Int. Adv. Otol. 2012; 8:(3) 345-353

ORIGINAL ARTICLE

Assesment of Castellani Solution's Ototoxic Effects on Guinea Pigs Using Otoacoustic Emission and Auditory Evoked Brainstem Potentials

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Objective: We planned to evaluate the ototoxic effects of Castellani's solution on guinea pigs when used intratympanically.

Materials and Methods: Fourteen female adult albino guinea pigs were used in this experiment. All treatments were performed in seven consecutive days using intratympanic injection. The first group comprised of left ears of seven guinea pigs and received 0.2 ml of gentamicin. The second group comprised the left ears of the other seven guinea pigs and received 0.1 ml of Castellani's solution. Contralateral ears of all these fourteen guinea pigs were used as negative control group and received 0.2 ml of saline solution intratympanically. DPOAEs and ABRs were recorded three times for each guinea pig, prior to intratympanic injection, on the tenth and twenty-first days after the injections.

Results: In Groups 1 and 2, DPOAE were absent on tenth and twenty-first days of the experiment. However, for Group 3, there was no significant change in both responses for the same time periods before and after the injections. On the tenth and twenty-first day of the experiment, in Groups 1 and 2, ABRs thresholds were found to be significantly increased. According to statistical analysis, significant differences were found between Groups 1 and 3 as well as between Groups 2 and 3.

Conclusion: It is concluded that Castellani's solution has ototoxic effect on guinea pigs. Thus, based on this conclusion, Castellani's solution can not be considered to be safe to use patients with tympanic membrane perforation.

Submitted : 18 March 2012 *Accepted : 01 October 2012*

Introduction

Trials related to the medications featured by both strong efficacy in the treatment of otomycosis and lack of ototoxic effect has long been continued. Otomycosis is a mycotic infection which preferentially develops in external ear canal (EEC). The incidence mentioned in the medical literature for this disease that is seen more frequently in summer times varies 5 to 30% [1-3].

EEC hygiene constitute the most important stage in the treatment of otomycosis. Besides, some topical and systemic antifungal medications can be applied [1-4]. Topical antifungal agents are administered directly or as being dropped on applicator to EEC after EEC clearance until suppression of the infection. The topical antifungal agents are subdivided into two categories as specific and nonspecific agents [1-5].

Chemical agents or medications can lead to structural disruption and functional defects in cochlea, cochlear nerve and vestibular system. The hearing loss expected to occur in topical ototoxicity is especially of sensorineural type (SNHL), due to close relationship of middle ear with the cochlea [6,7]. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) are utilized in monitoring ototoxicity [8]. DPOAEs are known to originating from outer hair cells and especially has specificity over 20 dB SPL in SNHL cases [9,10].

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Castellani solution (Cs), which is one of the nonspecific topical medications used in the treatment of fungal otitis externa has long been used. First developed by Aldo Castellani in 1905, the Cs might comprise ingredients in various concentrations, though the solution's ingredients have been defined as the same in many sources [11,12]. However, this solution has been used for a long time, it's potential ototoxic effects have not been thoroughly investigated. Cs comprised of 0.3 g basic fuchsin, 10 g resorcinol, 4 ml aceton, 10 ml 90% ethyl alcohol, 4 g phenol, 1 g boric acid and 100 ml of distilled water. The main objective of this study is to evaluate the possible ototoxic effect of Cs solution when administered intratympanically into the middle ear of albino guinea pigs.

Materials and Methods

This study was performed after obtaining approval from Animal Experimental Local Ethical Committee of Dokuz Eylul University Medical School. The experimental protocol was conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (DHEW publication NIH 85-23, revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD). Fourteen female adult albino guinea pigs weighing between 500 gr to 650 gr were included in the study. During the study, the subjects were hosted in the Department of Laboratory Animal Science of Dokuz Eylul University Medical School in the following conditions: 12-hour long day-light and 12-hour long darkness; 24 – 26°C temperature; ability to reach any time to water and seeds, along with feeding cabbage; with a background sound below 50 dB SPL. Otomicroscopic examinations were performed before intratympanic injections in order to check the presence of otitis media or tympanic membrane (TM) perforation; thus, those of external ear and middle ear pathologies were excluded from the study. DPOAEs and ABRs tests were utilized to evaluate ototoxicity.

Intratympanic injection

All intratympanic injections were applied at the same time through 7 days. 0.2 ml (40 mg/ml) Gentamisin (Gm) (Genta 40 mg amp, İE Ulagay-Menarini Group) was administered intratympanically into the left middle ears of 7 guinea pigs (Group 1, positive control group). 0.1 ml of specially-produced Cs solution was administered under sterile conditions into the left ears of remaining 7 subjects (Group 2). Moreover, 0.2 ml saline solution (Sf) injected intratympanically into the right ears of all guinea pigs again under sterile conditions (Group 3, negative control group). EECs and TMs of all guinea pigs were examined otomicroscopically before all injections as well as audiological testings.

Audiological testings

DPOAEs and ABRs were repeated 3 times before intratympanic injection, at time of the injection and tenth and twenty-first day of the experiment. Sample recordings of ABR tests obtained at the time of injection, and tenth and twenty first days of the experiment are presented in figures 1, 2 and 3. The tests were performed in a sound-insulated room at,

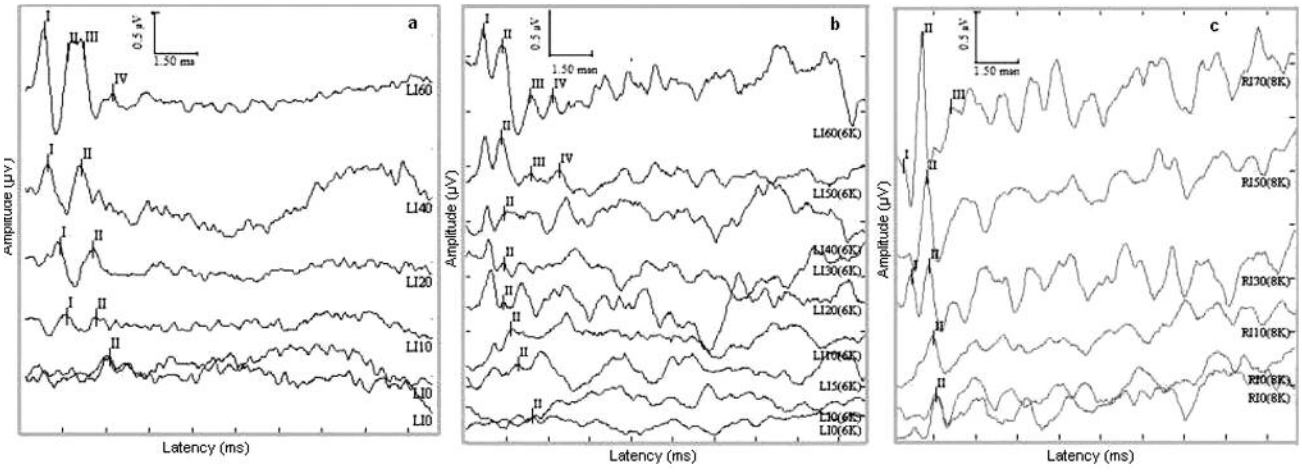

Figure 1. Samples of ABR traces recorded before intratympanic injections. **a;** Click stimulus responses, **b;** 6 kHz tone burst responses, **c;** 8 kHz tone burst responses, LI; left ipsilateral, RI; right ipsilateral.

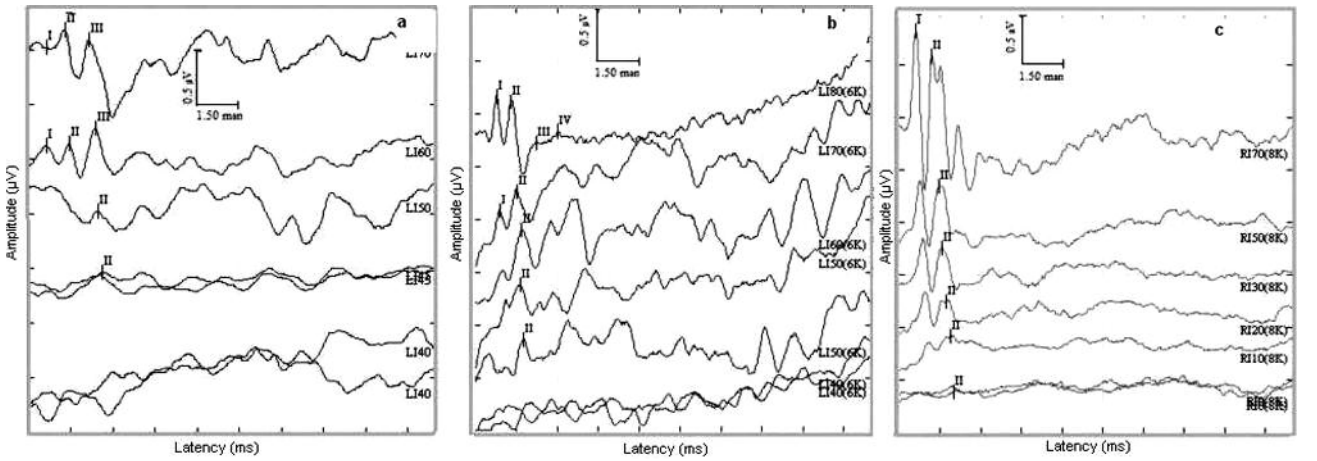

Figure 2. Samples of ABR traces obtained on tenth day after intratympanic injections. **a;** click responses for Gm, **b;** 6 kHz tone burst responses for Cs, **c;** 8 kHz tone burst responses for Sf, LI; left ipsilateral, RI; right ipsilateral.

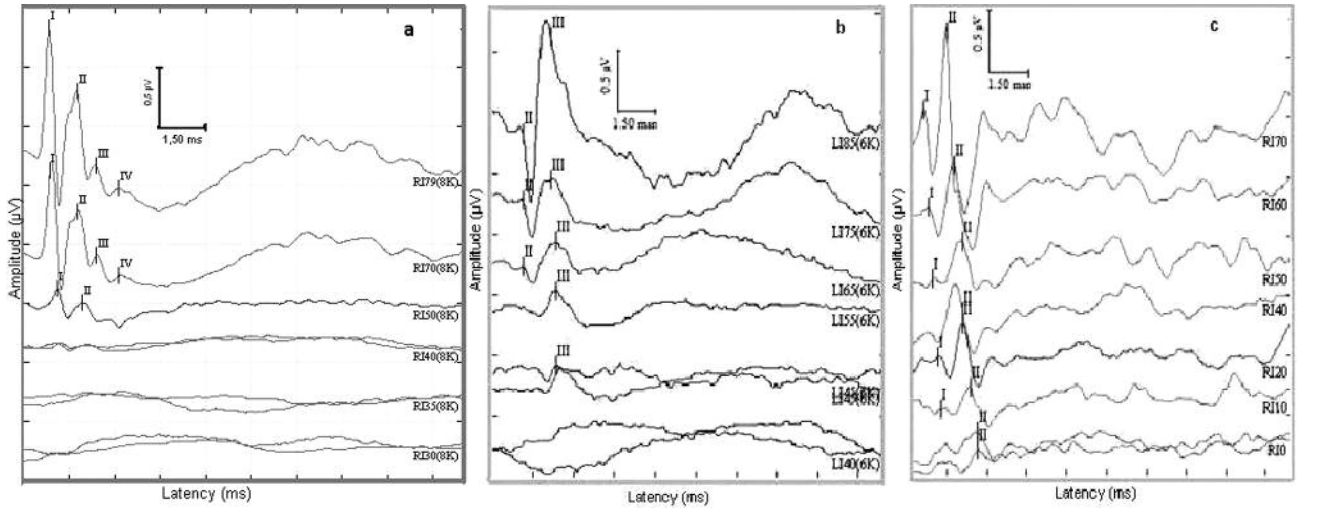

Figure 3. A sample of ABR traces obtained on twenty-first day after intratympanic injections. **a;** 8 kHz tone burst responses for Gm, **b;** 6 kHz tone burst responses for Cs, **c;** click responses for Sf, LI; left ipsilateral, RI; right ipsilateral.

temperature of 24-26°C. For ABRs recordings, ICS CHARTR EP Software V5.2 (GN Otometrics A/S, Denmark) system was used and the hearing thresholds were recorded. Four subcutaneous electrode were prepared as recording electrodes by soldering 22 gauge injector tip to each one of the standart gold-plated arched-electrodes. Two reference electrodes, one active electrode and one grounding electrode were placed to two retroauricular area, midline vertex and midline back, respectively [13]. For ABR testings, 6 kHz, 8 kHz tone burst and standart click stimuli were utilized. At least two recordings were obtained for the same stimulus in each ear (Table 1). The threshold levels were determined by commencing from a stimulus level of 90 dB and decreasing the level by 10 dB for supra threshold levels and by 5 dB for the levels near the threshold.

ILOv6 Otoacoustic emission device (Otodynamics Ltd, London, UK) was utilized for DPOAEs testings. In the DPOAE testings performed before the procedure and 10th and 21st days after the procedure, the ratio between f2 and f1 stimulus frequencies (f2/f1) were kept stable at 1.22. Moreover, the difference between L1 and L2 levels were kept stable at 10 dB SPL (L1 = 75 dB SPL, L2 = 65dB SPL). DPOAEs, at geometrik means, signal-noise ratios (SNR) at 1000, 1500, 2000, 3000, 4000, 6000 ve 8000 Hz were recorded.

Table 1. Stimulus and recording parameters applied for ABR testings

Stimulus type	Polarity	Transducer	Stimulus rate	Stimulus direction	Recording window	Amplifier gain	Recording filter	Frequency
Click	Alternating	Insert earphone	21.1/sec	Monoaural	15 msec	100 k	0,1 - 3 kHz	_
Tone Burst	Alternating	Insert earphone	31.1/sec	Monoaural	25 msec	100 k	0,05 - 1,5 kHz	6 kHz, 8 kHz

Anesthesia

100 mg/kg Ketamine (Ketalar vial, Pfizer) and 5 mg/kg Xysilazine (Basilazin %2, Bayer Drug industry and trade Co.inc) were administered by intraperitoneal route in order to provide anesthesia to the guinea pigs. %99 ether sulfuric (Diethyl Ether, Aktif Chemistry and Medical Devices Industry, Marketing, Domestic and Foreign Trade. Limited Company) were applied as maintenance anesthesia through respiratory system, monitoring the reflexes and motions of the subjects. After DPOAEs and ABRs tests performed on 21st day, sacrifications of the subjects were fulfilled by inhalating high dose of ether.

Statistical analysis

SPSS (Version 15.0 for Windows, SPSS Inc.) program was used for statistical analysis. Moreover, nonparametric tests also were utilized in statistical analysis due to the number of ears included in the study being below 30 as well as the presence of data not showing normal distribution and continuous alterations in the variables used. Wilcoxon Signed Ranks Test was utilized in all groups so as to evaluate both audiological tests results both before and after treatment (before intratympanic injection, on tenth and twenty-first days). Mann - Whitney U test was used to compare the results obtained from two different groups. Kruskal – Wallis test was used in order to compare the results obtained from three groups received simultaneous treatment.

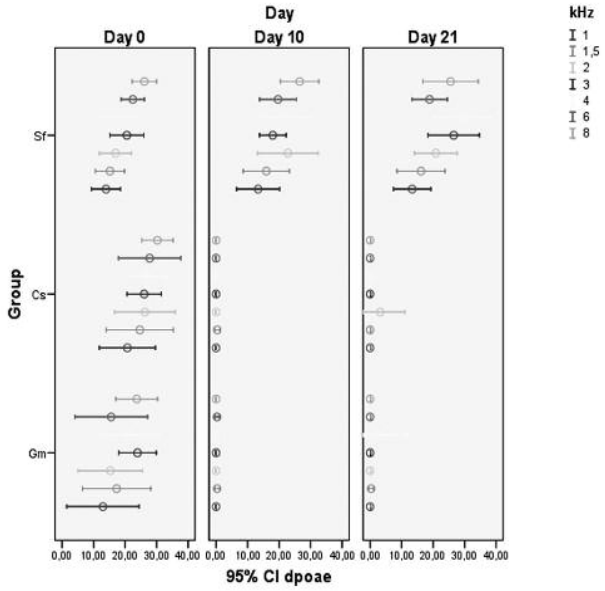

Figure 4. The mean values of DPOAE responses by days, calculated with Error Bar method.

RESULTS

One of the subjects in group 1 died on the tenth day of intratympanic injections due to anesthesia, so this subject was excluded from the statistical analysis. DPOAE responses could not be obtained in groups 1 and 2 on tenth and twenty-first days, while responses were obtained in group 3 (Table 2, Figure 4). When ABRs thresholds were compared to each other, significant increment in thresholds was observed in the ears of guinea pigs in group 1 and group 2 before the injections and on tenth and twenty-first days during the study (Table 3). Statistical analysis of the audiological parameters between groups 1 and 2 are presented in table 4 and these were found to be statistically significant. In addition, although significant difference was detected when ABR testholds before administration of saline solution and on twenty-first day were compared, this difference actually was below 5 dB in all tested ears (Figure 5). For this reason, it was not considered that this difference was related to ototoxicity. In the statistical analysis performed by using both of audiological test results, significant differences were found between groups 1 and 3 as well as between groups 2 and 3 (Table 5).

Table 2. Comparison of groups between each others before intratympanic injection, on 10th and 21st days following the commence of injections, based on DPOAE response parameters (mean +/- 1 SD values, median (Q1-Q3) values and p values of Kruskal-Wallis test).

	Group 1		Group 2		Group 3		
	Mn+/SD	**Md(Q1-Q3)**	**Mn+/SD**	**Md(Q1-Q3)**	**Mn+/SD**	**Md(Q1-Q3)**	**Kruskal Wallis Test**
1 kHz	12.96+/-10.96	15.45(0.00-21.47)	20.72 +/- 9.58	16.60(11.40-31.20)	13.33+/-9.79	12.30(6.65-17.70)	0.190
1.5 kHz	17.31+/-10.32	21.70(5.32-25.25)	24.68+/-11.48	23.20(16.90-37.60)	15.19+/-7.63	17.30(8.50-22.20)	0.213
2 kHz	15.35+/-9.77	16.35(4.87-25.17)	26.28+/-10.41	26.00(17.70-31.90)	16.97+/-8.44	18.00(7.95-22.30)	0.132
3 kHz	23.95+/-5.66	24.40(19.20-29.47)	26.05+/-5.86	27.60(21.70-31.10)	20.55+/-8.92	20.00(15.05-22.05)	0.97
4 kHz	21.58+/-8.87	21.95(16.42-28.17)	27.38+/-6.83	27.50(19.90-35.70)	17.77+/-9.96	17.20(7.00-28.65)	0.148
6 kHz	15.56+/-10.98	18.30(3.75-24.45)	27.80+/-10.71	29.80(20.10-37.20)	22.43+/-6.11	24.00(16.30-27.00)	0.111
8 kHz	23.70+/-6.29	24.05(17.82-29.45)	30.27+/-5.35	28.00(26.90-36.00)	26.12+/-6.43	27.00(20.20-32.25)	0.270
1 kHz	0	0	0	0	13.32+/-11.27	13.70(4.50-18.65)	< 0.001
1.5 kHz	0.30+/-0.73	0(0.00-0.45)	0.35+/-0.94	0	15.95+/-12.12	13.30(6.75-22.55)	< 0.001
2 kHz	0	0	0	0	22.80+/-15.93	19.90(7.25-39.15)	< 0.001
3 kHz	0	0	0	0	18.00+/-6.98	17.10(14.20-20.45)	< 0.001
4 kHz	0	0	0	0	22.83+/-13.10	20.40(11.25-29.50)	< 0.001
6 kHz	0.30+/-0.73	0(0.00-0.45)	0	0	19.65+/-9.68	18.70(14.20-27.70)	< 0.001
8 kHz	0	0	0	0	26.51+/-10.10	25.40(18.65-36.20)	< 0.001
1 kHz	0	0	0	0	13.33+/-9.79	12.30(6.65-17.70)	< 0.001
1.5 kHz	0.31+/-0.77	0(0.00-0.47)	0	0	16.16+/-12.59	10.20(7.50-28.60)	< 0.001
2 kHz	0	0	7.11+/-18.82	0	20.82+/-11.16	19.70(10.90-28.80)	< 0.001
3 kHz	0	0	0	0	26.54+/-13.51	22.10(17.10-39.70)	< 0.001
4 kHz	3.31 /- 0.12	0(0.00-4.97)	0	0	29.23+/-14.69	23.80(19.20-42.00)	< 0.001
6 kHz	0	0	0	0	18.86+/-9.28	19.50(17.80-24.95)	< 0.001
8 kHz	0	0	0	0	25.53+/-14.57	31.00(15.45-35.55)	< 0.001

Mn; mean value, SD; standart deviation, Md; Median value, Q-Q3; value of 25 and 75 percentiles.

Table 3. Comparison of groups between each others before intratympanic injection, on 10th and 21st days following the commence of injections, based on ABR thresholds (mean +/- 1 SD values, median (Q1-Q3) values and p values of Kruskal-Wallis test).

		Day 0			Day 10			Day 21		
		Click	6 kHz	8 kHz	Click	6 kHz	8 kHz	Click	6 kHz	8 kHz
Group 1	Mn+/SD	1.67 +/- 2.58	0	0	45.83 +/- 13.93	47.50 +/- 22.52	47.50 +/- 17.81	62.50 +/- 16.95	65.00 +/- 17.88	61.67 +/- 22.94
	Md(Q1-Q3)	0.00 (0.00-5.00)	0	0	47.50 (30.00-57.50)	42.50 (32.50-60.00)	50.00 (31.25-62.50)	60.00 (51.25-75.00)	67.50 (47.50-78.75)	67.50 (37.50-78.75)
Group 2	Mn+/SD	0	0	0	53.57 +/-16.51	50.00 +/- 22.54	46.43 +/- 22.30	60.71 +/- 10.17	45.71 +/- 11.70	45.00 +/- 10.40
	Md(Q1-Q3)	0	0	0	55.00 (45.00-70.00)	50.00 (50.00-70.00)	50.00 (45.00-60.00)	60.00 (55.00-70.00)	40.00 (40.00-55.00)	45.00 (40.00-55.00)
Group 3	Mn+/SD	2.69 +/- 5.99	1.92 +/- 5.60	1.54 +/- 5.54	3.85 +/- 5.82	3.46 +/- 5.54	2.69 +/- 5.63	5.00 +/- 5.40	4.23 +/- 5.34	4.23 +/- 5.34
	Md(Q1-Q3)	0.00 (0.00-2.50)	0	0.00 (0.00-5.00)	0.00 (0.00-5.00)	0.00 (0.00-5.00)	0.00 (0.00-5.00)	5.00 (0.00-5.00)	5.00 (0.00-5.00)	0.00 (0.00-5.00)
Kruskal Wallis Test		0.321	0.353	0.607	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Mn; mean value, SD; standart deviation, Md; Median value, Q-Q3; value of 25 and 75 percentiles.

Table 4. Comparison of DPOAE responses and ABR thresholds of the groups between each other by using of DPOAE Wilcoxon Signed Ranks Test (p values)

		DPOAE Frequancy							ABR Stimulus Type		
	Day	1 kHz	1.5 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz	Click	6 kHz Tone burst	8 kHz Tone burst
Group 1	0-10	0.068	0.028	0.028	0.028	0.028	0.043	0.028	0.027	0.028	0.027
	0-21	0.068	0.028	0.028	0.028	0.046	0.043	0.028	0.028	0.028	0.027
	10-21	1.000	0.655	1.000	1.000	0.317	0.317	1.000	0.026	0.066	0.072
Group 2	0-10	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.016	0.027
	0-21	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.017	0.018
	10-21	1.000	1.000	0.317	1.000	1.000	1.000	1.000	0.084	0.343	0.527
Group 3	0-10	0.485	0.972	0.221	0.173	0.152	0.422	0.807	0.083	0.102	0.083
	0-21	0.600	0.972	0.422	0.221	0.055	0.422	0.944	0.014	0.014	0.008
	10-21	0.780	0.650	0.753	0.075	0.173	0.917	0.807	0.083	0.414	0.102

Table 5. Significance values obtained as a result of comparisons between two groups by applying Mann-Whitney U test (p values).

		DPOAE Frequancy							ABR Stimulus Type		
	Day	1 kHz	1.5 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz	Click	6 kHz Tone burst	8 kHz Tone burst
Group 1 & Group 2	0	0.252	0.317	0.116	0.568	0.253	0.460	0.199	0.111	1.000	1.000
	10	1.000	1.000	1.000	1.000	1.000	0.280	1.000	0.387	0.304	1.000
	21	1.000	0.280	0.355	1.000	0.280	1.000	1.000	1.000	0.050	0.172
Group 1 & Group 3	0	0.930	0.599	0.726	0.114	0.599	0.293	0.456	0.820	0.324	0.497
	10	0.003	0.001	0.001	0.001	0.001	0.002	0.001	<0.001	<0.001	<0.001
	21	0.001	0.002	0.001	0.001	0.002	0.001	0.003	<0.001	<0.001	<0.001
Group 2 & Group 3	0	0.630	0.810	0.630	0.630	0.520	0.166	0.178	0.181	0.287	0.463
	10	0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.001	0.002
	21	<0.001	0.001	0.003	<0.001	<0.001	0.001	0.001	<0.001	<0.001	<0.001

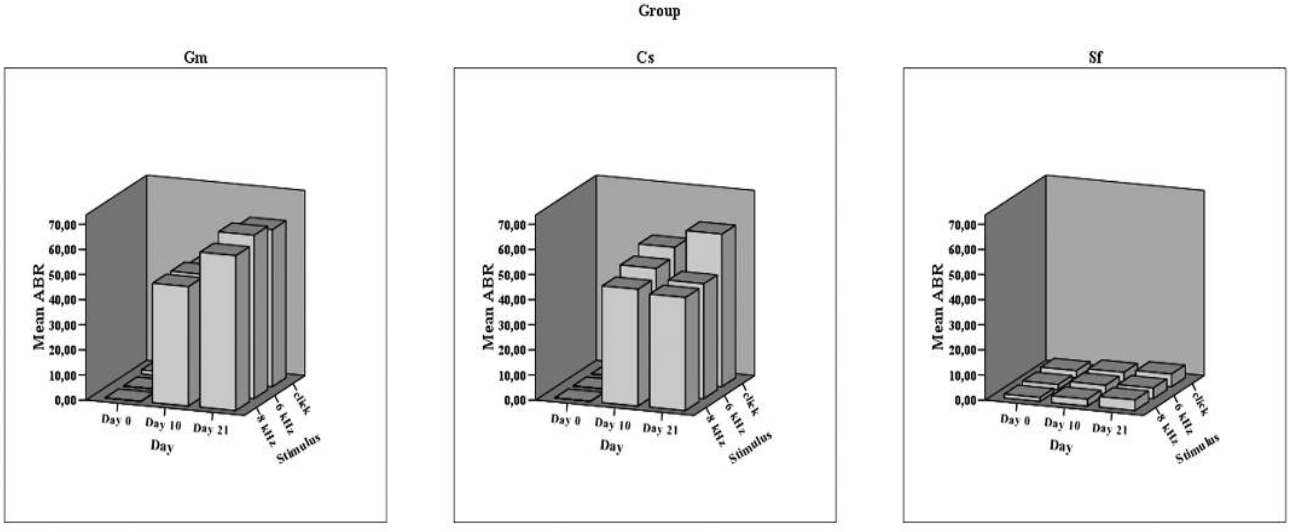

Figure 5. Mean values of ABR thresholds of the groups by days, according to 3-D Bar Charts method.

Discussion

In our study, ototoxic effects of Cs as a nonspesific ototopical antifungal agent on healthy adult albino guinea pigs were tested utilizing DPOAE and ABR tests after repeated intratympanic injections.

By intratympanic route, the ototopical agents applied can reach cochlea via the membrane of round foramen. The membran of round foramen has a three-layered with micropinocytotic vesicles and permits passage of albumin, peroxidase and many other materials. The passage through round foramen occurs by passive diffusion and both interepitelial and intraepitelial transportation [14,15].

There are some reasons why guinea pigs are experimented on in ototoxicity studies. The fact that the round foramen in human beings are thicker than that of guniea pigs causes the ototopical agents to exert their ototoxic effects less prominently. Moreover, the niche in the round foramen in guinea pig is located more superficially [16,17]. So, the fact that ototopically used agents produce ototoxicity in guinea pigs does not necessarily imply that they will also produce the same effect in human beings. In contrast, the probability for the agents that is not ototoxic in guinea pigs to be ototoxic in human beings is low. Although hearing range for guinea pigs is between 54 Hz-51.4 kHz, hearing acuity is higher in for frequencies between 1 kHz and 32 kHz [18]. Due to the above-mentioned reasons, guinea pigs were used as the test subjects.

In a study by Jinn et al on chinchilla, it was stated that acetic acid was quite ototoxic, and documented that the mixture consisting of polymyxin B and neomycin also had the same effect. [19]. Marsh and Tom showed that cresylate and acetic acid used ototopically on guinea pigs had ototoxic effects [20]. In a study perfomed with a mixture composed of 70% alcohol and 4% boric acid by Ozturkcan and the colleagues, it was shown that boric acid mixed with distilled water exerts no ototoxic effect [21]. Morizono and Sikora documented dehidratation, mucosal irritation along with cellular injury with application of ethyl alcohol in the middle ears of chinchillas and, also demonstrated inner ear injury is due to diffusion of ethyl alcohol through round foramen into the cochlea. These authors stated that 50% of ethyl alcohol in concentration induced reduction in cochlear microphonics; in contrast, that the concentrations above this might produce ototoxic effects [22]. In addition, alcohol can possibly potentiate the ototoxic effects of other medications. Even if alcohol of 90% concentration is used in Cs, when combined with distilled water, its concentration falls below 10%. Although there are some studies related to the two components found in Cs, we could find no sign of ototoxic effects of basic fuchsin, aceton, phenol and resorcinol. What matters in term of Cs ototoxicity is not to evaluate ototoxic effects of each ingredient along but in a combined form of Cs.

Ueda et al observed in guinea pig bulla that they could obtain DPOAE responses when it is filled with the less than 50% fluid, while they could not obtain any response when it is filled totally with fluid. In the same study, it was also shown that DPOAE signal-to-noise ratio decreased as the perforation diameter on tympanic membrane increased [23]. LeBourgeois 3rd and the colleagues compared DPOEA responses after perforated the tympanic membranes of guinea pigs in various diameters and documented that DPOAE responses were markedly decreasing as the perforation diameter increased markedly [24]. Zhao et al stated, in their study in patients with distinct middle ear pathology, that DPOAE responses could be obtained in the presence of middle ear effusions, while the responses might not be observed in patients with TM perforation, especially when the diameter of perforation is wide and hearing loss is obtained above 20 dB HL [25]. In our study, we observed no TM perforation and no findings related to otitis media in guinea pigs by otomicroscopic views before the tests. We preferred intratympanic enjection applications rather than a model wherein tympanic membrane is perforated in a fear that the later can incur poor outcomes over DPOAE results.

In the study by Gultekin and the colleagues, they operated by using topical Cs in tympanic membrane perforation model in rats [26]. In this research, a tympanic membrane perforation was created before treatment and DPOAE test results were evaluated both before and after the treatment. There was no statistically significant difference in the Cs group and, also, no ototoxic effect was documented. ABR thresholds were not evaluated in this research. Contrary to the above-mentioned study by Gultekin and the colleagues, we preferred guinea pigs which are known to be more sensitive to ototoxicity. Moreover, in order to provide cross-check between audiological

tets, two different testing system were utilized, namely ABR and DPOAE, in an attempt to verify each other.

We noticed no statistically significant difference when DPOAE responses obtained in 0th,10th and 21st days were compared in the group where intratympanic saline solution was administered. Even though there was statistically significant difference between the responses from ABR tests in 0th and 21st day, increment in threshold above 5 dB nHL was not detected. In this regard, the conclusion that no ototoxicity developed was infered from the fact that significant result was obtained by only a single test technique in negative control group.

Considering before injection test results, when DPOAE and ABR results from negative control group (i.e. saline injected group) and the Cs-applied group were compared to each other, no statistically significant difference was detected (table 5). In contrast, there was a statistically significant difference when the data on 10th and 21st days from these two groups were compared. When the Cs administered group 2 and Gm administered group 1 were compared to each other based on data on 0th, 10th and 21st days by using the same test, no statistically significant difference was found (table 5).

Even though responses in all frequencies were able to be obtain before injections in group 2, no response could be obtained to none of the frequencies of DPOAE test on 10th day. Statistically significant difference was detected when a comparison in group 2 is made between DPOAE and ABR values on 0th and 10th days and the values on 0th and 21st days, although no significant difference was found when the values obtained on 10th and 21st days were compared ($p>0.05$). Based on these findings, we can conclude that ototoxicity arise before the 10th day. Due to the fact that initially high frequencies are affected, albeit not essentially, in ototoxicity, we used 6 kHz and 8 kHz tone burst stimuli in addition to click stimulus for ABRs testings. Evaluation of even higher frequencies could not be fulfilled because of restriction of the test device used.

There was no significant difference between thresholds obtained in ABR tests on 10th and 21st days in guinea pigs where Gm was administered intratympanically. A similar conclusion were obtained when intratympanic Cs was applied. With these findings, we consider that the ototoxiciy occurring in the ears where Cs and Gm were administered commenced before 10th day of the study and proceeded beyond 21st day, after injections.

Conclusion

In the light of the aforementioned findings, it is concluded that Cs has ototoxic effects on guinea pigs. Assuming that Cs is ototoxic in guinea pigs and considering that they are more sensitive to ototoxicity than human beings, this does not necessarily mean that this result is a proof of ototoxicity in human beings. However, in accordance with the result obtained from this study, recommending Cs in patients with otomycosis and TM perforation will not be sensible.

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