitenin N-asetilsistein ve salisilat ile önlenmesi

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Objectives: In this study we investigated if CP induced ototoxicity could be prevented or reduced by the use of salicylate and N-acetylcysteine.

Patients and Methods: Fifty-four patients (28 females, 26 males; mean age 37±9.5 years; range 29 to 71 years) who had cisplatin chemotherapy due to solid organ tumors were enrolled in the study. The patients were randomized into three groups, with 18 patients in each group. The first group (control group) received cisplatin, second group received N-acetylcysteine (NAC; 600 mg/day) with cisplatin and the third group received salicylate (300 mg/day) with cisplatin. All patients evaluated audiologically including high frequency audiometry and auditory brainstem response.

Results: The cisplatin-induced ototoxic damage could be reduced in 10,000 and 12,000 Hz frequencies when N-acetylcysteine was added to the cisplatin therapy protocol. There was no decrease in the hearing loss levels of the patients who were receiving cisplatin with salicylate.

Conclusion: According to auditory brainstem response testing results, there was no difference detected between N-acetylcysteine or salicylate for the amelioration of cisplatin induced ototoxicity.

Key Words: Cisplatin; N-acetylcysteine; ototoxicity; salicylate.

Amaç: Bu çalışmada salisilat ve N-asetilsistein kullanımıyla cisplatin kaynaklı ototoksisitenin azaltılabilirliği ya da engellenebilirliği araştırıldı.

Hastalar ve Yöntemler: Solid organ tümörü nedeniyle cisplatin kemoterapi kullanan 54 hasta (28 kadın, 26 erkek; ort. yaş 37±9.5; dağılım 29-71 yıl) çalışmaya dahil edildi. Hastalar her bir grupta 18 kişi olacak şekilde rastgele üç gruba ayrıldı. İlk grup (kontrol grubu) sadece cisplatin aldı, ikinci grup N-acetylcysteine (NAC; 600 mg/gün) ile birlikte cisplatin aldı ve üçüncü grup salisilat (300 mg/gün) ile birlikte cisplatin aldı. Bütün hastalar yüksek frekans odyometri ve işitsel beyin sapı yanıtını içeren odyolojik incelemeyle değerlendirildi.

Bulgular: Cisplatin tedavi protokolüne N-asetilsisteine eklenmesiyle, 10.000 ve 12.000 Hz frekanslarındaki cisplatin kaynaklı ototoksik hasarın azaldığı görüldü. Cisplatine ile salisilat alan hastaların işitme kayıbı seviyelerinde herhangi bir azalma görülmedi.

Sonuç: Çalışmamızda işitsel beyin sapı yanıtı testi sonuçlarına bakıldığında ne N-asetilsistein ne de salisilat alan hastalar arasında cisplatin ototoksisitesini azaltma yönünden anlamlı fark bulundu.

Anahtar Sözcükler: Cisplatin; N-asetilsistein; ototoksisite; salisilat.

Received / *Geliş tarihi:* September 16, 2009 Accepted / *Kabul tarihi:* March 10, 2010

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Cis-Diamminedichloroplatinum is known as cisplatin (CP) and is commonly used as chemotherapeutic agent. Cisplatin had a wide range of use in cancer therapy since 1978. The ototoxic effects of CP are described as;[1]

1- It has been thought that free radicals which are toxic to organ of Corti increase remarkably. Cisplatins metabolic products are also thought to decrease calcium levels by changing the cell membrane.[2]

2- Some changes are seen in spiral ganglion cells and there is sporadic loss of some inner hair cells and loss of outer hair cells in the apical section of the cochlea.[3,4]

3- Cisplatin, by means of decreasing the metabolic activity of the hearing pathways, causes changes of the evoked brain stem potentials and retro-cochlear sensory neural hearing loss (SNHL) depending on the dose.[5]

4- Intracellular sulfhydryl groups of mitochondria and cytosolic fractions bind CP and disrupt DNA cross-links.[6]

5- It has been shown that CP inhibits the adenylate cyclase activity in the cochlear lateral wall and stria vascularis.[7]

Incidence of CP ototoxicity differs between 9 to 91% in the literature.[8]

This situation could depend on lack of diagnostic criteria consensus between physicians, treatment duration and drug dosage. Rybak et al.[6] made a detailed investigation on CP ototoxicity incidence and they found the incidence to be 69%.

Lots of agents were found to be preventive for CP ototoxicity. Lazorids (free oxygen radical cleaners), sodium thiosulphate, phosphomycin, dietyl carbomat, lypoic acid and 4-metylbensoic asid and agents like metalloenzym inhibitors have been proposed for preventing ototoxicity both in animals and humans. Recent studies have shown that D-methionine can prevent stria vascularis damage due to CP administration in rats.[9]

High frequency audiometry is a very well investigated method for ototoxicity monitoring. This test follows the rule which declares that agents such as aminoglycosides and CP affect high frequencies first, therefore hearing loss could be detected by standard audiometry.[10]

The auditory brainstem response (ABR) could be preferred to pure tone hearing threshold for early recognition of the ototoxic changes due to CP. Investigators suggested that using ABR to determine ototoxic changes earlier and the means through which it would be possible to prevent changes in the speech frequencies. However in these studies, ABR wasn't compared to high frequency audiometry and otoacustic emission before the threshold shifts.[11] Several agents were determined as otoprotectors for the CP induced ototoxicity. The aim of our study was to determine and monitor the ototoxicity among cancer patients recieving CP by using ABR and audiometry testing as well as to explore if the ototoxicity could be reducible or preventable by salicylate and N-acetylcysteine usage.

PATIENTS AND METHODS

Our study took place between September 2005 and September 2006 in the Dicle University Faculty of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery and Department of Medical Oncology. Fifty-four patients who had solid organ tumors and used CP for the treatment were divided into three groups with 18 subjects in each group. Only CP was given to the first group (control group; 10 females, 8 males; mean age 36±10.5 years; range 29 to 66 years), N-acetylcysteine (600 mg/day) with CP was given to the second group (11 females, 7 males, mean age 34±8.5 years; range 31 to 69 years), and for the third group (7 females, 11 males, mean age 43±9.5 years; range 38 to 71 years) salicylate (300 mg/day) was given with CP. The ototoxicity reducing capabilities of salicylate and N-acetylcysteine were investigated in CP receiving subjects.

In all groups high frequency audiometry and ABR were done just before and 38 to 42 days after the CP therapy.

Madsen-made pure tone audiometry was used for measuring the patients' hearing levels. The auditory brainstem response records were done by Saphire 2A EP system in a room which is electrically and acoustically isolated.

Three silver-silver chloride disc electrodes adjusted for recording the ABR. Four-KΩ or less were applied to midline of forehead, the ipsilateral mastoid as a negative impulse maker and to the vertex as a positive impulse maker and interelectrode resistance.

Rarefaction clicks of 100-μs duration were used as acoustic stimuli. Responses to 2048 click stimuli

were recorded in each ABR sequence; recorded signals were filtered and investigated via computed band pass filter to keep them in the 100 and 2,000 Hz range. High amplitude muscular activities were eliminated for preventing the errors.

Sampling was stopped during huge amounts of muscle artifact formations on the oscilloscope monitor. A stable duration and absolute 90 dB sound pressure level (SPL) was given in repeating records and TDH39 headphones were used for comparisons. In all the patients, a 40 dB pure tone sound masking was applied to the contralateral ear to have a true answer to the click stimulations on the ipsilateral side. All the patients were tested with 10 and 50 per second clicks.

Statistics

A t-test was used for each group before and after audiometric frequency specific hearing thresholds, also I-III, I-V and III-V inter pike latency comparisons of ABR testing were done. Independent-samples t-test was used to compare parameter changes pre and post CP use in control and the other two groups.

RESULTS

Audiometric and ABR findings of the first group before and 38 to 42 days after receiving one round of CP are shown in table 1 and 2. Tables 1 and 2 were compared via t-test. There was no significant difference in ABR measurements, however in audiometric measurements there was a significant difference in high frequencies in both ears' both air and bone conductions ($p<0.005$) In other frequencies there were not any significant difference ($p>0.005$).

N-acetylcysteine was added to the CP therapy for the second group. Audiometric and ABR results of the second group before and 38 to 42 days after the first round of CP therapy are seen in tables 3 and 4. T-test was used for the comparison of the results. There were no significant differences among the ABR results, however in the audiometric results, there were significant differences among the high frequencies (10,000 Hz - 12,000 Hz) in both air and bone conductions ($p<0.005$). Other frequencies did not show any significant difference ($p>0.005$).

Salicylate was added to the CP therapy of the third group. Audiometric and ABR results of the third group before and 38 to 42 days after the first round of CP therapy are seen in tables 5 and 6. t-test was administrated for the comparison of ABR and audiometric results, but there were no significant difference among all parameters in the third group ($p>0.005$).

The changes before and after CP therapy are compared between the first and the other groups separately by independent-samples t-test (Table 7-10). There were no significant results among the ABR findings. In audiometric results, we found a significant decrease in NAC using group's both air and bone conduction levels in 10,000 Hz and 12,000 Hz frequencies compared to the first group ($p<0.005$). There was no significant difference detected between the salicylate using group and the control group ($p>0.005$).

DISCUSSION

Cisplatin is still among the most effective chemotherapeutic agents for most organ cancers, especially head and neck cancers, for both children and adults.[12] The most important side effect of CP is ototoxicity, and other known side effects include nephrotoxicity, gastrointestinal toxicity due to mucositis, bone marrow suppression and peripheral neuropathy. Cisplatin induced nephrotoxicity could be minimized by hydration and salt loading but CP induced ototoxicity is not affected by these attempts.[13]

The ototoxicity of CP is bilateral, progressive, generally irreversible, and occurs in high frequencies, most frequently 4,000 - 12,000 Hz. Functional studies have declared that CP could cause cochlear damage in both high dosages and cumulative doses. These results are validated by ABR and electrocochleographic tests that measure endocochlear potentials.[14] The hearing loss happens mostly in the higher frequencies, because the ototoxicity due to CP usually damages the basal turn of the cochlea. We also saw an obvious ototoxic effect after receiving CP in our study. When the audiometric data of pre and post CP usage compared we saw a statistically significant hearing loss especially in the high frequencies ($p<0.005$).

Ototoxicity as a common side effect reduces the quality of life and limits the therapy protocol. By ameliorating the ototoxicity and nephrotoxicity side effects of CP, it could be widely used in higher doses as a potent chemotherapeutic agent. Outer hair cells do not have regenerative ability in mammals, so otoprotection is important. Sitoprotective

Table 1. High frequency audiometry results of group 1

	dB (Hz.)	Group 1 Before cisplatin (mean±SD)	Group 1 After cisplatin (mean±SD)	t	*p*
Right ear bone conduction	10,000	68.4±7.8	82.3±8.9	4.965	0.000
	12,000	70.9±7.7	81.8±8.9	3.894	0.000
Right ear air conduction	10,000	64.4±9.1	79.8±9.0	3.794	0.001
	12,000	66.9±7.7	81.8±8.9	3.894	0.000
Left ear bone conduction	10,000	60.3±8.7	72.1±7.9	4.630	0.00
	12,000	65.0±10.0	82.9±9.0	4.379	0.00
Left ear air conduction	10,000	63.6±10.7	79.8±10.5	3.467	0.001
	12,000	64.4±9.1	79.8±9.0	3.794	0.001

dB: Decibel; SD: Standart deviation; t: t-test.

agents were used as otoprotectors: these agents were firstly NAC then sodium thiosulfate, D-methionine, vitamin E, vitamin B, erdosteine, Caffeic acid phenethyl ester, aminoguanidine, glutathione, pantoteic acid, lipoic acid, coenzyme-A, melanocyt stimulating hormone, magnesium, 4-Methylthiobenzoic acid and sodium salicylate.[15-22]

Standard audiometric methods like high frequency audiometry, summation by ABR testing which show the 1st wave changes as a mark

Table 2. Auditory brainstem response results of group 1

	dB	Latency	Group 1 (msn) Before cisplatin (mean±SD)	Group 1 (msn) After cisplatin (mean±SD)	t	*p*
Right ear 10 pps	90	I-III	2.1±0.2	2.2±0.2	1.463	0.153
		I-V	4.1±0.3	4.1±0.3	0.497	0.622
		III-V	1.9±0.2	1.9±0.3	0.775	0.444
	80	I-III	2.1±0.3	2.1±0.2	0.361	0.721
		I-V	4.1±0.4	4.1±0.4	0.176	0.861
		III-V	2.0±0.3	2.0±0.3	0.101	0.920
	70	I-III	2.0±0.4	2.1±0.4	1.436	0.160
		I-V	3.8±0.6	3.9±0.6	0.489	0.628
		III-V	2.2±0.4	2.1±0.2	0.820	0.418
Right ear 50 pps	90	I-III	2.2±0.4	2.3±0.4	0.409	0.685
		I-V	4.1±0.3	4.2±0.5	0.685	0.538
		III-V	2.0±0.2	1.9±0.4	1.124	0.269
	80	I-III	2.2±0.4	2.3±0.3	0.720	0.477
		I-V	4.2±0.4	4.2±0.4	0.135	0.894
		III-V	2.0±0.2	1.9±0.1	1.786	0.083
	70	I-III	2.3±0.3	2.4±0.3	0.935	0.357
		I-V	4.1±0.5	4.1±0.4	0.000	1.000
		III-V	1.9±0.2	1.8±0.3	1.589	0.121
Left ear 10 pps	90	I-III	2.2±0.3	2.2±0.1	0.020	0.984
		I-V	4.1±0.4	4.1±0.3	0.409	0.685
		III-V	1.9±0.2	1.9±0.3	0.284	0.778
	80	I-III	2.2±0.2	2.2±0.2	0.046	0.964
		I-V	4.2±0.2	4.1±0.3	0.783	0.439
		III-V	2.0±0.3	2.0±0.3	0.104	0.918
	70	I-III	2.2±0.4	2.2±0.3	0.091	0.928
		I-V	4.1±0.2	4.1±0.4	0.525	0.603
		III-V	2.0±0.2	1.9±0.5	0.598	0.554
Left ear 50 pps	90	I-III	2.1±0.2	2.1±0.3	0.253	0.802
		I-V	4.2±0.3	4.1±0.3	0.844	0.405
		III-V	2.0±0.3	1.9±0.4	1.161	0.254
	80	I-III	2.2±0.3	2.3±0.4	0.795	0.432
		I-V	4.1±0.3	4.2±0.3	0.652	0.519
		III-V	2.0±0.3	1.9±0.3	0.592	0.558
	70	I-III	2.1±0.3	2.2±0.4	0.887	0.381
		I-V	4.1±0.3	4.2±0.5	1.062	0.296
		III-V	2.0±0.2	2.0±0.4	0.175	0.862

dB: Decibel; SD: Standart deviation; t: t-test.

Table 3. High frequency audiometry results of group 2

	dB (Hz.)	Group 2 Before cisplatin + NAC (mean±SD)	Group 2 After cisplatin + NAC (mean±SD)	t	*p*
Right ear bone conduction	10,000	65.3±9.1	71.8±9.0	3.794	0.001
	12,000	68.9±7.7	73.8±9.0	3.894	0.000
Right ear air conduction	10,000	60.4±9.1	70.8±9.0	3.794	0.001
	12,000	61.9±7.7	71.8±9.0	3.894	0.000
Left ear bone conduction	10,000	56.5±6.2	62.7±6.6	4.267	0.000
	12,000	59.1±7.8	65.4±8.0	3.164	0.003
Left ear air conduction	10,000	60.3±8.7	69.1±7.9	4.630	0.000
	12,000	65.0±10.0	74.9±9.0	4.379	0.000

dB: Decibel; NAC: N-acetylcysteine; SD: Standart deviation; t: t-test.

for cochlear damage, and electrocochleography, which measures the cochlear microphonic potentials, could be used for detecting the affects of the otoprotectors on both animals and humans.

The most important benefits of otoacustic emissions (OAEs) are its objectivity and its non-invasive capacity to inspect the primary stages of the sound process to identify and evaluate the biomechanical activity of the outer hair cells. Otoacustic emissions

Table 4. Auditory brainstem response results of group 2

	dB	Latency between waves	Group 2 (msn) Before cisplatin + NAC (mean±SD)	Group 1 (msn) After cisplatin + NAC (mean±SD)	t	*p*
Right ear 10 pps	90	I-III	2.3±0.3	2.3±0.3	0.409	0.685
		I-V	4.3±0.4	4.2±0.4	0.014	0.989
		III-V	2.0±0.2	1.9±0.2	0.224	0.824
	80	I-III	2.3±0.2	2.2±0.2	0.096	0.924
		I-V	4.3±0.3	4.3±0.3	0.077	0.939
		III-V	2.0±0.3	2.0±0.3	0.000	1.000
	70	I-III	2.2±0.3	2.2±0.3	0.246	0.807
		I-V	4.2±0.4	4.3±0.4	0.031	0.975
		III-V	2.1±0.2	1.9±0.5	1.046	0.303
Right ear 50 pps	90	I-III	2.2±0.3	2.3±0.2	1.003	0.323
		I-V	4.3±0.4	4.3±0.3	0.059	0.953
		III-V	2.0±0.2	2.0±0.2	0.000	1.000
	80	I-III	2.2±0.2	2.2±0.3	0.210	0.835
		I-V	4.3±0.3	4.3±0.4	0.126	0.901
		III-V	2.1±0.2	2.1±0.2	0.000	1.000
	70	I-III	2.2±0.6	2.3±0.3	0.724	0.474
		I-V	4.3±0.4	4.3±0.4	0.026	0.979
		III-V	2.0±0.3	2.0±0.3	0.000	1.000
Left ear 10 pps	90	I-III	2.3±0.2	2.3±0.2	0.000	1.000
		I-V	4.2±0.3	4.2±0.3	0.000	1.000
		III-V	1.9±0.3	1.9±0.3	0.000	1.000
	80	I-III	2.2±0.5	2.3±0.2	0.877	0.387
		I-V	4.3±0.4	4.0±1.0	1.108	0.276
		III-V	2.0±0.4	2.0±0.2	0.294	0.771
	70	I-III	2.2±0.2	2.2±0.2	0.098	0.923
		I-V	4.2±0.4	4.3±0.4	0.213	0.832
		III-V	2.0±0.3	2.1±0.3	0.576	0.568
Left ear 50 pps	90	I-III	2.3±0.3	2.3±0.3	0.000	1.000
		I-V	4.2±0.3	4.1±0.7	0.627	0.535
		III-V	1.9±0.3	1.9±0.3	0.000	1.000
	80	I-III	2.3±0.3	2.3±0.3	0.183	0.856
		I-V	4.0±1.0	1.0±0.5	0.918	0.365
		III-V	1.9±1.9	1.9±0.3	0.000	1.000
	70	I-III	2.3±0.3	2.2±0.3	0.183	0.856
		I-V	4.2±0.4	4.2±0.4	0.112	0.911
		III-V	2.0±0.3	2.0±0.3	0.000	1.000

dB: Decibel; NAC: N-acetylcysteine; SD: Standart deviation; t: t-test.

Table 5. High frequency audiometry results of group 3

	dB (Hz.)	Group 3 Before cisplatin + Salicylate (mean±SD)	Group 3 After cisplatin + Salicylate (mean±SD)	t	*p*
Right ear bone conduction	10,000	76.4±8.5	80.9±7.5	1.645	0.109
	12,000	77.2±8.6	81.2±7.6	1.436	0.160
Right ear air conduction	10,000	79.7±8.3	86.5±5.2	1.441	0.007
	12,000	77.5±8.4	84.7±6.0	2.892	0.007
Left ear bone conduction	10,000	75.6±8.4	79.1±5.9	1.444	0.158
	12,000	79.2±7.7	83.2±7.7	1.561	0.128
Left ear air conduction	10,000	74.7±11.6	65.3±10.8	2.487	0.018
	12,000	76.1±8.6	70.3±12.1	1.631	0.114

dB: Decibel; SD: Standart deviation; t: t-test.

are considered as an ototoxicity monitor because of their features.

Ekborn et al.[23] used OAEs and electron microscopy in their studies to investigate the effects of protective agents against the CP's ototoxicity but they found that animals with or without CP therapy were not suitable for OAE measurements. By means of this study it has been declared that OAE is not appropriate to test ototoxicity in experimental animals and

Table 6. Auditory brainstem response results of group 3

	dB	Latency between waves	Group 3 (msn) Before cisplatin + salicylate (mean±SD)	Group 3 (msn) After salicylate + cisplatin (mean±SD)	t	*p*
Right ear 10 pps	90	I-III	2.0±0.2	2.2±0.1	2.60	0.013
		I-V	4.1±0.5	4.1±0.4	0.087	0.932
		III-V	2.0±0.5	2.0±0.4	0.037	0.971
	80	I-III	2.1±0.2	2.1±0.2	0.139	0.890
		I-V	4.2±0.5	4.2±0.4	0.043	0.966
		III-V	2.1±0.4	2.0±0.4	0.514	0.611
	70	I-III	2.1±0.2	2.1±0.2	0..806	0.426
		I-V	4.1±0.5	4.1±0.5	0.137	0.892
		III-V	2.2±0.5	2.0±0.4	0.913	0.368
Right ear 50 pps	90	I-III	2.2±0.2	2.2±0.2	0.076	0.940
		I-V	4.1±0.3	4.1±0.3	0.016	0.987
		III-V	1.9±0.2	1.9±0.2	0.142	0.888
	80	I-III	2.2±0.2	2.3±0.3	0.771	0.446
		I-V	4.2±0.3	4.2±0.2	0.228	0.821
		III-V	2.0±0.2	2.0±0.2	0.288	0.775
	70	I-III	2.1±0.2	2.0±0.2	0.780	0.441
		I-V	4.1±0.4	4.2±0.3	0.291	0.773
		III-V	2.1±0.3	2.1±0.2	0.694	0.492
Left ear 10 pps	90	I-III	2.3±0.2	2.3±0.3	0.211	0.834
		I-V	4.2±0.5	4.1±0.5	0.422	0.675
		III-V	1.9±0.3	1.9±0.3	0.035	0.972
	80	I-III	2.3±0.3	2.2±0.2	0.626	0.535
		I-V	4.2±0.4	4.1±0.3	0.376	0.710
		III-V	2.1±0.6	1.9±0.2	1.153	0.257
	70	I-III	2.1±0.3	2.2±0.2	0.307	0.760
		I-V	4.3±0.4	4.2±0.4	1.151	0.258
		III-V	2.1±0.4	2.0±0.3	1.199	0.239
Left ear 50 pps	90	I-III	2.3±0.4	2.3±0.3	0.133	0.895
		I-V	4.3±0.4	4.2±0.4	0.999	0.325
		III-V	2.0±0.3	1.9±0.2	0.959	0.344
	80	I-III	2.3±0.3	2.1±0.3	1.421	0.164
		I-V	4.3±0.5	4.1±0.6	1.039	0.306
		III-V	2.1±0.4	2.1±0.4	0.141	0.888
	70	I-III	2.3±0.4	2.2±0.4	0.277	0.783
		I-V	4.4±0.6	4.2±0.6	0.598	0.553
		III-V	2.1±0.3	1.9±0.3	1.478	0.149

dB: Decibel; SD: Standart deviation; t: t-test.

Table 7. High frequency audiometry results of group 1 and 2

	dB (Hz.)	Group 1 After cisplatin (mean±SD)	Group 2 After NAC + cisplatin (mean±SD)	t	*p*
Right ear bone conduction	10,000	82.3±8.9	71.8±9.0	4.965	0.000
	12,000	81.8±8.9	73.8±9.0	3.894	0.000
Right ear air conduction	10,000	79.8±9.0	70.8±9.0	3.794	0.001
	12,000	81.8±8.9	71.8±9.0	3.894	0.000
Left ear bone conduction	10,000	77.1±7.9	62.7±6.6	4.630	0.00
	12,000	82.9±9.0	65.4±8.0	4.379	0.00
Left ear air conduction	10,000	79.8±10.5	69.1±7.9	3.467	0.001
	12,000	79.8±9.0	74.9±9.0	3.794	0.001

dB: Decibel; SD: Standart deviation; t: t-test.

electrophysiological tests are needed in such studies.[24]

The studies about prevention of CP induced ototoxicity are usually done on animals. There are not enough clinical studies done on patients who received CP. The most important factor among difficulties is the human cochlea could have some degeneration due to age, environmental factors, acquired ear diseases. Having homogeneous study models with humans is not as easy as with animals. This situation is so important especially in measuring conduction velocity of hearing nerves by ABR. That is why we tried to make CP receiving patients as homogeneously (age, sex, cochlear wellness) as possible.

High frequency audiometric tests have been used for ototoxicity monitoring for a long time. This test is been recommended every two or three days as a sensitive method for the early diagnosis of ototoxicity.[7,25]

Conventional audiometric tests are needed at the beginning of the CP therapy to have a basal value of hearing for the follow-ups. Previous hearing looses could not protect the patient from later ototoxicity events.[26]

The auditory brainstem response could be preferred to pure tone hearing threshold for early diagnosis of CP induced ototoxic changes. In a study that included normal subjects, the 5th wave latency shifts were detected in two of nine patients who received CP in two chemotherapy sequences. There were no pure tone threshold changes until the end of 5th and 6th chemotherapy sequences. But in this study, ABR wasn't compared to high frequency audiometric tests and OAEs which could show us the ototoxicity before the threshold shifts of ABR.[27]

N-acetylcysteine is L-cysteine's N-acetyl derivative and has a potent anti-oxidant activity. It has been shown that NAC provokes the de-novo synthesis of glutathione and NAC is effective in long term cell protection.[28] It could directly bind the platinum molecule and form a complex which is helpful to the protection mechanism. It has been reported that NAC could ameliorate CP induced ototoxicity in guinea pigs by decreasing the loss of outer hair cells (OHC) in vitro. This effect of NAC did not reduce the anti-tumor activity of CP, however there are still some concerns about this.[29,30]

Our study results also parallels the literature: we saw that by using NAC, CP induced hearing loss could be significantly decreased. In our study in the second group, we saw that hearing loss levels in both 10,000 Hz and 12,000 Hz

Table 8. High frequency audiometry results of group 3

	dB (Hz.)	Group 1 After cisplatin (mean±SD)	Group 3 After salicylate + cisplatin (mean±SD)	t	*p*
Right ear bone conduction	10,000	82.3±8.9	80.9±7.5	1.645	0.109
	12,000	81.8±8.9	81.2±7.6	1.436	0.160
Right ear air conduction	10,000	79.8±9.0	86.5±5.2	1.441	0.007
	12,000	81.8±8.9	84.7±6.0	2.892	0.007
Left ear bone conduction	10,000	77.1±7.9	79.1±5.9	1.444	0.158
	12,000	82.9±9.0	83.2±7.7	1.561	0.128
Left ear air conduction	10,000	79.8±10.5	65.3±10.8	2.487	0.018
	12,000	79.8±9.0	70.3±12.1	1.631	0.114

dB: Decibel; SD: Standart deviation; t: t-test.

significantly decreased (p<0.005). This unpleasant effect of NAC is not as obvious as sodium salicylate and D-methionine but it is chemically similar to these agents and it deserves some investigation.[31]

Sodium salicylate as aspirin is a widely used medication and after entering the organism it rapidly transfers to salicylate. It could be used as high as 4-8 g/day in diseases such as rheumatoid arthritis, and in this dose it is known that it could cause sensorineural hearing loss and tinnitus. It is also reported that salicylate in low doses could make the hydroxyl radicals ineffective and could make cell protection by affecting the apoptotic process.[32]

Cytoprotective effect of sodium salicylate occurs in three ways;

1- It is an iron chelator and binds the toxic radicals,

2- It oxidized in the tissues with 2, 3 dihydroxy benzoate,

3- It decreases the tumor necrosing factor (TNF) production which is important in preventing the nephrotoxicity of CP.[32]

Salicylate usage for prevention of CP induced ototoxicity is a novel idea with increasing numbers of studies in this field. Sodium thiosulfate and D-methionine are accepted as the protectors of CP induced ototoxicity but also these reduce the anti-tumor effect of CP chemotherapy. This is probably through the thiol groups binding directly to the CP molecules. However, it is proven that salicylate does not have reduction effects on the

Table 9. Auditory brainstem response results of group 1 and 2

	dB	Latency between waves	Group 1 (msn) After cisplatin (mean±SD)	Group 2 (msn) After NAC + cisplatin (mean±SD)	t	*p*
Right ear 10 pps	90	I-III	2.2±0.2	2.28±0.3	1.463	0.153
		I-V	4.1±0.3	4.27±0.4	0.497	0.622
		III-V	1.9±0.3	1.93±0.2	0.775	0.444
	80	I-III	2.1±0.2	2.28±0.2	0.361	0.721
		I-V	4.1±0.4	4.25±0.3	0.176	0.861
		III-V	2.0±0.3	1.96±0.3	0.101	0.920
	70	I-III	2.1±0.4	2.24±0.2	1.426	0.160
		I-V	3.9±0.6	4.25±0.4	0.489	0.628
		III-V	2.1±0.2	1.93±0.5	0.820	0.418
Right ear 50 pps	90	I-III	2.3±0.4	2.31±0.2	0.409	0.685
		I-V	4.2±0.5	4.31±0.3	0.685	0.538
		III-V	1.9±0.4	2.01±0.2	1.124	0.269
	80	I-III	2.3±0.3	2.19±0.3	0.720	0.467
		I-V	4.2±0.4	4.29±0.4	0.135	0.894
		III-V	1.9±0.1	2.05±0.2	1.786	0.083
	70	I-III	2.4±0.3	2.31±0.3	0.935	0.357
		I-V	4.1±0.4	4.33±0.4	0.000	1.000
		III-V	1.8±0.3	2.02±0.3	1.589	0.121
Left ear 10 pps	90	I-III	2.2±0.1	2.27±0.2	0.020	0.984
		I-V	4.1±0.3	4.18±0.3	0.409	0.743
		III-V	1.9±0.3	1.91±0.3	0.284	0.778
	80	I-III	2.2±0.2	2.29±0.2	0.046	0.964
		I-V	4.1±0.3	4.01±1.0	0.783	0.439
		III-V	2.0±0.3	1.99±0.2	0.104	0.918
	70	I-III	2.2±0.3	2.21±0.2	0.091	0.928
		I-V	4.1±0.4	4.26±0.4	0.525	0.603
		III-V	1.9±0.5	2.05±0.3	0.598	0.554
Left ear 50 pps	90	I-III	2.1±0.3	2.31±0.3	0.253	0.802
		I-V	4.1±0.3	4.13±0.7	0.844	0.405
		III-V	1.9±0.4	1.92±0.3	1.161	0.254
	80	I-III	2.3±0.4	2.28±0.3	0.795	0.432
		I-V	4.2±0.3	0.95±0.4	0.652	0.519
		III-V	1.9±0.3	1.87±0.3	0.592	0.558
	70	I-III	2.2±0.4	2.24±0.3	0.887	0.381
		I-V	4.2±0.5	4.23±0.4	1.062	0.296
		III-V	2.0±0.4	1.98±0.3	0.175	0.862

dB: Decibel; NAC: N-acetylcysteine; SD: Standart deviation; t: t-test.

Table 10. Auditory brainstem response results of group 1 and 3

	dB	Latency between waves	Group 1 (msn) After cisplatin (mean±SD)	Group 3 (msn) After salicylate + cisplatin (mean±SD)	t	*p*
Right ear 10 pps	90	I-III	2.2±0.2	2.3±0.3	0.409	0.685
		I-V	4.1±0.3	4.3±0.4	0.014	0.989
		III-V	1.9±0.3	1.9±0.2	0.224	0.824
	80	I-III	2.1±0.2	2.3±0.2	0.096	0.924
		I-V	4.1±0.4	4.3±0.3	0.077	0.939
		III-V	2.0±0.3	2.0±0.3	0.000	1.000
	70	I-III	2.1±0.4	2.2±0.3	0.246	0.807
		I-V	3.9±0.6	4.3±0.4	0.031	0.975
		III-V	2.1±0.2	1.9±0.5	1.046	0.303
Right ear 50 pps	90	I-III	2.3±0.4	2.3±0.2	1.003	0.323
		I-V	4.2±0.5	4.3±0.3	0.059	0.953
		III-V	1.9±0.4	2.0±0.2	0.000	1.000
	80	I-III	2.3±0.3	2.2±0.3	0.210	0.835
		I-V	4.2±0.4	4.3±0.4	0.126	0.901
		III-V	1.9±0.1	2.1±0.2	0.000	1.000
	70	I-III	2.4±0.3	2.3±0.3	0.724	0.474
		I-V	4.1±0.4	4.3±0.4	0.026	0.979
		III-V	1.8±0.3	2.0±0.3	0.000	1.000
Left ear 10 pps	90	I-III	2.2±0.1	2.3±0.2	0.000	1.000
		I-V	4.1±0.3	4.2±0.3	0.000	1.028
		III-V	1.9±0.3	1.9±0.3	0.000	1.043
	80	I-III	2.2±0.2	2.3±0.2	0.877	0.387
		I-V	4.1±0.3	4.0±1.0	1.108	0.276
		III-V	2.0±0.3	2.0±0.2	0.294	0.771
	70	I-III	2.2±0.3	2.2±0.2	0.098	0.923
		I-V	4.1±0.4	4.3±0.4	0.213	0.832
		III-V	1.9±0.5	2.1±0.3	0.576	0.568
Left ear 50 pps	90	I-III	2.1±0.3	2.3±0.3	0.000	1.000
		I-V	4.1±0.3	4.1±0.7	0.627	0.535
		III-V	1.9±0.4	1.9±0.3	0.000	1.000
	80	I-III	2.3±0.4	2.3±0.3	0.183	0.856
		I-V	4.2±0.3	1.0±0.5	0.918	0.365
		III-V	1.9±0.3	1.9±0.3	0.000	1.000
	70	I-III	2.2±0.4	2.2±0.3	0.183	0.856
		I-V	4.2±0.5	4.2±0.4	0.112	0.911
		III-V	2.0±0.4	2.0±0.3	0.000	1.000

dB: Decibel; SD: Standart deviation; t: t-test.

anti-tumor agents.[33] Hyppolito et al.[14] investigated the effects of salicylate on CP induced ototoxicity in rats; additional to the electrophysiological tests they also registered each cochlear turns OHC counts by postmortem histological examination with sitocochleogram method. According to this study, they declared that by using CP, outer hair cells of basal and middle turn of cochlea were affected the most. Our study could not find any statistically significant decrease of hearing loss in CP induced ototoxicity by using both ABR and audiometric tests.

In our study there were no differences detected between NAC and Salicylate use for CP induced ototoxicity. Our findings differ from the ABR test results in animals, because CP's effecting mechanism is primarily on outer hair cells of cochlea, and audiometry is much more sensitive than ABR in showing the damage of outer cells. First wave latency of ABR reflects cochlear

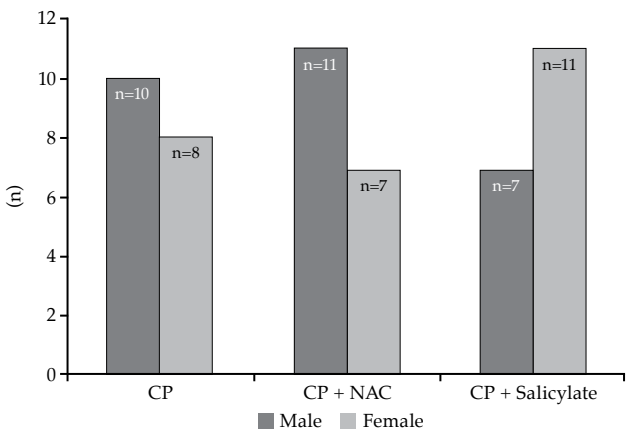

Figure 1. *Male - female rates in groups.*

pathologies but there could be patient related factors affecting the ABR results. Uncontrolled parameters [decreased serum albumin, hemoglobin (Hb), hematocrit (Htc), levels and erythrocyte counts] could increase the effect of CP induced ototoxicity. For a better reflection of ABR changes in CP using patients; we should eliminate the systemic and ear related diseases (acoustic trauma, otosclerosis, Meniere's disease, other ototoxic factors, presbycusis, etc...)

In our study we found that CP induced hearing loss could be reduced in 10,000 and 12,000 Hz frequencies when NAC was added to the CP therapy protocol. This result is parallel to the literature but it is still uncertain whether NAC usage will affect CP's anti-tumor ability or not.

Salicylate usage to prevent CP induced ototoxicity is a new topic, but there has been an increase in studies in this field. Unlike NAC, salicylate does not affect the anti-tumor ability of CP, which is an advantage of its usage for ototoxicity. We could not find any decrease in the hearing loss levels of the patients who were receiving CP with salicylate in our study. Some of the factors such as differences between animal and human cochleas, poverty of CP receiving patients, that causes in cooperation in the audiometric tests, differences of patient histories and difficulties of arranging homogeneous study groups, could be impeding us to determine the exact hearing loss levels clinically. Further clinical studies are needed in this topic.

According to the ABR testing results there were no differences detected between NAC receivers nor salicylate for the amelioration of CP induced ototoxicity in our study.

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