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Evaluation of the effect of gemcitabine on cochlea by otoacoustic emission in experimental animal model

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Abstract. – OBJECTIVES: In this study, we aimed to search whether gemcitabine – a commonly used antimetabolite type antineoplastic agent-has ototoxic effect.

STUDY DESIGN AND SETTING: An experimental animal research.

SUBJECTS AND METHODS: We evaluated the effect of gemcitabine on hearing through its possible effect on cochlea by using otoacoustic emission method on experimental rat model. For this purpose 16 healthy adult male Wistar albino rats were used and these were divided into 4 groups. The rats in the 1st, 2nd, 3rd and the 4th groups were given 40-160-240 and 320 mg/kg of intraperitoneal gemcitabine respectively. Distortion product otoacoustic emission measurements on both ears of each rat were performed before and 1 week after administration of gemcitabine. The mean of signal to noise ratio of emission values obtained from each rat before and after the drug administration were calculated.

RESULTS: Rats in the 1st group had no statistically significant difference at the emission rates before and after gemcitabine administration. A statistically significant decrease was observed in the emission rates at emission values of only 5 kHz in the 2nd group, at 4-6 kHz in the 3rd group, at 4-5-6-8 kHz in the 4th group.

CONCLUSIONS: Gemcitabine causes a decrease in otoacoustic emission values on experimental rat models at high doses and especially at high frequencies. Larger clinical and laboratory studies are needed to determine whether this decrease in emission rates are permanent and whether it has any permanent effect on hearing.

Key Words:
Gemcitabine, Otoacoustic emission, Hearing, DPOAE, Ototoxicity.

Introduction

The main principle of chemotherapy used in the treatment of cancer is to eliminate or to limit the growth and reproduction of the tumor cell without damaging the normal cells of the patient. However, the selectivity of these antineoplastic agents against the cancer cell is low. They destroy the normal cells which reproduce quickly as well as the cancer cells and, therefore, damage certain systems within the body. The vestibulocochlear system which enables hearing and balance is one of them.

Hearing loss is a major health problem which results in a decrease in finance, labour and quality of life. Ototoxicity is a general term which is used to define the damage which emerges in the cochlear and vestibular organ as a result of various therapeutic agents and chemical materials. Today, ototoxicity is a major cause of hearing losses and balance disorders. The symptoms of ototoxicity emerge as a result of prolonged clinical usage of some therapeutic agents. There are many heavy metals, antibiotics, antineoplastics, anti-inflammatory or diuretic medicines which are known to have ototoxic effects[1,2]. The main complaints that arise upon exposure to such agents are tinnitus, hearing loss, and vertigo. Tinnitus is the most frequent, and generally the first symptom. Hearing loss is of sensory-neural type, generally bilateral and symmetrical. This loss may be temporary or permanent, based on the dose.

In this study, we evaluated whether Gemcitabine, which is an antineoplastic agent of the antimetabolite class, has ototoxic effects. For this purpose, we assessed the possible effect of Gemcitabine on cochlea by using the otoacoustic emission (OAE) method through the rat model.

Materials and Methods

The study was performed on 16 healthy adult male Wistar albino rats divided into 4 groups. The weights of the rats ranged between 271 to 408 g. They were kept in an environment which was dark for 12 hours and illuminated for 12 hours. The temperature of the medium was 21 centigrade Cel-

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sius. The rats were left free to eat food and drink water. Animal use and care were in accordance with the principle of the Declaration of Helsinki and approved by the Animal Experiment Committee of the Zonguldak Karaelmas University (protocol number B.30.2.Z.K.U.0.01.00.00/21).

In order to have reliable results, all rats were evaluated by a baseline otoscopic examination. Rats with a clean external ear channel and a normal eardrum were included in the study. The rats in the 1st, 2nd, 3rd and 4th groups were exposed to 40-160-240 and 320 mg/kg of intraperitoneal (IP) gemcitabine respectively. Since the emission results of the right and left ears are independent from each other, distortion product otoacoustic emission (DPOAE) measurements were performed bilaterally, before and 1 week after the administration of gemcitabine. 50 mg/kg of intramuscular ketamine hydrochloride was applied to all rats before the procedure. The rats which died/developed otitis during the study was excluded and new rats were included instead. The rats from which no emissions were received were also excluded from the study. Subsequently, the average of the OAE values obtained for every single rat was calculated and the signal-to-noise ratio (SNR) frequency curves were drawn. During the study, the background noise was lower than 50 dB.

OAE's resulting from distortion were measured with the Otodynamics Ltd. DPE choport IL-O292 through the use of neonate probe. The measurements were performed by placing the probe in the external ear after the head of the animal was brought to a horizontal position. DPOAE's (2f1-f2 cubic distortion product components) were measured in the General Diagnostic mode with the ILOv6 (Otodynamics ltd) equipment. The ratio between the f2 and f1 frequencies (f2/f1) was kept at 1.22. The amplitude of the stimulus was L1 for f1 frequency and L2 for f2 frequency. The difference between the L1-L2 levels was kept at 10 dB SPL (L1=65 dB SPL, L2=55dB SPL). The DPOAEs were measured at the 2f1-f2 frequency with the microphone in the external ear-canal and were recorded at 1001, 1501, 2002, 3003, 4004, 6006, and 7996 frequencies at the geometrical means of f1 and f2. The average time of the test was 60 seconds for each subject. DPOAE amplitude 3 dB higher than the noise threshold was considered meaningful. The evaluation of DPOAE results were based on SNR's that formed at the geometrical means of f1 and f2 which are 2f1-f2 cubic distortion products or in other words at 1001, 1501, 2002, 3003, 4004, 6006 and 7996 Hz frequency bands. SNR's have been reported to be more reliable than the DPOAE amplitudes for the assessment of DPOAE responses[2]. In our study, the means of these SNR's have been calculated for each rat and the SNR frequency curves have been drawn.

Statistical Analysis

The averages and standard deviations (SD's) of DPOAE results have been calculated. The differences between DPOAE results before and after the administration of gemcitabine were statistically examined through the Wilcoxon test. $p < 0.05$ was considered statistically significant.

Results

Four different groups included in the study, the test results and the results of the statistical analysis of these by using the Wilcoxon test are shown in the tables. We did not observe any statistically significant difference in the emission results in the 1st group before and after the application of 40 mg/kg IP gemcitabine (Table I, Figure 1). In the 2nd group where 160 mg/kg gemcitabine was applied, a statistically meaningful decrease in emission values was observed at 5 kHz after the application of the drug in comparison to before (Table II, Figure 2). A similar decrease was observed at 4-6 kHzs in the 3rd group, and at 4-5-6-8 kHzs in the 4th group of rats after the application of the drugs (Tables III, IV; Figures 3, 4).

Discussion

In this study, the ototoxic effects of gemcitabine, a chemotherapeutic agent of the antimetabolite class, was examined following its IP application to healthy adult male Wistar albino rats at different amounts in a single dose through DPOAE measurements and the results have been examined.

Gemcitabine, which is a pyrimidine antimetabolite, is used primarily against ovarian cancer, non-small cell (NSCLC) and small cell (SCLC) lung cancers, and also against pancreatic, bladder, renal and colorectal carcinomas and certain leukemic malignancies[3]. Although it is a relatively new chemotherapeutic agent, many pre-clinical, clinical, and animal studies have been carried out with this agent. These studies have mainly focused on the mechanisms of effect of gemcitabine, its pharmacokinetic, pharmacodynamic effects and its toxicities[3].

In general, the principal side effect of gemcitabine is myelotoxicity. This effect is not marked

Table I. Group 1 (40 mg/kg IP gemcitabine): There was no statistically significant difference in the DPOAE results at any frequency before and after the administration of gemcitabine.

Frequencies (kHz)	SNR's in dB before gemcitabine (mean ± 2 SD)	SNR's in dB after gemcitabine (mean ± 2 SD)	p
1	-5.61 ± 8.28	-8.73 ± 5.49	> 0.05
1.5	-2.92 ± 3.46	-4.10 ± 4.23	> 0.05
2	-5.00 ± 3.58	-7.97 ± 4.88	> 0.05
3	-3.05 ± 3.40	-2.36 ± 4.81	> 0.05
4	3.35 ± 6.96	-0.57 ± 9.01	> 0.05
5	12.05 ± 6.33	2.96 ± 11.55	> 0.05
6	23.72 ± 7.31	11.11 ± 14.63	> 0.05
8	30.10 ± 5.14	24.12 ± 13.41	> 0.05

SNR: Signal-to-noise ratio, IP: Intraperitoneal.

but is moderate[4]. In a study carried out by Yang et al[5], courses of treatment was applied to 28 patients with hepatocellular cancer. Course of treatment consisted of a 4-week treatment process, according to which a dose of 1250 mg/m^2 gemcitabine was given once a week for 3 subsequent weeks, followed by a one-week interval. According to National Cancer Institute Common Toxicity Criteria, 3rd or 4th grade myelotoxicity was observed in the patients participating in this study. The incidence of leukopenia was 10.7%, anemia was 14.3%, thrombocytopenia was 10.7% and hepatoxicity was 14.3%. Thrombocytopenia was determined as the dose-limiting effect in Yang's study. In another study conducted on 82 cases by Anderson et al[6], anemia was observed in 4 patients (5%), thrombocytopenia in 1 patient (1%), leukopenia in 6 patients (7%), and neutropenia in 18 patients (22%), elevated liver enzymes were observed in 10 patients (12%).

Gemcitabine is eliminated by undergoing deamination particularly in kidneys and liver. It may cause some degree of hepatotoxicity. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the blood may increase at different rates. In a study carried out by Martin et al[7], AST and ALT levels of two different patient groups to whom different treatment protocols were applied increased by 9.2% and 7.2% respectively.

Renal toxicity is not significantly high during gemcitabine usage. In a study carried out by Anderson et al[6], no renal toxicity was observed in any of the patients. In another study of 39 patients, Javed et al[8] applied low doses of gemcitabine followed by radiotherapy in cases with grade 3-4 squamous cell carcinoma in the headneck region, and nephrotoxicity was not observed as a side effect.

Gemcitabine may lead to elevated creatine levels through its low nefrotoxic effect; however, this effect may be associated with co-morbid diseases and dehydration. In various studies, although grade 3-4 nausea or vomiting was the most frequently observed non-hematological side effect of gemcitabine treatment, this problem is eliminated by giving serotonin antagonist drugs during the treatment[9]. Hair loss, mucositis, gastrointestinal system side effects are infrequent side effects of gemcitabine[4]. There are very rare complications

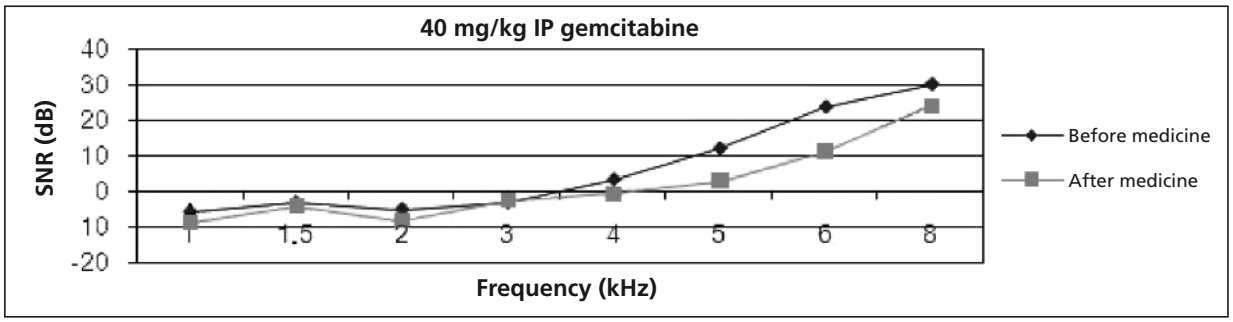

Figure 1. Group 1 (40 mg/kg IP gemcitabine). Distortion product otoacoustic emision results. X-axis is Frequency (kHz); y-axis is signal-to-noise ratio (dB). SNR: Signal-to-noise ratio, IP: Intraperitoneal.

Table II. Group 2 (160 mg/kg IP gemcitabine): A statistically significant decrease in the emission values was observed at 5 kHz following the administration of gemcitabine when compared to the phase before the medication.

Frequencies (kHz)	SNR's in dB before gemcitabine (mean ± 2 SD)	SNR's in dB after gemcitabine (mean ± 2 SD)	*p*
1	-2.32 ± 4.41	-9.18 ± 8.26	> 0.05
1.5	-4.41 ± 5.46	-0.76 ± 3.18	> 0.05
2	0.48 ± 6.56	-2.21 ± 5.58	> 0.05
3	5.76 ± 7.38	-1.35 ± 7.80	> 0.05
4	12.51 ± 4.49	9.22 ± 6.23	> 0.05
5	23.35 ± 5.28	15.46 ± 5.09	< 0.05*
6	24.43 ± 4.64	26.21 ± 3.99	> 0.05
8	21.16 ± 3.93	22.82 ± 5.09	> 0.05

SNR: Signal-to-noise ratio, IP: Intraperitoneal.

reported in case presentations such as vascular toxicity and tumor lysis syndrome[10,11].

The recommended application of gemcitabine is by slow intravenous infusion for 30 minutes[3] However, other application methods have also been used in the literature. Putte et al[12] applied gemcitabine to the lungs of rats with pulmonary metastases through perfusion and observed a long survey as a result of this application. They did not observe any acute or major complication in long term. In various experimental studies, gemcitabine has been applied both intravenously and intraperitoneally[13]. In our study we applied gemcitabine intraperitoneally which is safe and more accessible.

As far as we know, there are no studies in the literature which mentions the ototoxic effects of gemcitabine besides the side effects which we have listed.

In clinical studies ototoxicity is defined as loss of hearing exceeding 15dB in two or more frequencies, exceeding 20dB in one or more frequency or exceeding 15dB in any frequency[14]. Ototoxicity is generally a phenomenon which presents iatrogenically. Therefore, clinicians need to know whether any medicine they prescribe has ototoxic potential. If predisposing factors for ototoxicity are detected or when a medicine with ototoxic potential is started, the patient must be followed closely, ototoxic side effects must be determined early and necessary measures must be taken. This issue also has medicolegal importance.

Stimulation of the cochlea with 2 different frequencies results in the overlapping of these waves which propagate in the inner ear and leads to a lower-amplitude response in the tones where these two waves interact, which is called DPOAE. DPOAEs are stimulated OAE'e which enable the assessment of the cochlea objectively and they constitute an easy, reliable, and a rapid method which can be used in the examination of functions of certain frequency regions in the cochlea[15].

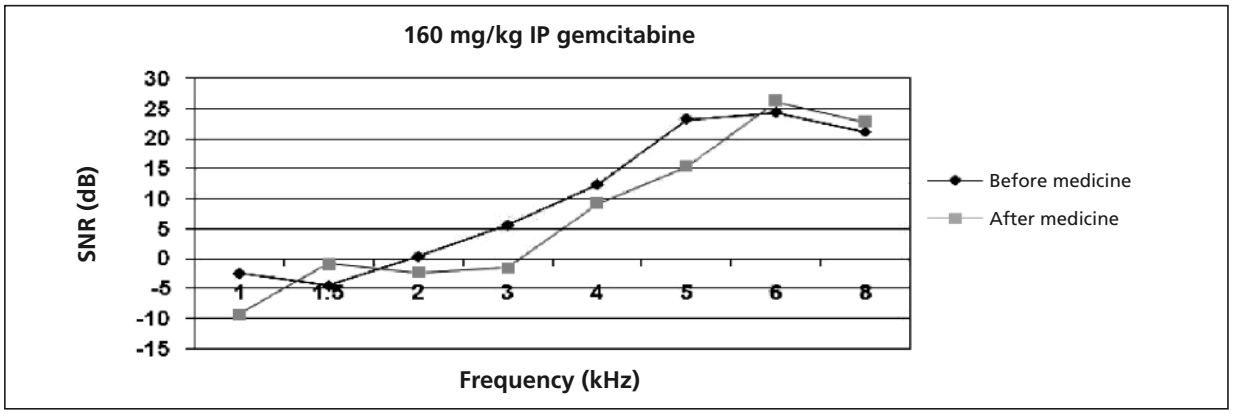

Figure 2. Group 2 (160 mg/kg IP gemcitabine). Distortion product otoacoustic emission results. X-axis is Frequency (kHz); y-axis is signal-to-noise ratio (dB). SNR: Signal-to-noise ratio, IP: Intraperitoneal.

Table III. Group 3 (240 mg/kg IP gemcitabine): A statistically significant decrease in the emission values was observed at 4 and 6 kHz following the administration of gemcitabine in comparison to the phase before the medication.

Frequencies (kHz)	SNR's in dB before gemcitabine (mean ± 2 SD)	SNR's in dB after gemcitabine (mean ± 2 SD)	p
1	-3.37 ± 4.15	-7.33 ± 3.93	> 0.05
1.5	-9.65 ± 7.48	-7.46 ± 4.61	> 0.05
2	-6.76 ± 9.20	-3.76 ± 5.75	> 0.05
3	-0.58 ± 5.91	-4.71 ± 5.18	> 0.05
4	2.02 ± 6.37	-3.56 ± 3.34	< 0.05*
5	4.56 ± 7.56	-1.73 ± 7.37	> 0.05
6	19.90 ± 12.25	0.38 ± 5.25	< 0.05*
8	22.57 ± 13.31	12.47 ± 7.96	> 0.05

SNR: Signal-to-noise ratio, IP: Intraperitoneal.

Table IV. Group 4 (320 mg/kg IP gemcitabine): A statistically significant decrease in the emissions was observed at 4 – 5 – 6 – 8 kHz in comparison to the phase before the medication.

Frequencies (kHz)	SNR's in dB before gemcitabine (mean ± 2 SD)	SNR's in dB after gemcitabine (mean ± 2 SD)	p
1	-5.68 ± 5.37	-7.85 ± 4.22	> 0.05
1.5	-5.75 ± 6.94	-10.71 ± 7.42	> 0.05
2	-5.63 ± 8.08	-3.98 ± 5.57	> 0.05
3	0.40 ± 7.07	-2.17 ± 3.42	> 0.05
4	14.41 ± 7.84	-2.60 ± 4.83	< 0.05*
5	16.97 ± 6.41	-5.50 ± 6.67	< 0.05*
6	30.21 ± 3.32	7.00 ± 5.95	< 0.05*
8	32.58 ± 4.12	21.27 ± 5.05	< 0.05*

SNR: Signal-to-noise ratio, IP: Intraperitoneal.

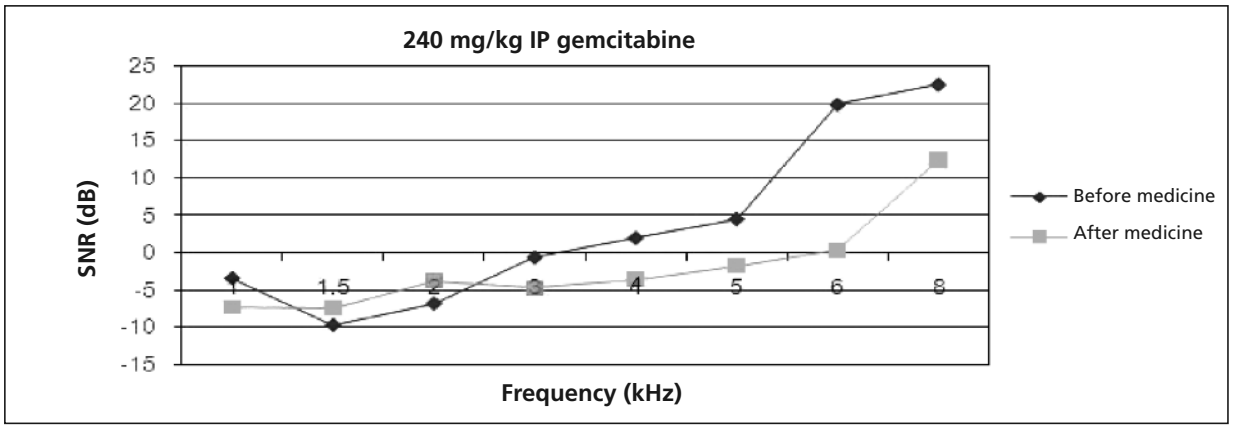

Figure 3. Group 3 (240 mg/kg IP gemcitabine). Distortion product otoacoustic emission results. X-axis is Frequency (kHz); y-axis is signal-to-noise ratio (dB). SNR: Signal-to-noise ratio, IP: Intraperitoneal.

The first medicine whose ototoxic effect was reported is quinine. Temporary deafness related to quinine was reported by Morton in 1684. Feldman discovered streptomycin in 1946 and later it was discovered that it caused loss of hearing and vestibular disorders. After the discovery of the otoxic effects of other aminoglycoside antibiotics, studies concerning ototoxicity gained momentum. In various subsequent studies, ototoxic medicines were shown to have cochleatoxic effects. For exam-

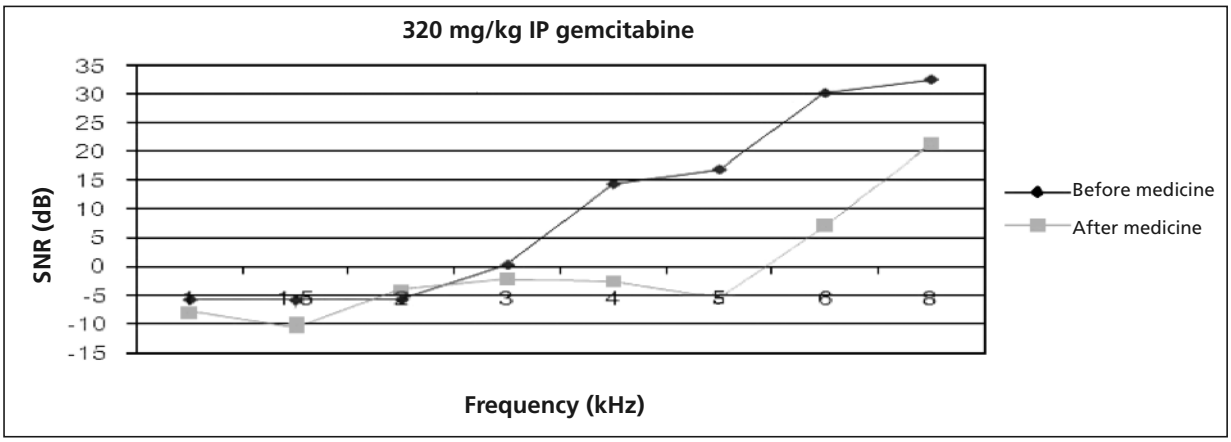

Figure 4. Group 4 (320 mg/kg IP gemcitabine). Distortion product otoacoustic emission results. X-axis is Frequency (kHz); y-axis is signal-to-noise ratio (dB). SNR: Signal-to-noise ratio, IP: Intraperitoneal.

ple, it was discovered that cysplatin caused ototoxicity by influencing the outer hair cells[16], this led to the conclusion that the damage in the inner ear could be demonstrated through OAE's. Plinkert and Kröber[17] discovered a 30% decrease in the emission amplitudes in 31% of the patients whom they gave 100 mg/m^2 cysplatin although no audiological changes were observed. In a study by Robinette and Glattke[18], it was stated that OAE's were more susceptible to decreases in cochlear functions compared to pure tone audiometry (PTA). They demonstrated that after cysplatin treatment a hearing loss was detected through PTA at frequencies above 3000 Hz, but with DPOAE a significant decrease was discovered at frequencies below 3000 Hz. Thus, they stated that DPOAE was a more sensitive method for detecting the changes in the cochlear functions in cases of ototoxicity compared to PTA.

In another study[19], children who were treated with cysplatin and/or carboplatin were examined with both PTA and DPOAE at normal hearing frequencies (0.5-8 kHz) and at high frequencies (9-16 kHz). During the treatment, bilateral ototoxicity was detected in 20 (62.5%) out of 32 children in conventional frequencies through audiometry. 26 (81.3%) of them showed bilateral decrease in the DPOAEs. 17 children were examined by high frequency audiometry and ototoxicity was detected at high frequencies in 16 (94.1%) of the patients. As a result of this pilot study, it was stated that high frequency audiometry and DPOAE measurements detected changes in auditory functions in patients who were exposed to platinum derived antineoplastic agents earlier than conventional audiometric measurements.

Recommended dose range for Gemcitabine is 800-1250 mg/m^2. It is applied by intravenous infusion within 30 minutes. The maximum tolerable dose[20] was determined to be 2400 mg/m^2. The doses which we used in our study were determined by considering the minimum and maximum doses used in human beings. The dose which corresponded to the dose used in human beings was calculated with respect to the surface area of the rats' body and the appropriate dose for every rat was applied intraperitoneally. 1 week after the application of the medicine, 4 of the rats in the group to which 320 mg/kg was applied and 3 of the rats to which 240 mg/kg was applied died. The control emissions of the dying rats had been done. In a similar study where 320 mg/kg intravenous gemcitabine was applied to rats, all of the rats died in a week, whereas the morbidity rate in the rats to whom gemcitabine was applied through isolated lung perfusion was much more lower[12].

The greatest problem encountered during the measurement of OAE in rats is the placement of the probe in the external ear canal of the rat, which is very narrow. For this reason, the rat must be well-sedated and a baby probe must be used for emission measurements. Many rats required a few adjustments in order to place the probe in the outer ear to receive ideal emission responses. The emission amplitudes and noise thresholds of measurements carried out at different times may vary. SNR is more reliable than DPOAE amplitudes while evaluating the DPOAE responses. We adopted this ratio in our study.

In our study, we compared the DPOAE results of the subjects before and after the application of the pre-determined doses of gemcitabine with Wilcoxon test. Consequently, we did not observe any statistically significant difference in the emission results in the 1st group before and after

the application of 40 mg/kg IP gemcitabine (Table I). In the 2nd group where 160 mg/kg gemcitabine was applied, a statistically meaningful decrease in emission values was observed at 5kHz after the application of the medicine in comparison to before (Table II). A similar decrease was observed at 4-6 kHzs in the 3rd group, and at 4-5-6-8 kHzs in the 4th group of rats after the application of the medicine (Table III, IV).

Conclusions

In our study which was performed on an experimental animal model, we concluded that gemcitabine which has a widespread clinical usage resulted in a decrease in DPOAE values in the rat model, this decrease increased in proportion with the amount of the dose and it displayed this effect particularly at high frequencies. More comprehensive clinical and laboratory studies are needed to determine whether this decrease in emission values that emerge following the application of gemcitabine is permanent or not.

References

1) Wright A, Forge A, Kotecha B. Ototoxicity. Gleeson M editors. Scott-Brown's Otolaryngology vol. 3. UK: Butterworth-Heinemann, London, 1997.
2) Lamm K, Lamm H, Arnold W. Effect of hyperbaric oxygen therapy in comparison to conventional or placebo therapy or no treatment in idiopathic sudden hearing loss, acoustic trauma, noise-induced hearing loss and tinnitus. A literature survey. Adv Otorhinolaryngol 1998; 54: 86-99.
3) Ryan DP, Lynch TJ, Grossbard ML, Seiden MV, Fuchs CS, Grenon N, Baccala P, Berg D, Finkelstein D, Mayer RJ, Clark JW. A phase I study of gemcitabine and docetaxel in patients with metastatic solid tumors. Cancer 2000; 88: 180-185.
4) Vogelzeng N, Stadler W. Gemcitabine and other new chemotherapeutic agents for the treatment of metastatic bladder cancer. Urology 1999; 53: 243-250.
5) Yang TS, Lin YC, Chen JS, Wang HM, Wang CH. Phase II study of gemcitabine in patients with advanced hepatocellular carcinoma. Cancer 2000; 89: 750-756.
6) Anderson H, Thatcher N, Walling J, Hansen H. A phase I study of a 24 hour infusion of gemcitabine in previously untreated patients with inoperable non-small-cell lung cancer. Br J Cancer 1996; 74: 460-462.
7) Martin C, Lund B, Anderson H, Thatcher N. Gemcitabine: once-weekly schedule active and better tolerated than twice-weekly schedule. Anticancer Drugs 1996; 7: 351-357.
8) Javed AA, Shaharyar A, Shah IH, Shah MA, Ansari TN, Faheem M, Mehmood H, Khan MS, Afridi MA, Rasool S. A phase II Study of Gemcitabine Concurrent with Radiation in Locally Advanced Squamous Cell Carcinoma of Head and Neck: a trial of the cancer research group pakistan. ASCO Annual Meeting Proceedings (Post-Meeting Edition). J Clin Oncol 2006; 24(18S): (June 20 Supplement).
9) Khaled HM, Hamza MR, Mansour O, Gaafar R, Zaghloul MS. A phase II study of gemcitabine plus cisplatin chemotherapy in advanced bilharzial bladder carcinoma. Eur J Cancer 2000; 36(Suppl 2): 34-37.
10) Holstein A, Batge R, Egberts EH. Gemcitabine induced digital ischaemia and necrosis. Eur J Cancer Care (Engl) 2010; 19: 408-409.
11) Lin CJ, Lim KH, Cheng YC, Chen HH, Wu CJ. Tumor lysis syndrome after treatment with gemcitabine for metastatic transitional cell carcinoma. Med Oncol 2007; 24: 455-457.
12) Van Putte BP, Hendriks JM, Romijn S, Pauwels B, Friedel G, Guetens G, De Bruijn EA, Van Schil PE. Isolated lung perfusion with gemcitabine in a rat: pharmacokinetics and survival. J Surg Res 2003; 109: 118-122.
13) Morgan RJ, Synold TW, Xi B, Lim D, Shibata S, Margolin K, Schwarz RE, Leong L, Somlo G, Twardowski P, Yen Y, Chow W, Tetef M, Lin P, Paz B, Koczywas M. Phase I trial of intraperitoneal gemcitabine in the treatment of advanced malignancies primarily confined to the peritoneal cavity. Clin Cancer Res 2007; 13: 1232-1237.
14) Brummett RE, Morrison RB. The incidence of aminoglycoside antibiotic-induced hearing loss. Arch Otolaryngol Head Neck Surg 1990; 116: 406-410.
15) Wake M, Anderson J, Takeno S, Mount RJ, Harrison RV. Otoacoustic emission amplification after inner hair cell damage. Acta Otolaryngol 1996; 116: 374-381.
16) Barron SE, Daigneault EA. Effect of cisplatin on hair cell morphology and lateral wall Na,K-ATPase activity. Hear Res 1987; 26: 131-137.
17) Plinkert P, Krober S. Early detection of cisplatin-induced ototoxicity using evoked otoacoustic emissions. Laryngorhinootologie 1991; 70: 457-462.
18) Robinette M, Glattke T. Otoacoustic Emissions. In: Roeser V, Hosford D editors. Audiology diognosis. New York: Thime Medical Publishers 2000; pp. 503-526.
19 Knight KR, Kraemer DF, Winter C, Neuwelt EA. Early changes in auditory function as a result of platinum chemotherapy: use of extended high-frequency audiometry and evoked distortion product otoacoustic emissions. J Clin Oncol 2007; 25: 1190-1195.
20) Fossella FV, Lippman SM, Shin DM, Tarassoff P, Calayag-Jung M, Perez-Soler R, Lee JS, Murphy WK, Glisson B, Rivera E, Hong WK. Maximum-tolerated dose defined for single-agent gemcitabine: a phase I dose-escalation study in chemotherapy-naive patients with advanced non-small-cell lung cancer. J Clin Oncol 1997; 15: 310-316.