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ORIGINAL ARTICLE

Does Aluminum Cause Ototoxicity in Rats?

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Background: Aluminum (Al) is a nonessential and toxic metal to which humans are frequently exposed. Except one study which revealed adverse effects of serum Al levels on the auditory functions in hemodialysis patients, there is not any other study on the effects of Al on auditory functions.

Study design: Acute and chronic effects of Al on rat auditory system were investigated in that randomized controlled study.

Methods: Forty five male Sprague-Dawley rats were included. Rats were divided into six groups according to the dose and route of Aluminum chloride ($AlCl_3$): in groups A (n=7), B (n=9), and C (n=9), intraperitoneally (IP) $AlCl_3$ was injected in doses of 1mg/kg, 5mg/kg, and 80mg/kg, respectively; in control group (group K, n=6), saline was injected IP; in groups D (n=7) and E (n=7) oral $AlCl_3$ was administered in doses of 5mg/kg, and 50mg/kg, respectively. OAE measurement was performed for four times in IP $AlCl_3$ groups; before and on the 1st, 7th, and 14th days after aluminum administration. In oral group OAE measurement was performed before and on the 1st, 2nd, and 3rd months after aluminum administration. The distortion product otoacoustic emissions (DPOAEs) at 2f1-f2 were recorded and analyzed. Histological examination of the cochlea was performed.

Results: DPOAE measurements of all groups before and after $AlCl_3$ administrations were not statistically different. Histological examination revealed normal stria vascularis, spiral ganglion and organ of corti in all groups.

Conclusion: Neither acute nor chronic administration of $AlCl_3$ in aforementioned doses and routes caused neither clinical nor histological ototoxicity.

Key words: Aluminum; ototoxicity

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Introduction

Acquired hearing impairment may be caused by different ototoxic substances that cause transitory or definitive alteration in auditory and vestibular functions [1,2]. In addition to the drug-related ototoxicity, damage to the hearing apparatus may occur from exposure to other physical agents including heavy metals such as lead and mercury [1,2]. Aluminum (Al) is a nonessential and toxic metal to which humans are frequently exposed [3]. While oral exposure to Al occurs through ingestion of Al-containing pharmaceuticals, foods and water, parenteral exposure can occur via contaminated total parenteral nutrition, intravenous solutions, or contaminated dialysates [3]. It is known as a neurotoxin in both human and animal models [4]. Recently, Chu et al [4] investigated the effects of serum Al levels on the auditory functions in hemodialysis patients, who are known to have high aluminum levels, and found that serum Al level correlated with the severity of hearing impairment [4].

As there is not any other study on the effects of Al on auditory functions, we conducted an experimental study investigating acute and chronic effects of the aforementioned metal in rat auditory system.

Materials and Methods

Animal groups and experimental conditions

Forty-five male Sprague-Dawley rats weighting 250-350 g were included in the study. Before and during the experiment rats were fed with standard rat chow and

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tap water ad libitum. They were maintained in a 12 h light/12 h dark cycle at 21˚C. Rats were randomly divided into six groups according to the dose and route of Aluminum chloride ($AlCl_3$) administration as mentioned below.

Test protocol

$AlCl_3$ administration was performed via oral or intraperitoneal (IP) route in different doses. In groups A (n=7), B (n=9), and C (n=9), intraperitoneally (IP) $AlCl_3$ was injected in doses of 1mg/kg, 5mg/kg, and 80mg/kg, respectively. In control group (group K, n=6), same amount of saline was injected IP. In groups D (n=7) and E (n=7) oral $AlCl_3$ was administered in doses of 5mg/kg, and 50mg/kg, respectively. Oral and IP $AlCl_3$ (Sigma A-3017) administrations were made by adding to regular drinking water and in the form of diluted in saline, respectively.

Otoacoustic emission measurements

Otoacoustic emission (OAE) measurements were made on the right ear of each animal with normal otoscopic findings. Rats without replicable OAEs were discarded from this study. OAE measurement was performed for four times in IP $AlCl_3$ groups; before and on the 1st, 7th, and 14th days after aluminum administration. In oral group OAE measurement was performed before and on the 1st, 2nd, and 3rd. months after aluminum administration. And in control group measurement was performed for two times; before and at the end of study.

The distortion product otoacoustic emissions (DPOAEs) at 2f1-f2 were recorded and analyzed utilizing the ILO-96 cochlear emission analyzer (Otodynamics, London). DPOAEs were measured as DP-gram, where the intensity levels of the primary tones were held constant and DPOAE data were recorded for different frequency regions, from 1 to 6.3 kHz (1001, 1257, 1587, 2002, 2515, 3174, 4004, 5042 and 6348 Hz) and plotted as a function of f2. Unfortunately, the frequencies higher than 6,299 Hz could not be recorded because of the limits of the commercially available OAEs analyzer manufactured for use in humans. DP-grams, the intensities of primary stimuli, were set as equilevel (L1=L2) at 65 dB. The frequencies of the primary stimuli (f1 and f2) were adjusted as f2/f1=1.21. The earphone probe inserted into the animal's outer ear canal contained two separate speakers producing different primary tones. By decreasing the primary tone amplitudes from 75 to 36 dB SPL (sound pressure level) in 3-dB steps, the detection threshold and supra-threshold measures in the form of input/output (I/O) functions were obtained. The DPOAEs were measured and recorded as an average of four separate spectral averages of each stimulus condition. Using similar averaging techniques, the level of the noise floor was measured at the frequency that was 50 Hz above the DPOAE frequency. For both the DP-gram and I/O functions, if the DPOAEs at 2f1-f2 were ≥3 dB above the noise floor level at the 2f1-f2+50 Hz frequency, the emitted responses were accepted. All measurements were accomplished under brief anesthesia induced by an intramuscular injection of ketamine (30 mg/kg) mixed with xylazine (6 mg/kg). DPOAE measurements were taken after otoscopic examination of the rats on each testing day to exclude middle ear pathology. All of the animals were tested by an ILO 96 cochlear emission analyzer (Otodynamics, London) prior to the treatment to determine the baseline hearing status. DP-grams between the 1 to 6.3 kHz and I/O functions at 3, 4, 5 and 6 kHz were recorded, and the detection thresholds of each frequency for I/O functions were noted. Separate threshold and I/O function plots were constructed on each test day for each group of subjects. Second DPOAE measurements were taken on day 5 of the therapy in all groups. The same test protocol was carried out on days 10 and 15 of the study. Body weights were recorded regularly throughout the treatment period on DPOAE testing days.

Histological methods

Anesthesia was induced with intramuscular injection of ketamine (30 mg/kg) mixed with xylazine (6 mg/kg), and the rats were sacrificed by decapitation after the last DPOAE measurements. After the temporal bones were taken and fixed in 10% formaldehyde, they decalcified in 10% ethylenediaminetetraacetic (EDTA) solution with pH 7. The cochlea was separated from the clemented temporal bones and intersected from cochlear modiolus to set up paraphine blocks after routine histological processes were done. Sections were taken from paraphine blocks in 5 µm thickness and colored with hematoxiline-eosin. The section were analyzed with Leica DFC 280 Light microscope and photographed with Leica Q Win image analysis system.

Statistical methods

The non-parametric tests were used for the results of the study. Results of repeated measurements were analyzed statistically by Friedman's two-way ANOVA test to determine differences in amplitudes of DPOAEs and corresponding noise floor differences and thresholds for each frequency. In all analyses, the SPSS 13.0 statistical analysis program was utilized, and $P<0.05$ was defined as the level of significance.

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Results

DPOAE I/O measurements of IP $AlCl_3$ groups (Group A, B, C), and control group (Group K) were shown in Figs. 1, 2, 3, and 4, respectively ($p> 0.05$). DPOAE I/O amplitudes in 3000, 4000, 5000 and 6000 Hz frequencies were measured by decreasing the primary tone amplitudes from 75 to 36 dB SPL in 3-dB steps. DPOAE I/O measurement was performed in IP $AlCl_3$ groups; before and on the 1st, 7th, and 14th days after aluminum administration. In control group; before and at the end of study (Figs 1,2,3,4).

DP-gram measurements of IP AlCl3 groups (Group A, B, C), oral $AlCl_3$ groups (Group D, E) and control group (Group K) were shown in Fig. 5 ($p> 0.05$). DP-grams between the 1 to 6.3 kHz were recorded in IP $AlCl_3$ groups (Group A, B and C); before and on the 1st, 7th, and 14th days after aluminum administration. In oral groups (Group D and E); before and on the 1st, 2nd, and 3rd. months after aluminum administration. In control group (Group K) measurement was also performed for two times; before and at the end of study.

The statistical analysis showed no significant differences in I/O amplitudes and DP-gram measurement sessions of the whole study between groups ($p> 0.05$).

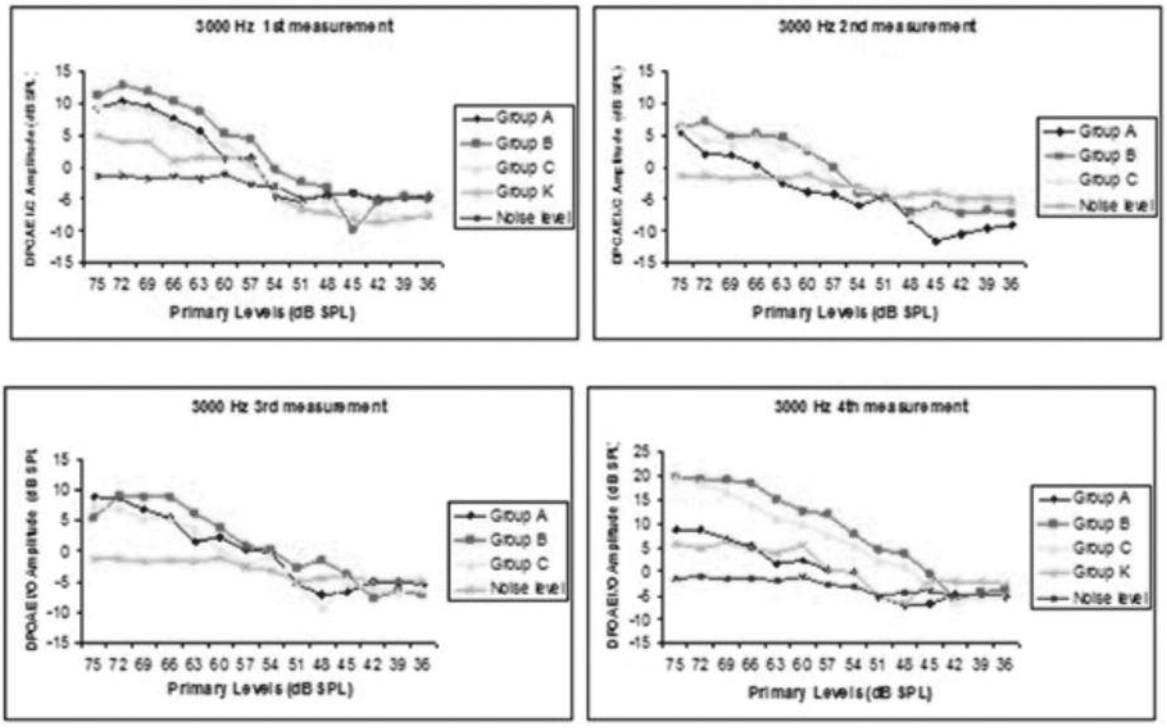

Figure 1. DPOAE I/O amplitudes in 3000 Hz were measured by decreasing the primary tone amplitudes from 75 to 36 dB SPL in 3-dB steps in IP $AlCl_3$ administrated groups (Group A, B and C) and control group (Group K). DPOAE I/O measurement was performed four times in IP $AlCl_3$ groups; before and on the 1st, 7th, and 14th days after aluminum administration. In control group measurement was performed for two times; before and at the end of study.

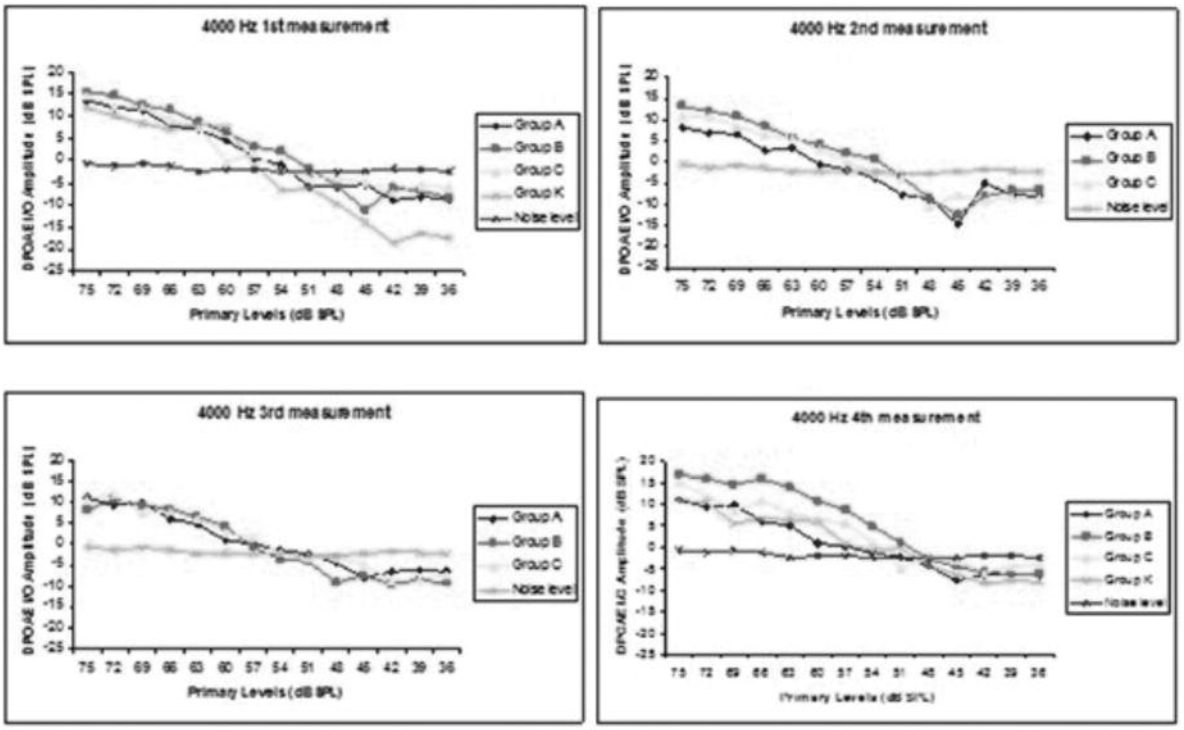

Figure 2. DPOAE I/O amplitudes in 4000 Hz were measured by decreasing the primary tone amplitudes from 75 to 36 dB SPL in 3-dB steps in IP $AlCl_3$ administrated groups (Group A, B and C) and control group (Group K).

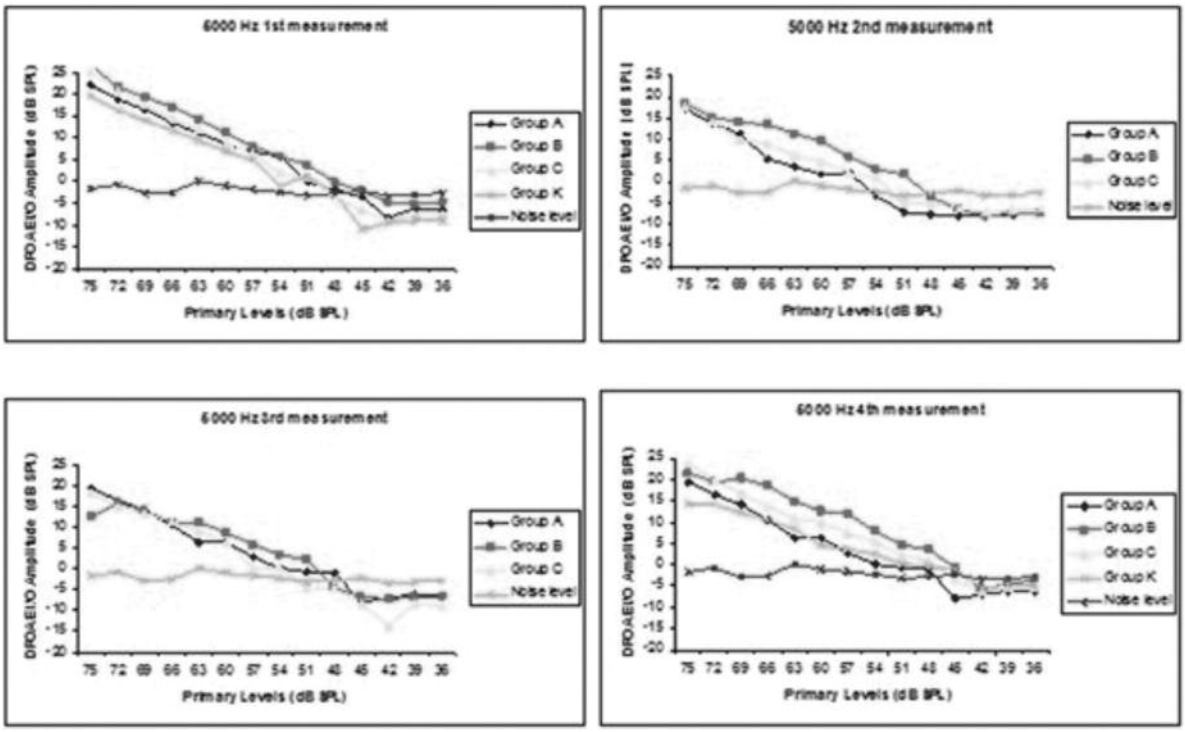

Figure 3. DPOAE I/O amplitudes in 5000 Hz were measured by decreasing the primary tone amplitudes from 75 to 36 dB SPL in 3-dB steps in IP AlCl3 administrated groups (Group A, B and C) and control group (Group K).

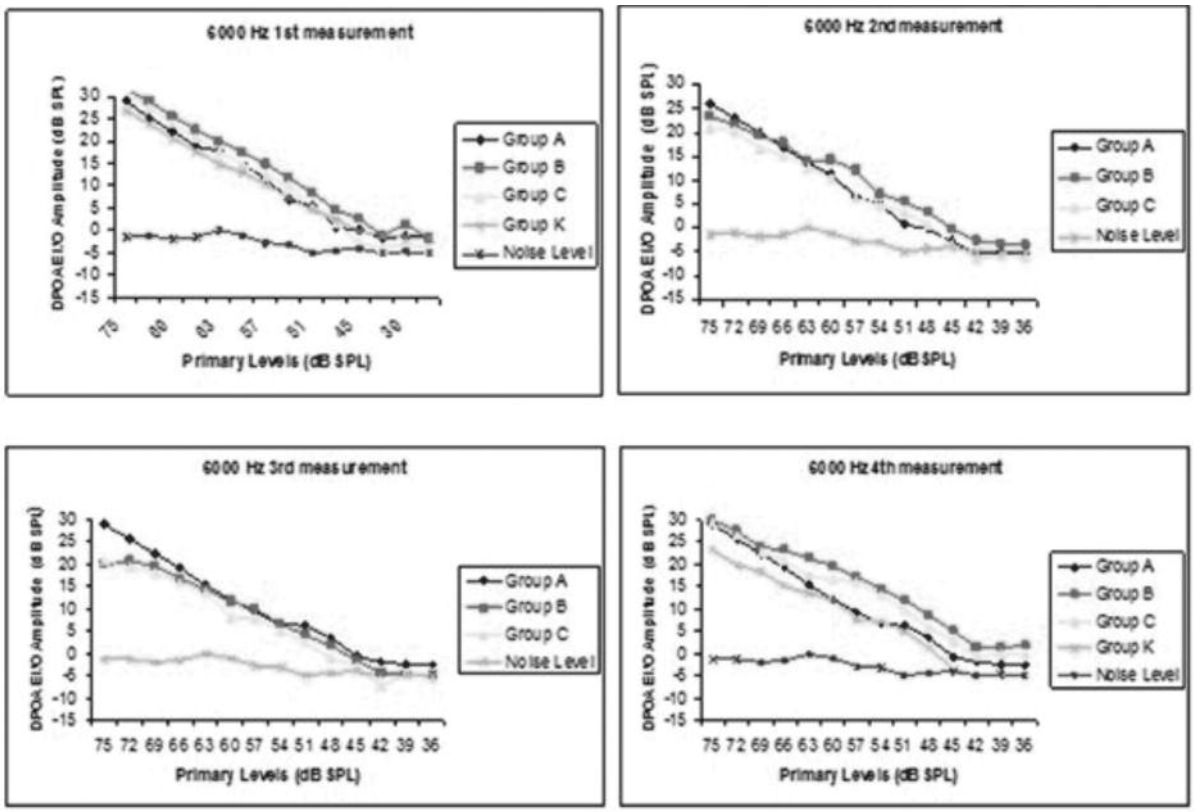

Figure 4. DPOAE I/O amplitudes in 6000 Hz were measured by decreasing the primary tone amplitudes from 75 to 36 dB SPL in 3-dB steps in IP $AlCl_3$ administrated groups (Group A, B and C) and control group (Group K).

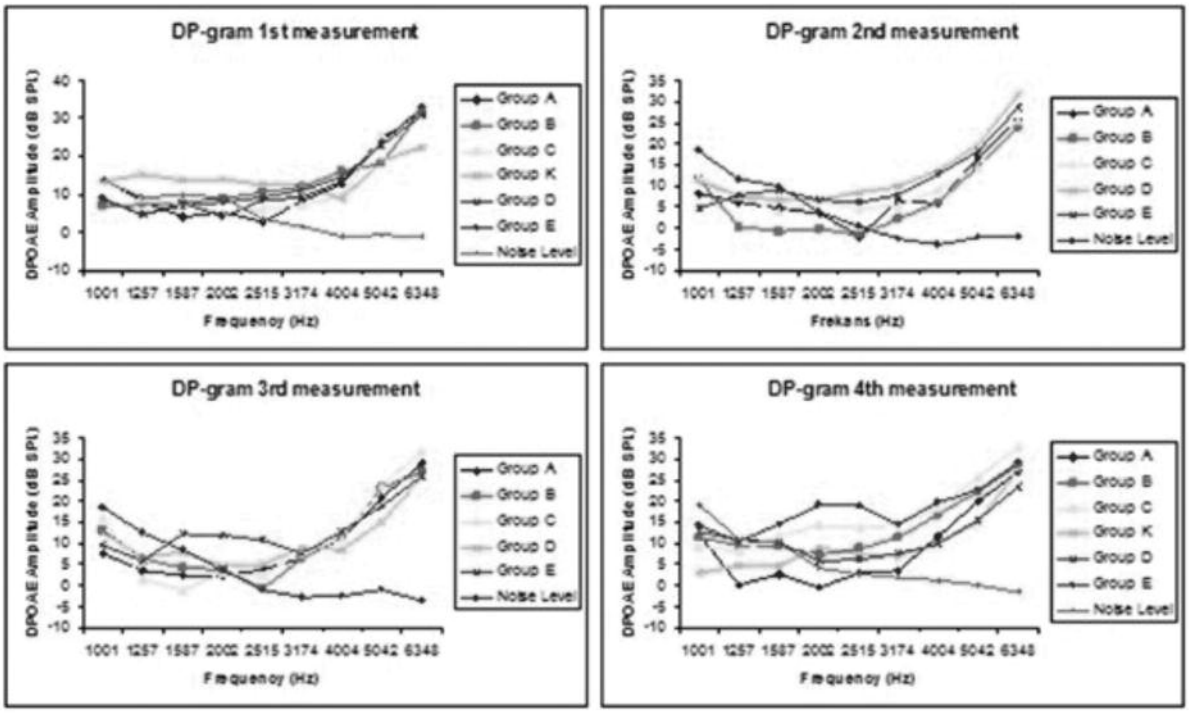

Figure 5. DP-grams between the 1 to 6.3 kHz and I/O functions at 3, 4, 5 and 6 kHz were recorded for four times in IP $AlCl_3$ groups (Group A, B and C); before and on the 1st, 7th, and 14th days after aluminum administration. In oral groups (Group D and E); before and on the 1st, 2nd, and 3rd. months after aluminum administration. In control group (Group K) measurement was performed for two times; before and at the end of study.

Histological examination revealed normal stria vascularis, spiral ganglion and organ of corti both in control group and all other groups that were given $AlCl_3$ (Figs. 6A, 6B, and 6C).

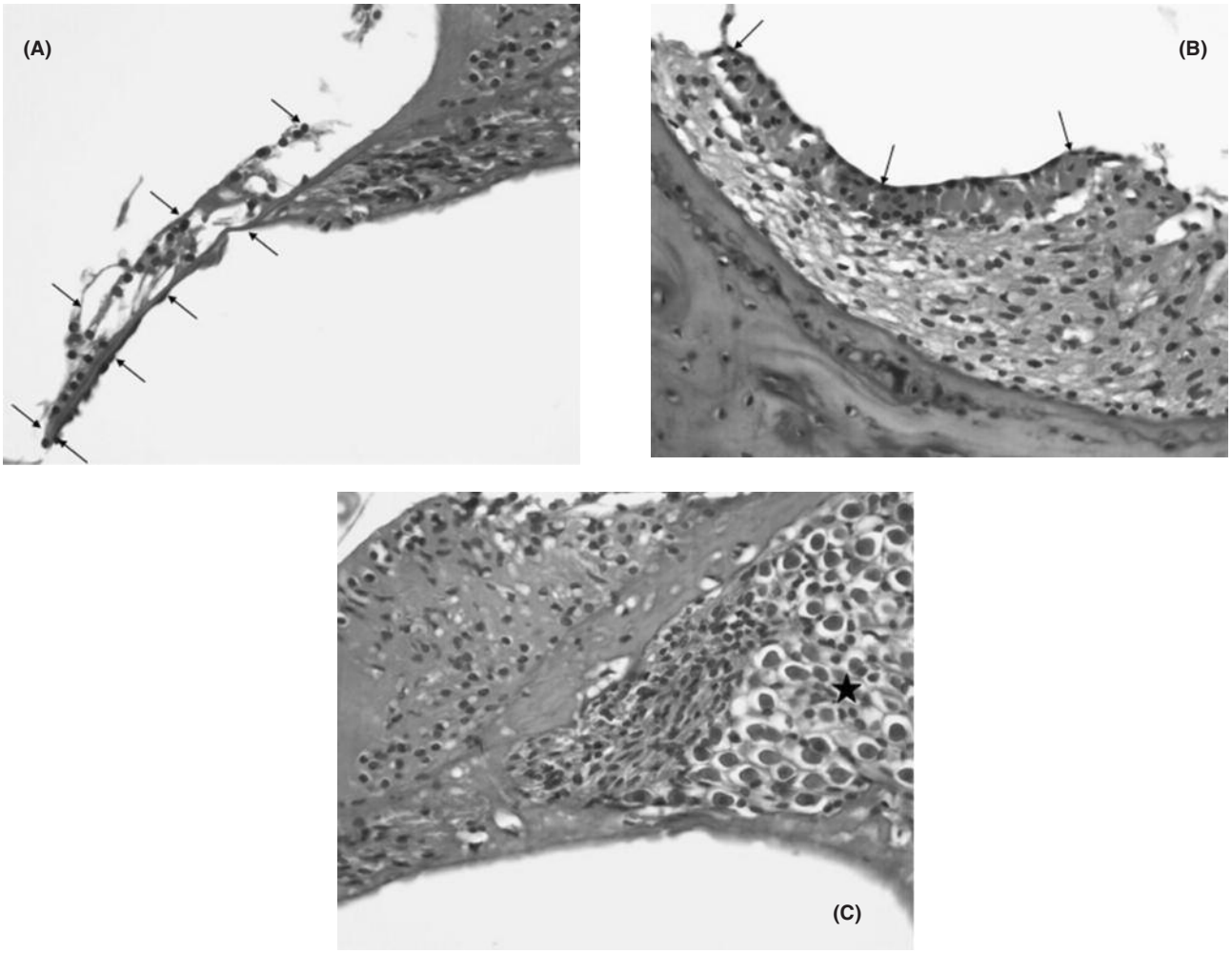

Figure 6. A-Corti organ (arrows), **B-**stria vascularis (arrows), and C-spiral ganglion (star) of group **C** (80 mg/kg $AlCl^3$ injected group). Normal histological findings. H-E, X40.

Discussion

It is known that aluminum is a neurotoxic agent which passes blood brain barrier easily but is cleared with difficulty from central nervous system, which is probably due to the slowness of neuron cycle [4]. In view of this information, it can be said that brain is a target organ for aluminum. It is thought that aluminum accumulation in the brain may play a key role in dialysis encephalopathy and probably in Alzheimer disease. [5,6]

Inner ear is a specialized nerve tissue sensitive to many toxins and ischemic events. Our study based upon the hypothesis that aluminum may have a detrimental effect on hearing functions disclosed that aluminum did not influence hearing in rats irrespective of dose, administration method and whether it is short or long term. Yet, in the study of Chu et al [4] on 40 dialysis patients with accumulation of aluminum, pure tone audiometry, otoacoustic emission and brain stem hearing responses were examined and hearing loss at high frequencies was found to be the most marked pathological effect. In the aforementioned study, worse hearing levels were determined in pure tone audiometry and lower amplitudes were observed in 3

KHz and 4 KHz. Age was found to be an important determinant factor, and after age was matched, it was established that aluminum levels were not correlated with 3 K and 4 K measurements, pure tone audiometry measurements and ABR results, but measurements at 2 K correlated with aluminum levels [4].

The reason why aluminum did not have any adverse effect on hearing in our study may be as follows; 1) The doses that we administered were not high enough to produce this effect, 2) In rats, metabolism of aluminum and its excretion from kidney decreases its toxicity, 3) Hearing measurement technique which we used was not able to detect hearing loss in rats, 4) Aluminum has no adverse effects on hearing.

In the neurotoxicologic studies that have been carried out on rats with aluminum so far, doses used, aluminum component and ways of administration were different. The lowest dose of aluminum which exerts noticeable side effects was reported to be 100mg Al/kg/day [7,8] However, at this level, no histological damage was observed in brain or spinal cord [7]. This was supported by another study in which a higher dose (130 mg Al/kg/day) was used [9]. However, in another study, by Florence [10] it was demonstrated that with the dose of 84 mg/kg histopathological changes may occur in astrocytes and neurons. The lowest dose required for the side effect to appear may vary between different species of animals and may also be related to the chemical form of aluminum [11]. In addition, in various studies it was reported that personal threshold values may also show variation [11]. Mameli et al [11] stated that the lowest dose producing side effect was 43.3 mg/kg/day. In the mentioned study, the side effect established was disturbance in vestibuloocular reflex. Normally aluminum is excreted by healthy kidney. However, in chronic renal failure, it was reported that serum values increased after the intake of over 35mg/kg aluminum hydroxide [11]. All of the above data indicate that different doses are required for the production of different side effects. Although the doses that we used varied widely, they may have been inadequate to produce hearing loss.

When aluminum salts were inoculated to intrathecal areas and cerebral cortexes of experimental studies, animals exerted different effects depending on the species, age, administration time, dose and route of administration. Aluminum should pass blood brain barrier so that it can produce neurotoxic effects [12]. Aluminum given by oral route does not lead to encephalopathy. In rats, compared to cat, dog and rabbits, brain aluminum concentrations even more than six fold higher did not result in encephalopathy [13]. The fact that our experimental animals were rats may account for the lack of expected effect. However, in human beings, especially in patients undergoing hemodialysis, it is thought that aluminum accumulation due to total parenteral nutrition and employment of aluminum based phosphorus linking gels is the most important cause of osteodystrophia and encephalopathy [5]. In those patients, high aluminum values were shown in brain, cerebrospinal fluid, serum and hair [14]. In the brains of hemodialysis patients with such problems, no specific finding could be demonstrated [5]. In the present study, aluminum encephalopathy findings developed in none of the animals. While aluminum encephalopathy occurs in human with renal failure, it is not seen in animals, which is ascribed to blood brain barrier variation developing owing to renal failure [15]. Similarly, the fact that aluminum affected hearing in hemodialysis patients, but we could not show this in our study can be explained by the employment of healthy rats in our study. First evidence of neurotoxicity induced by aluminum was described in long term hemodialysis patients developing neurodegenerative syndrome.

It is known that excessive amount of aluminum is excreted in normal individuals [16]. It is estimated that daily aluminum intake is 2-6 mg /day for children and 6-14mg/day for adults and thought that in normal excretion with urine doses far over the above ones can be tolerated. For the development of aluminum neurotoxicity, brain concentrations should be over 10-20 fold of normal concentrations. In the study of Mameli et al [11], it was demonstrated that chronic aluminum exposure leads to high concentrations in nervous tissue even if it does not change blood concentrations. However, these findings contradict with the results of the study of Golub et al [17] in which aluminum was used at the dose of 130 mg/kg day, but high aluminum concentrations could not be demonstrated in the brain. The idea that aluminum itself changes blood brain barrier and this metal is accumulated in nervous tissue is accepted by some investigators [18-21].

It was demonstrated that aluminum induced insoluble amyloid beta protein accumulation, impaired inositolphosphate system and calcium regulation, and organelle cell function (lysosomal and mitochondrial activity), differentiation (cell specific protein synthesis) and cell skeleton organisation and altered neurotransmission [22] in experimental studies. It affects neurotransmission by increasing glutamin concentration in hippocampus and neocortex and by influencing colinergic, noradrenergic and gabaergic systems [18,23-25].

The intraneural accumulation of aluminum was demonstrated using Scanning electron microscope and spectrometry [13]. In two patients with dialysis encephalopathy, blood, hair and brain aluminum values were shown to be much higher than normal values. In these cases, neurofibrillary material was detected in the cytoplasm of cortical neuron. Neurotoxicity associated with high brain aluminum concentrations has an impact on axonal transport, neurotransmitter and receptor activation and dendritic morphology. It is thought that aluminum influences brain enzyme activity (acetylcholinesterase, catecholamine balance) and microsomal and ribosomal protein synthesis [13]. Another probable reason for the lack of adverse effect of aluminum on hearing may be that we may have used a measurement technique, which was not accurate enough. In the early detection of hearing loss induced by ototoxic drugs, high frequency audiometry is known to be a sensitive technique and many investigators think that hearing monitorisation should be carried out before the development of degenerative events in cochlea. However, there is no consensus on the interpretation of results. Park [26] suggested that pure tone audiometry and acoustic reflexes should be investigated following high frequency audiometry. Fausti et al [27,28] proposed a test procedure using five frequencies (8kHz, 9kHz, 10kHz, 12.5kHz and 16kHz). Otoacoustic emissions show the response of external hair cells in the cochlea, i.e. they are involved with cochlear bioactive mechanism. As evoked otoacoustic emission can measure a larger field of frequency and higher frequencies, it is a more effective method in detecting ototoxicity [29]. The most important drawback of the otoacoustic emission employed in our study was that it could make measurements as high as 6300 Hz, namely it was not able to measure hearing losses that may have occurred at higher frequencies. Therefore, we do not know whether the doses we used had any adverse effect on hearing at frequencies over 6300 Hz.

Finally, the lack of hearing loss in our study may result from the case that aluminum is actually not ototoxic. The only report regarding the ototoxicity of aluminum in the literature is by Chu et al [4]. Ototoxicity was found in hemodialysis patients with chronic renal failure. It is known in these patients that many different metabolites and toxins accumulate apart from aluminum. In these patients, the rate of sensorineural hearing loss is higher than the general population [30] Bazzi et al [31] reported this incidence to be 77 % in their study. Audiometric findings found in this patient group are usually high frequency hearing losses [33-35]. It has been suggested that there are a few etiological factors associated with hearing loss in chronic renal failure. These are, ototoxic drugs, electrolyte imbalance, hyper tension, D vitamin deficiency, and hemodialysis itself [31,35-38]. Adler et al [39] found decrease in Na-K ATPase activity in the inner ear of guinea pigs with uremia and demonstrated that this correlated with serum creatinin levels. There are few histopathological studies on the status of inner ear during renal failure; Charachon et al. [40] reported demyelinisation in preganglionic cochlear fibers and cell loss in spiral ganglion at the rate of 25 %. Moreover, anatomic changes in labyrinth such as collapse of endolymphatic system, edema and atrophy, were also reported [41]. Correlation between hearing losses and albumin and calcium may account for sedimentation. Bergstrom et al [35] could not find any correlation between cochlear strial accumulation and calcium metabolism. After age variable was matched, Antonelli et al [34] established that in this age group, measurements showing reflex conduction from cochlea to cochlear nucleus yielded faulty results thinking that axonal uremic neuropathy led to subclinical dysfunction in 8th nerve.

Conclusion

In conclusion, in the present study which aimed to investigate the probable effect of aluminum on hearing functions, no statistically significant effect was found. It was determined that aluminum was not ototoxic or did not lead to ototoxicity at the doses we administered to rats. In order to draw firm conclusions, new studies

using otoacoustic emission measurements at higher frequencies and or higher doses of aluminum are required.

Acknowledgements

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