Original Article

Does melatonin alleviate ototoxic effect caused by administration of cisplatin?

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Abstract

Aim: In this study, it was aimed to experimentally investigate the protective effects of melatonin in the cisplatin-induced ototoxicity.
Material and Methods: Ten Wistar-albino rats were included in the study. Two equal groups were generated randomly as cisplatin and melatonin groups. Rats' underwent Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emission (DPOAE) testing before the drug administration and the results were recorded. Both tests were repeated 72 hours after the cisplatin administration in all rats.
Results: Significant difference was found between the I-IV interval values before the treatment and after the treatment both in cisplatin and melatonin group. As well as the significant difference in hearing threshold value changes, statistically, significant differences in ABR-I and ABR-IV interval variations were also seen between the cisplatin and melatonin groups. A statistically significant decrease was found between the initial and final control SNR (signal-to-noise ratio) levels within the cisplatin group in the evaluations at 2000Hz, 3000Hz and 4000Hz. Statistically, significant differences were observed between SNR levels when the melatonin group was compared with the cisplatin group.
Conclusion: Melatonin appears to reduce cisplatin-induced ototoxicity in rats. Although, the use of supplementary therapies targeting to reduce the toxic effects in clinical studies is still a controversial point.

Keywords: Melatonin; cisplatin; ototoxicity; DPOAE; ABR.

INTRODUCTION

Cisplatin is an important and frequently used antineoplastic drug in several neoplasms including head and neck tumors, gastrointestinal system tumors, and central nervous system cancers. Despite its success in cancer therapy, dose limitation can be necessary due to ototoxicity, kidney toxicity, digestive system toxicity, peripheral neuropathy and myelosuppression (1,2). Although there are animal and human studies claiming that cisplatin ototoxicity involves spiral ganglion cells, stria vascularis and outer and inner hair cells in the organ of Corti (3,4), the ototoxicity mechanism of cisplatin is still not clear. Consequently, the hearing loss secondary to cisplatin use are generally bilateral, progressive, irreversible, and high-frequency sensorineural type accompanied by tinnitus. Cisplatin dose and dosing frequency are associated to the hearing loss (3,5,6).

Different anti-oxidant agents have been used to reduce or prevent cisplatin-induced ototoxicity, but there is not an approved therapy for this. Melatonin is mainly secreted by the pineal gland and reduces oxidative stress acting as a direct free radical scavenger and an indirect antioxidative effector that provokes antioxidant enzymes (7). It can potentially protect inner ear hair cells from the ototoxicity of free radicals produced by cisplatin (8).

In the diagnosis of ototoxicity, audiological tests such as distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) were used in animal studies, and they should be noted as the gold standards for this assessment (9).

In this study, it was aimed to determine the protective

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effects of melatonin on cisplatin-induced ototoxicity in rats via DPOAE and ABR tests.

MATERIAL and METHODS

Animals

Experimental animal study approval (decree number 2011-112-426) was received from the Ankara University local ethical board for animal experiment. In this experimental research, a total of 10 (20 ears) 6- to 9-mo-old, male, healthy, Wistar-Albino rats, with an average weight of 310 g (260-350), were used, taken from the healthy animal laboratory. After carrying of the animals, the rats were left to rest for 7 days under the suitable conditions, to comply with the new environment. The temperature was set between 20–22°C and the humidity was 65–70%, with a 12-h day-night cycle. The rats were fed with dry baits and tap water excluding drug administration times.

Drugs

Before the examination of rats, 70 mg/kg intraperitoneal (i.p.) ketamine hydrochloride (Ketalar; Eczacıbaşı Parke-Devis, İstanbul, Turkey) and 7.5 mg/kg i.p. Xylazine hydrochloride (Alfazyne; Alfasan International B.V. Woerden, Holland) were used to anesthesia

All the rats' tympanic membranes and external auditory canals were examined. Ears with cerumen were removed. Moreover, the rats with an outer ear canal infection, tympanic membrane perforation and/or opacification, or infection in the middle ear were excluded from the study. Two rat groups formed randomly, and the rats' ABR and DPOAE tests were performed both before the study and 72 hours after cisplatin administration under general anesthesia. The rats were heated up with warm pads. Cisplatin group (as control group) (n=5)); the rats were administered i.p. cisplatin 15 mg/kg (Cipintu; Koçsel İlaç İstanbul, Turkey) by slow infusion.

Melatonin group (as study group) (n=5); the rats were administered i.p. melatonin 10 ml/kg (Sigma Aldrich; St.Louis, MO., USA), followed by i.p. 15mg/kg cisplatin 30 min later.

DPOAE measurements

DPOAE's values were recorded and analyzed. GSI AUDIOscreener device (Grason-Stadler, Minnesota, USA) was used to measure the 2f1-f2 cubic distortion product components. The f2/f1 ratio was stabilized at 1.22. The intensity of levels was taken as L1 (65 dB SPL) for the f1 frequency and as L2 (55 dB SPL) for the f2 frequency. The measurement of distortion product otoacoustic emissions were recorded with a microphone at a frequency of 2f1-f2 in the external ear canal, frequencies of 2, 3 and 4 kHz at geometrical averages of f1 and f2 were used.

In the assessment of DPOAE results, an SNR created at a geometrical average of 2f1-f2 cubic distortion products at 2000, 3000 and 4000 Hz frequency bands were used. In the evaluation of DPOAE responses, the SNR was regarded to be more dependable than DPOAE amplitudes.

ABR measurements

Medelec Audiostar Evoked Response Audiometers (Oxford Instrument-UK) were used to record the auditory brainstem response. The external ear canal probes that were sized for neonatal were plugged in the external ear canal of the measurement side. Disposable monopolar and subdermal stainless-steel needle electrodes were fixed on the mastoid area as a negative electrode, vertex as a positive electrode, and dorsum as a ground electrode. The impedances of electrodes were adjusted to provide that they were less than 2 k Ω. A white noise mask was used to contralateral of the stimulated ear aiming to evaluate each ear individually.

Stimulations were produced in the first ten milliseconds, and each click was filtered (100–3000 Hz). The stimulation level started from 90 dB HL and decreased in steps of 10 dB. The lowest stimulation intensity at which a visible and reproducible ABR response was observed is defined as the threshold of hearing. A mean of 1500 clicks/stimulus was applied for all levels.

Statistical analysis

The data were analyzed by SPSS (Statistical Package for Social Science) for Windows 11.5. Shapiro Wilk test was used in order to determine whether the distribution of continuous variables was close to normal. Descriptive statistics were shown as the mean ± standard deviation. During the analysis of the data, variance analysis was used in the Repeated Measurements.

Bonferroni Corrected dependent t-test was used to determine the significance of the change in the initial and final control I-IV intervals, threshold dB and emission measurements within the groups and p<0.025 were considered statistically significant. Student's t-test evaluated the significance of the difference between the groups. P <0.05 were considered statistically significant. Bonferroni Correction was performed to control the Type I error in all the possible multiple comparisons.

RESULTS

Pre-treatment DPOAE and ABR results examination revealed that there was not a significant difference between the two groups (p>0.05).

| Table 1. Pre-and post-treatment ABR* wave I-IV intervals and hearing thresholds			
Parameters	**Pre-treatment**	**Post-treatment**	**p**
Interval (mean±SD;ms)			
Cisplatin(n=10)	3.24±0.12	4.42±0.17	<0.001
Melatonin(n=10)	3.42±0.15	3.56±0.24	0.069
Hearing threshold (mean±SD;dB)			
Cisplatin(n=10)	28.0±4.47	72.0±7.58	<0.001
Melatonin(n=10)	31.5±6.98	45.0±9.18	<0.001
(ABR: Auditory brainstem response; SD: standard deviation)			

Table 1 includes the before and after treatment mean wave ABR-I−ABR-IV interval values. When the pre- and after treatment values of the cisplatin group were examined it was seen that wave ABR-I and ABR-IV interval differences were statistically significant (p<0.025). The variation in wave ABR-I and ABR-V interval differences were statistically significant between the melatonin group and the cisplatin group (p<0.05, Figure 1).
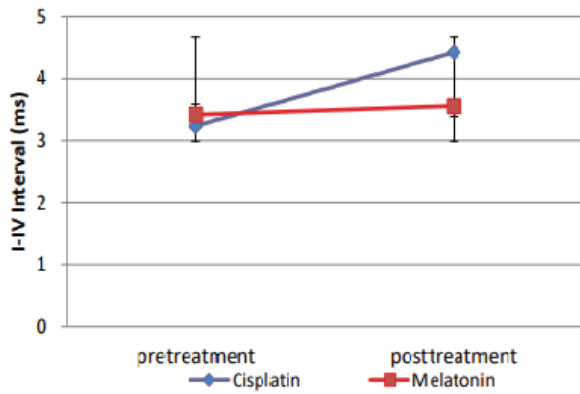
Figure 1. The change in ABR I-IV interval of groups

Cisplatin administration caused a very large range of ABR threshold changes. As shown in Table 1, however, there were not any statistically significant differences between pre- and after treatment ABR threshold values (p>0.025). Figure 2 includes ABR hearing threshold value changes of the two groups and there was a statistically significant difference between the melatonin group and cisplatin group (p<0.05).
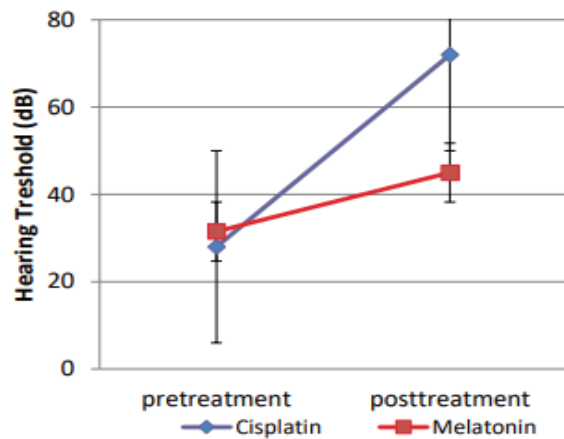

Figure 2. The change in ABR hearing thresholds of groups

Table 2 presents the mean pre- and after treatment SNR's. Comparison of the initial versus final SNR pointed out a significant decline in the cisplatin group for evaluations at 2000, 3000 and 4000Hz (p<0.008, Figure 3). However, no significant differences were detected in the melatonin group at all frequencies (p>0.008).

Regarding the signal-to-noise ratio, a statistically significant difference was noticed between the melatonin and cisplatin group (p<0.017).

Table 2. Pre-and post-treatment DPOAE* signal-to-noise ratios

Parameters	Pre-treatment	Post-treatment	p
2000 Hz (mean±SD;dB)			
Cisplatin	14.15±2.87	-7.99±1.81	<0.001
Melatonin	12.96±2.30	4.33±5.49	0.030
3000 Hz (mean±SD;dB)			
Cisplatin	14.64±2.88	-4.70±0.99	<0.001
Melatonin	13.56±4.14	9.65±3.68	0.174
4000 Hz (mean±SD;dB)			
Cisplatin	23.26±3.31	8.57±1.88	0.002
Melatonin	21.00±5.87	20.41±3.32	0.696

(DPOAE: Distortion product otoacoustic emission; SD: standard deviation)
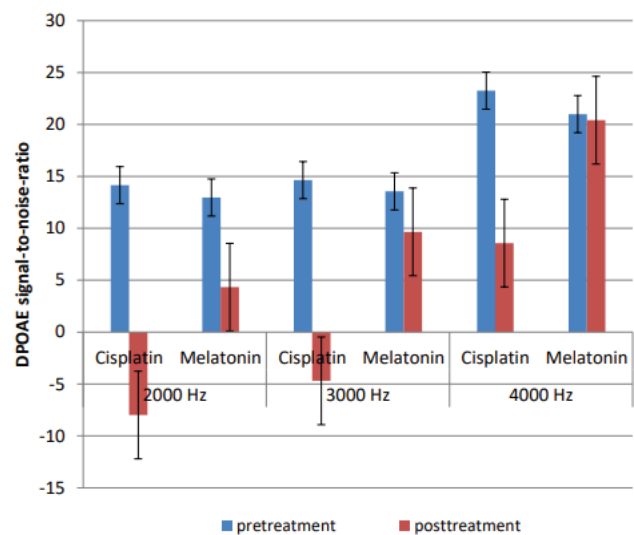
Figure 3. The change in DPOAE SNR of groups

DISCUSSION

Cisplatin is a highly potent chemotherapeutic agent which is used for a variety of tumors, (e.g. head and neck cancers) but its side effects can limit the therapeutic use in high doses (10). Ototoxicity is the main side effect of cisplatin that reduces the patients' quality of life (11). Compared to nephrotoxicity, which can be treated by hydration and medications such as diuretics, ototoxicity is an irreversible and cumulative effect. (12).

In the past studies, several antioxidant agents such as D-methionine (9), N-acetylcysteine (13), glutathione ester (14), sodium salicylate (15) and 4-methylthiobenzoic acid (16) were administrated to avoid cisplatin-induced inner ear injury; each of whom resulted with different success rates.

The mechanism of cisplatin-induced ototoxicity is not well established. However, it is observed that, in particular, superoxide anions, free radicals cause depletion of intracellular antioxidants. The major substrate of the antioxidant pathway is glutathione. A significant decrease

in glutathione levels is and free radical production are noted in cisplatin-given rats. Chemoprotective agents prevent ototoxicity, by providing direct antioxidant activity besides its effect to increase glutathione level (17).

In the relevant studies, it has been reported that patients whose malignancies were treated with cisplatin, had lower serum antioxidant levels. The studies showed that vitamin C, vitamin E, ceruloplasmin and uric acid levels decrease after each cisplatin administration and reach back to the normal level before the next treatment (18).

The melatonin is, mainly secreted rhythmically by the pineal gland, a tryptophan derivate. Melatonin receptors are present in numerous structures/organs, including cochlea (19,20). Current articles have demonstrated that melatonin possesses additional characteristics such as anti-inflammatory, anti-oxidant and free radical scavenger effects (21,22). Melatonin provides these effects by altering the activities of superoxide dismutase, glutathione GSH peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase: The enzymes which increase the total antioxidative defense capacity (23). Under in vivo conditions of high oxidative stress, melatonin has shown to be superior to vitamins E and C by means of decreasing the oxidative damage (19).

In previous rat model studies investigating cisplatin-induced ototoxicity, more expensive and advanced evaluation procedures such as ABR, DPOAE and electrocochleography were used (24). Nowadays, ABR and DPOAE assessment are commonly used in clinical researches (5). However, our study assessment utilizes ABR threshold values and wave ABR-I and ABR-IV intervals and SNR's in the DPOAE.

The current debate is the identification of Auditory Brainstem Response thresholds in animal models. Similar to prior studies, in our research, it is detected that wave ABR-II had a large amplitude which disappeared as the stimulation intensity decreased. Wave ABR-II has been used to determine the hearing threshold levels in experimental studies (24). Kamimura et al. suggested that the wave ABR-IV and ABR-V complex created in humans is similar to the wave ABR-IV complex in rats (16). Our study results showed that wave ABR-III has a small amplitude and commonly occurs at the descendant leg of wave ABR-II. A identifiable positive peak was confirmed as wave ABR-III (24).

The protective effects of melatonin in ototoxicity have been researched in some experimental studies. Erdem et al. showed that low dose melatonin (0,4mg/kg) protect the inner ear, although high dose melatonin facilitated amikacin-induced ototoxicity (25). Furthermore, Demir et al. reported that it was statistically significant that the high frequencies in DPOAE and ABR were protected with the use of transtympanic melatonin (26). Recently Ye et al. reported that an intramuscular application of melatonin in guinea pigs significantly preserved the cochlea from gentamicin ototoxicity (27). In a similar experimental model, Lopez-Gonzalez et al. study the effects of melatonin and some antioxidants on DPOAE measurements in rats treated with cisplatin (28). They pointed out that melatonin pretreatment is associated with a significant prevenion of cisplatin-induced ototoxicity. In this study, ABR threshold value variations and wave ABR-I and ABR-IV interval variations of the melatonin group were significantly reduced compared to the control group. According to the DPOAE, SNR melatonin had a protective effect at all frequencies. These results collectively imply that the use of single dose melatonin can prevent cisplatin-induced ototoxicity. The limitation of this experimental study is the abscence of measurements of the oxidative damage in the cochlear tissue.

CONCLUSION

In this study, it is noted that melatonin made a significant contribution to prevent and reduce experimental cisplatin-induced ototoxicity. Our results marked that melatonin could be used safely before cisplatin treatment but before using animal model findings in clinical studies, further prospective and randomized researches on larger series should be done.

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