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ORIGINAL ARTICLE

The Effect of Selenium on Amikacin-Induced Ototoxicity

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Purpose: The aim of this study was to investigate the possible protective effects of selenium on amikacin-induced ototoxicity in rats.

Materials and Method: In this study, 32 healthy Wistar albino rats were used as test animals. After measurements of distortion-product otoacoustic emissions (DPOAE), the rats were divided into four groups. The rats in the "control" group were injected with intramuscular 1 ml/kg/day 0.9% NaCl (saline) for 28 days. The rats in the "amikacin" group received intramuscular 200 mg/kg/day amikacin for 28 days. The rats in the "Amikacin+Selen60" group received intramuscular 200 mg/kg/day amikacin and intraperitoneal low dose of selenium (60 μg/kg/day) for 28 days. The rats in the "Amikacin+selenium 300" group received intramuscular 200 mg/kg/day amikacin and intraperitoneal high dose of selenium (300 μg/kg/day) for 28 days. The experimental groups were evaluated with DPOAE at day 1, day 14 and day 28.

Results: In the "amikacin" group, there were significant hearing loss in DPOAE audiogram (DPgram) measurements at the days 14 and day 28 compared to day 1 ($p < 0.01$). In both "amikacin+selenium60" (group 3) and "amikacin+selenium300" (group 4), there were significant hearing loss in day 14 compared to day 1 ($p<0.01$); there were also significant hearing loss in day 28 compared with day 14 ($p<0.01$).

Differences in ototoxicity in the Dpgrams were assessed between the groups (repeated measures ANOVA and post-hoc Tukey test); there were no significant difference in all frequencies between "amikacin" group, "amikacin+selenium60" group and "amikacin+selenium300" group ($p > 0.05$).

Conclusion: A statistically significant hearing loss was detected in all groups. The long duration of selenium treatment were assessed in rats and both the time and the dose of selenium had no the protective effects on amikacin-induced ototoxicity.

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Introduction

Ototoxicity is the cellular degeneration and functional deterioration in the cochlear and/ or vestibular tissue due to drugs and therapeutic agents [1]. Sensitivity of the inner ear to various chemical substances has been well known for centuries. First, quinine and salicylates have been reported as the cause of tinnitus, hearing loss and vestibular disorders [2]. Today, ototoxicity is significant cause of hearing loss and balance disorders.

Ototoxicity is an important clinical problem and aminoglycoside ototoxicity accounts 5-10% of all sensorineural hearing loss [3]. An apoptotic form of cell death mediates aminoglycoside ototoxicity. Aminoglycosides destroy the sensory epithelium, especially outer hair cells in the cochlea [4]. The main symptoms in ototoxicity are hearing loss, vertigo, imbalance and frequently tinnitus. Ototoxicity symptoms may begin immediately after drug intake or may also develop in the next days

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or weeks [5]. Symptoms may be permanent or temporary. The ototoxic hearing loss is always sensorineural type [6]. There are currently no recommendations for pre or post-treatment therapies to prevent ototoxicity associated with aminoglycoside antibiotics. Anti-oxidant agents have been tested to prevent aminoglycoside ototoxicity [7].

Selenium (Se) is an essential element that is required for the growth of humans and animals. Selenium is one of the antioxidant system elements that protect the cell membranes from the oxidative damaged of peroxides in the lipid metabolism [7,8]. Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes.

Selenium deficiency was examined in cancers, diabetes, HIV / AIDS and serious or chronic diseases such as tuberculosis. However, selenium supplementation has not been established to reduce incidence or mortality of any diseases in the randomized controlled clinical trials on humans [7-9].

The purpose of this study is to evaluate the possible dose-dependent protective effects of antioxidant properties of selenium on the inner ear functions.

Materials And Methods

Animals

This study was carried out in Istanbul University Experimental Medical Research Institute (DETAM) animal laboratory on 32 healthy adult Wistar Albino rats. The rats were kept in an environment of 12 hours darkness and 12 hours of day light, 21^{o} Centigrade temperature and a background noise below 50dB, with free access to food and water.

Anesthesia

After otoscopic examination, those free of any pathology were included in the study. After anesthesia with intramuscular ketamine hydrochloride (0.45 mg·kg− 1) and xylacine (5 mg·kg− 1), DPOAE measurements were accomplished and rats with no measurable emissions were excluded.

Experimental design

The rats included in the study were evaluated in four groups: *"control"* group 1 (n=8), *"amikacin"* group 2 (n=8), *"amikacin+selenium^{60}"* group 3 (n=8), and *"amikacin+selenium^{300}"* group 4 (n=8).

"Control group" (n=8) received 1ml/kg/day intramuscular 0. 9 % NaCl (saline) for 28 days. *"Amikacin group"* (n=8) received 200 mg/kg/day intramuscular amikacin for 28 days. *"Amikasin+selenium^{60}"* group (n=8) received to 200 mg/kg/day intramuscular amikacin and 60 µg/kg/day (low dose) intraperitoneal selenium for 28 days. *"Amikacin+selenium* group (n=8) received 200 mg/kg/day intramuscular amikacin and 300 µg /kg/day (high dose) intraperitoneal selenium for 28 days. The DPOAE measurements were repeated in day 1, day 14 and day 28. The three sets of measurements were compared within the groups and between the groups.

DPOAE measurements

In this study DPOAE (2f1-f2 cubic distortion product compounds) were used to investigate the emissions. For this purpose, the Otodynamics Ltd. ILOv6 instrument was employed with the smallest rubber tympanometry probe attached to the tip of the instrument probe. Distortion product gram (DP-gram) measurements were performed and recorded at 1001,1184, 1416, 1685, 2002, 2380, 2832, 3369, 4004, 4761, 5652, 6726, 7996 Hz. Emission values were under the noise threshold at 1001,1184, 1416, 1685, 2002, 2380, 2832, 3369 and were excluded from the study.

The noise level for both Dpgram and I/O functions was measured at frequencies 50 Hz above the DPOAE frequencies. During measurements at 2f1-f2 frequency, the OAEs ≥3 dB above the noise intensity was accepted as positive. The test was finalized after recording up to the highest level the responses reached in both measurements.

Statistics

The data of this study were assessed for statistical analysis with NCSS (Number Cruncher Statistical System) 2007 & PASS 2008 Statistical Software (Utah, USA). Repeated measures analysis of the variances (repeated measures ANOVA) and post hoc Tukey as the comparison test were used to compare between the groups for emissions analysis. Paired samples t-test was used within the group to evaluate the normally distributed data. Significance level were set as $p < 0.05$.

Results

The experimental rats well tolerated intramuscular amikacin and selenium. Weight loss or excessive weight gain was not observed. Dpgram and I/O levels of the conveinient rats were recorded after successful otoscopic examination as follows.

In day 1, there were statistically significant difference between post-treatment 4000 Hz measurements for the groups ($p < 0.01$) (Table 1) . As a result of the Post-Hoc Tukey HSD in order to determine difference between the groups; *"Control"* group and *"Amikacin"* group hearing levels were significantly higher than *"Amikacin+Selen^{60}"* group (p:0.030; p:0.005) (Table 2).

In day 14 and day 28, there were statistically significant difference between the groups in 4000 Hz hearing levels ($p < 0.01$) (Table 1). As a result of the Post-Hoc Tukey HSD in order to determine difference between the groups; *"Control"* group hearing levels were significantly higher than *"Amikacin"*, *"Amikacin+Selen^{60}"* and *"Amikacin+Selen^{300}"* groups (p:0.001; p:0.001; p:0.001). (Table 2)

In day 1, there were no statistically significant difference between post-treatment 4761 Hz measurements for the groups ($p > 0.05$).

In day 14 and day 28, there were statistically significant difference between post-treatment 4761 Hz measurements for the groups ($p < 0.01$) (Table 3). *"Control"* group hearing levels were significantly higher than *"Amikacin"*, *"Amikacin+Selen^{60}"* and *"Amikacin+Selen^{300}"* groups (p: 0.001; p: 0.001; p: 0.001) (Table 4).

Table 1. Evaluation of DPOAE at 4004 Hz on day 1, day 14 and day 28

4004 Hz	Control	Amikacin	Selen ^{60}	Selen ^{300}	++p
	Mean±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Day 1	28.48±7.46	29.91±6.06	22.25±5.23	27.25±5.79	0.006**
Day 14	27.72±7.51	18.43±3.81	15.90±2.63	19.66±4.78	0.001**
Day 28	27.31±7.37	16.57±2.93	15.25±2.42	18.38±3.32	0.001**

Repeated measures ANOVA ***p<0.01*

Table 2. Evaluation of DPOAE between the groups at 4004 Hz (Post-Hoc)

4004 Hz	Day 1	Day 14	Day 28
Control - Amikacin	0.916	0.001**	0.001**
Control - Selen ^{60}	0.030*	0.001**	0.001**
Control - Selen ^{300}	0.942	0.001**	0.001**
Amikacin - Selen ^{60}	0.005**	0.486	0.836
Amikacin - Selen ^{300}	0.621	0.900	0.662
Selen ^{60} - Selen ^{300}	0.114	0.159	0.205

Post-Hoc Tukey HSD test **p<0.05* ***p<0.01*

Table 3. Evaluation of DPOAE at 4761 Hz on day 1, 14 and 28

4761 Hz	Control	Amikacin	Selen 60	Selen 300	++p
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Day 1	32.01±6.39	36.30±3.55	32.76±6.20	36.43±5.87	0.062
Day 14	32.22±6.31	21.61±4.44	20.10±3.94	23.93±4.01	0.001**
Day 28	32.09±6.66	18.41±3.07	18.07±2.71	21.06±2.33	0.001**

Repeated measures ANOVA ***p<0.01*

In day 1, there were no statistically significant difference between post-treatment 5652 Hz measurements for the groups in (p>0.05) (Table 5).

In day 14 and day 28, there were statistically significant difference between post-treatment 5652 Hz measurements for the groups (p <0.01) (Table 5). As a result of the Post-Hoc Tukey HSD in order to determine difference between the groups; *"Control"* group hearing levels were significantly higher than *"Amikacin", "Amikacin+Selen^{60}"* and *"Amikacin+Selen^{300}"* groups (p:0.001; p:0.001; p:0.008) (Table 6); In day 28, *Amikacin+Selen^{300}"* group were also higher than *"Amikacin+Selen^{60}"* group (p:0.051) (Table 6).

In day 1, there were no statistically significant difference between post-treatment 6726 Hz measurements for the groups (p>0.05) (Table 7).

In day 14 and day 28, there were statistically significant difference between post-treatment 6267 Hz measurements for the groups (p <0.01) (Table 7). As a result of the Post-Hoc Tukey HSD in order to determine difference between the groups; *"Control"* group hearing levels were significantly higher than *"Amikacin", "Amikacin+Selen^{60}"* and *"Amikacin+Selen^{300}"* groups (p:0.001; p:0.001; p:0.001) (Table 8).; In day 28, *Amikacin+Selen^{300}"* group were also higher than *"Amikacin+Selen^{60}"* group (p:0.057) (Table 8).

Table 4. Evaluation of DPOAE between the groups at 4761 Hz (Post-Hoc)

4761 Hz	Day 1	Day 14	Day 28
Control - Amikacin	0.148	0.001**	0.001**
Control - Selen ^{60}	0.982	0.001**	0.001**
Control - Selen ^{300}	0.129	0.001**	0.001**
Amikacin - Selen ^{60}	0.295	0.809	0.996
Amikacin - Selen ^{300}	1.000	0.516	0.266
Selen ^{60} - Selen ^{300}	0.263	0.116	0.175

Post-Hoc Tukey HSD test **p<0.05* ***p<0.01*

Table 5. Evaluation of DPOAE on 5652 Hz on day 1, day 14 and day 28)

5652 Hz	Control	Amikacin	Selen 60	Selen 300	++p
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Day 1	40.25±8.12	44.46±4.95	39.61±6.47	43.12±7.04	0.143
Day 14	37.98±8.31	28.28±6.14	22.93±5.39	29.88±7.32	0.001**
Day 28	36.50±9.62	24.21±5.24	18.91±3.53	24.88±5.70	0.001**

Repeated measures ANOVA ***p<0.01*

Table 6. Evaluation of DPOAE between the groups at 5652 Hz (Post-Hoc)

5652 Hz	Day 1	Day 14	Day 28
Control - Amikacin	0.300	0.001**	0.001**
Control - Selen ^{60}	0.993	0.001**	0.001**
Control - Selen ^{300}	0.628	0.008**	0.001**
Amikacin - Selen ^{60}	0.186	0.136	0.102
Amikacin - Selen ^{300}	0.942	0.913	0.991
Selen ^{60} - Selen ^{300}	0.460	0.300	0.051

Post-Hoc Tukey HSD test **p<0.05* ***p<0.01*

In day 1, there were no statistically significant between post-treatment 7996 Hz measurements for the groups (p>0.05) (Table 9).

In day 14 and day 28, there were statistically significant difference between post-treatment 7996 Hz measurements for the groups (p <0.01) (Table 9). As a result of the Post-Hoc Tukey HSD in order to determine difference between the groups; *"Control"* group hearing levels were significantly higher than *"Amikacin"*, *"Amikacin+Selen^{60}"* and *"Amikacin+Selen^{300}"* groups (p:0.001; p:0.001; p:0.001) (Table 10).

Discussion

Ototoxic effects of aminoglycoside antibiotics have been well known since the discovery of the first aminoglycoside antibiotic "streptomycin". The drugs in this group have different rates of ototoxic effects. Kahlmeter et al [9] investigated 10,000 patients for the toxic effect of aminoglycosides on the cochlea and found 8.6 % gentamicin, 13.9 % amikacin, 6.1 % tobramycin and 2.4 % netilmicin ototoxicity.

Aminoglycosides causes progressive damage in the outer hair cells of the cochlea, especially at the basal

Table 7. Evaluation of DPOAE at 6726 Hz on day 1, day 14 and day 28

6726 Hz	Control	Amikacin	Selen 60	Selen 300	++p
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Day 1	30.89±6.55	32.98±6.21	32.56±3.76	34.71±4.72	0.271
Day 14	28.84±6.47	22.06±5.24	21.08±3.99	26.01±5.42	0.001**
Day 28	29.35±6.07	19.56±3.07	18.54±3.13	23.01±4.60	0.001**

Repeated measures ANOVA ***p<0.01*

Table 8. Evaluation of DPOAE between the groups at 6726 Hz (Post-Hoc)

6726 Hz	Day 1	Day 14	Day 28
Control - Amikacin	0.698	0.004**	0.001**
Control - Selen ^{60}	0.821	0.001**	0.001**
Control - Selen ^{300}	0.203	0.445	0.001**
Amikacin - Selen ^{60}	0.996	0.955	0.913
Amikacin - Selen ^{300}	0.804	0.172	0.130
Selen ^{60} - Selen ^{300}	0.679	0.056	0.057

Post-Hoc Tukey HSD test **p<0.05* ***p<0.01*

Table 9. Evaluation of DPOAE at 7996 Hz on day 1, day 14 and day 28

7996 Hz	Control	Amikacin	Selen 60	Selen 300	++p
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Day 1	37.08±4.05	38.41±5.10	38.05±3.65	39.06±6.35	0.716
Day 14	36.36±4.26	24.38±6.93	23.0±5.76	28.41±7.10	0.001**
Day 28	35.23±4.78	21.60±3.98	18.96±2.39	24.01±5.56	0.001**

Repeated measures ANOVA ***p<0.01*

Table 10. Evaluation of DPOAE between the groups at 7996 Hz (Post-Hoc)

7996 Hz	Day 1	Day 14	Day 28
Control - Amikacin	0.870	0.001**	0.001**
Control - Selen ^{60}	0.944	0.001**	0.001**
Control - Selen ^{300}	0.667	0.003**	0.001**
Amikacin - Selen ^{60}	0.997	0.919	0.323
Amikacin - Selen ^{300}	0.982	0.255	0.402
Selen ^{60} - Selen ^{300}	0.938	0.070	0.080

Post-Hoc Tukey HSD test *$p<0.05$ **$p<0.01$

parts towards the apical parts. This also explains the formation of high-frequency hearing loss initially [10].

Studies of the mechanism of aminoglycoside ototoxicity have shown that apoptotic pathways due to the formation of oxygen free radicals are responsible for the death of the outer hairy cells [3]. In experimental studies, the ototoxic effects of gentamicin on the hairy cells were restricted by providing glutathione in the diet [11].

Selenium is one of the antioxidant system elements that protect the cell membranes from oxidative damage [12]. Selenium is a cofactor for glutathione peroxidase in the reduction of the glutathione mechanism. This study was conducted to prevent the formation of free radicals, increasing the cofactor in the environment that increases the formation of reduced anti-oxidant glutathione.

Some studies are available in the literature that antioxidants may prevent oxidative damage. Weijl et al. [13] administered the antioxidant vitamins C, E and selenium in 48 patients with cisplatin chemotherapy and did not find a significant difference compared with the placebo group. However, the hearing threshold in the each of the three antioxidants groups decreased less compared to placebo group than cisplantin group. Also in animal experiments, the application of selenium with gentamicin treatment prevented the renal damage by reducing oxidative stress caused by free oxygen radicals [14, 15].

The effect of glutathione co-therapy on the expression of gentamicin ototoxicity was tested in pigmented guinea pigs and this study suggested that glutathione protected against ototoxicity by interfering with the cytotoxic mechanisms [16].

A hospital-based case–control study with 294 individuals was conducted. All case subjects had average hearing over 25 decibels. Selenium was found to be inversely associated with the hearing threshold, and it was suggested as an antagonist to lead ototoxicity [17]. However, little literature exists evaluating the effectiveness of selenium on aminoglycoside ototoxicity.

In this study, we investigated the effect of low-dose and high-dose selenium on ototoxicity. Although selenium is an essential trace element, it is toxic if taken in excess. Rats received 300-microgram selenium as high doses because exceeding the tolerable upper intake level of 400 micrograms per day can lead to selenosis [18].

Rats were divided into 4 groups and measurements were performed on day 1, day 14 and day 28 with DPOAEs. We used distortion-product otoacoustic emissions (DPOAE) to evaluate hearing loss levels. The reliability of DPOAE as an objective test method with useful frequencies in the evaluation of cochlear functions has been demonstrated [19]. DPOAE is easily applicable even in very small experimental animals. Hotz et al. [20] reported that TEOAE is a useful method for monitoring ototoxic side effects.

As the results of the DPOAEs in the groups, "control" group had no statistically significant hearing loss in all frequencies compared with day 1 and day 14 ($p>0.05$). There were also no significant hearing loss in day 14 and day 28 ($p>0.05$). This shows that ototoxicity was not observered.

In "amikacin" group, there were significant hearing loss in all frequency in day 14 according to the day 1 ($p<0.01$); there were also significant hearing loss in day

28 compared with day 14 ($p<0.01$). This showed that ototoxicity increased with the cumulative effects of amikacin.

In both "amikacin+selenium^{60}" and "amikacin+selenium^{300}", there were significant hearing loss in day 14 compared to day 1 ($p<0.01$); there were also significant hearing loss in day 28 compared with day 14 ($p<0.01$). This showed that the low dose and high dose selenium with amikacin had no protective effect according to the pre and post treatment hearing levels.

As the results of the DPOAEs between the groups *(repeated measures ANOVA and post-hoc Tukey test*), there were no significant differences in all frequencies between "amikacin" group, "amikacin+selenium^{60}" group and "amikacin+selenium^{300}" group (Tables 1-10).

In this study, the long duration of treatment were assessed in rats and both the time and the dose of selenium had no the protective effects on amikacin-induced ototoxicity.

A large number of human studies have been performed to improve knowledge of the influence of selenium on the origin and development of several degenerative diseases. Controversy about the optimum dietary level of this element to cure or prevent diseases such as cirrhosis, cancer, diabetes, or cardiovascular pathologies remains [18].

This study has shown that selenium had no anti-oxidant mechanism on cochlea although selenium prevents oxidative damage in many organs. The protective effect of selenium on amikacin-induced ototoxicity is the first of its kind. The effects of quantities of selenium consumed in daily life upon hearing are among questions that remain to be answered.

Many studies have shown antioxidant activities for selenium, but the long duration of treatment were assessed in rats and both the time and the dose of selenium had no the protective effects on amikacin-induced ototoxicity in this study,.

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