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Protective Effects of Betaine on the Cochlea in Cisplatin-Induced Ototoxicity

Sisplatin Kaynaklı Ototoksisitede Betainin Koklea Üzerindeki Koruyucu Etkileri

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ABSTRACT Objective: Cisplatin is a platinum-based compound used to treat various cancers; however, its severe side effects, such as ototoxicity, limit its clinical use. Ototoxicity can lead to permanent damage, particularly affecting the organ of Corti. This study aimed to histopathologically evaluate the protective effect of betaine, known for its antioxidant and antiinflammatory properties, against cisplatin-induced ototoxicity. **Material and Methods:** Forty female Wistar albino rats were used with the approval of the Erciyes University Animal Experiments Local Ethics Committee. The rats were randomly assigned to 4 groups (n=10): Sham, Betaine, Cisplatin, and Cisplatin+Betaine. Betaine hydrochloride (250 mg/kg/day) was administered orally once daily for 30 days, while cisplatin (8 mg/kg/week) was administered intraperitoneally once weekly for 4 weeks. Cochlear tissues were collected, fixed, and processed for histopathological evaluation. **Results:** Cisplatin-induced ototoxicity led to structural deformities in the cochlea, particularly in the organ of Corti at the basal turn. In the Cisplatin+Betaine group, the organ of Corti exhibited a more preserved structure, with maintained integrity of the stria vascularis and tectorial membrane. Statistically, the Cisplatin group showed significantly reduced stria vascularis and basilar membrane thickness, tectorial membrane length, and inner and outer hair height. In the Cisplatin+Betaine group, these parameters improved, with a significant increase observed in tectorial membrane length across all cochlear turns and outer hair cell height in the apical turn. **Conclusion:** This study demonstrates the protective role of betaine against cisplatin-induced ototoxicity. While the findings are promising for ototoxicity prophylaxis, further studies are warranted.

Keywords: Betaine; cisplatin; cochlea; ototoxicity

ÖZET Amaç: Sisplatin, birçok kanserin tedavisinde kullanılan bir platin bazlı bileşiktir; ancak ototoksisite gibi ciddi yan etkileri tedavi kısıtlılığı oluşturmaktır. Ototoksisite, özellikle korti organını kalıcı olarak etkileyebilir. Bu çalışmada, antioksidan ve antiinflamatuar özelliklere sahip olduğu bilinen betainin, sisplatine bağlı ototoksisiteye karşı koruyucu etkisi histopatolojik olarak incelenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmada, Erciyes Üniversitesi Hayvan Deneyleri Yerel Etik Kurul onayı doğrultusunda 40 adet Wistar albino dişi sıçan kullanıldı. Sıçanlar rastgele 4 eşit gruba (n=10) ayrıldı: Kontrol, Betain, Sisplatin ve Sisplatin+Betain. Deneyler sırasında sıçanlara betain hydrochloride (250 mg/kg/d) 30 gün boyunca (günde 1 kez) oral yolla ve sisplatin (8 mg/kg/d) 4 hafta (haftada 1 kez) intraperitoneal olarak uygulandı. Koklear dokuları toplandı, fiksasyon yapıldı ve histopatolojik değerlendirme yapıldı. **Bulgular:** Sisplatin ototoksisitesine bağlı olarak kokleanın, özellikle de bazal dönüşte yer alan korti organında deformasyonların meydana geldiği belirlendi. Sisplatin+Betain grubunda ise korti organının daha düzenli bir yapıya sahip olduğu, stria vaskülaris ile tektorial membranın bütünlüğünün korunduğu gözlendi. İstatistiksel sonuçlar da; sisplatin grubunda, stria vaskülaris ve baziler membran kalınlığı, tektorial memebran uzunluğu, dış ve iç saçlı hücre uzunluklarının anlamlı olarak azaldığı görüldü. Sisplatin+Betain grubunda ise artış meydana geldiği ancak bu artışın sadece kokleanın tüm dönüşlerindeki tektorial membran uzunluğunda ve kokleanın apeks dönüşündeki dış saçlı hücre uzunluğunda anlamlı olduğu tespit edildi. **Sonuç:** Bu çalışma, betainin sisplatin kaynaklı ototoksisiteye karşı koruyucu rolünü ortaya koymaktadır. Bulgular ototoksisite profilaksisi için umut verici olsa da, daha ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Betain; sisplatin; koklea; ototoksisite

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Cisplatin, a platinum-based compound whose biological properties were discovered in 1965, is one of the first-line antitumor agents.[1] Today, it is frequently used in the treatment of various malignancies, including bladder, head and neck, lung cancers, ovarian and testicular.[2] However, cisplatin may be limited due to its diverse side effects. Repeated cisplatin administration during cancer treatment can accumulate in certain parts of the body, leading to toxic effects such as nephrotoxicity, ototoxicity, hepatotoxicity, peripheral neuropathy, myelosuppression, and retinopathy.[3,4]

Cisplatin is converted into the active form within the cell, reacting with nucleotides and amino acids. This process leads to DNA cross-linking and protein misfolding, triggering apoptosis in cancer cells.[5] However, since this effect is not specific to cancer cells, cisplatin also impacts healthy tissues, resulting in serious side effects.[4] In normal cells, glutathione can mitigate the toxic effects of cisplatin by either preventing its interaction with DNA or neutralizing DNA-cisplatin mono-adducts.[6] However, under oxidative conditions characterized by the presence of reactive oxygen species, the protective role of intrinsic glutathione is diminished.[7] In cisplatin-induced ototoxicity, spiral ganglion neurons, sensory hair cells, as well as the spiral ligament and stria vascularis of the cochlea are primarily affected.[8] Cochlear damage and hearing loss caused by cisplatin involve processes in which oxidative stress plays a complex role. Excessive reactive oxygen species production triggers mitochondria-mediated apoptosis, leading to damage in cochlear hair cell DNA, proteins, and lipids.[9] Furthermore, cisplatin exacerbates lipid peroxidation and oxidative damage by reducing the levels of antioxidant enzymes, such as glutathione, superoxide dismutase, and catalase.[10]

In current treatments, reducing the dose of cisplatin or switching to an alternative therapy is not always feasible. Therefore, exploring interventions that can prevent or treat hearing loss caused by cisplatin chemotherapy is crucial. Although sodium thiosulfate, an antioxidant drug, is recommended for the prevention of cisplatin-induced hearing loss, many patients still suffer from hearing impairment.[11,12] In addition to sodium thiosulfate, options such as N-acetylcysteine, coenzyme Q10, and are available in the literature, but their outcomes remain limited.[13,14]

Betaine is a naturally occurring, non-toxic, and stable compound found in animals and plants. It is also present in dietary sources such as spinach, wheat, and seafood and can be endogenously synthesized through choline metabolism.[15] Its low cost and non-toxic nature make it suitable for human consumption. Betaine, through its three methyl groups, plays a role in the transmethylation process, facilitating the conversion of homocysteine to methionine.[16] Additionally, it is a crucial osmoprotectant that accumulates in cells under osmotic stress, protecting proteins and enzymes without disrupting their function.[17] Various studies have demonstrated that betaine possesses antioxidant, anti-inflammatory, and osmoprotective properties.[18] However, to the best of our knowledge, there are no studies investigating the protective or therapeutic role of betaine against cisplatin-induced ototoxicity, one of the common side effects of cisplatin. In this context, the present study is a pioneering effort to histopathologically investigate the protective effects of betaine against cisplatin-induced ototoxicity.

MATERIAL AND METHODS

ETHICS AND SUBJECTS

Experimental studies were conducted according to the Guide for the Care and Use of Laboratory Animals, ensuring the humane treatment of all animals. The study received approval from the Erciyes University Animal Experiments Local Ethics Committee (date: December 5, 2024, no: 24/23905). In this study, 40 female Wistar albino rats (8-10 weeks old, weighing 200-250 grams) were obtained from the Erciyes University Experimental Research Application and Research Center (DEKAM). The rats were fed a standard pellet diet and provided with water. They were housed in plastic cages under controlled conditions, with a temperature of 22±2˚C and a 12-h dark-light cycle throughout the experiment.

STUDY GROUPS

The rats were randomly divided into four equal groups (n=10): Sham, Betaine, Cisplatin, and cisplatin+betaine. Betaine hydrochloride (B3501-100G,

Sigma-Aldrich, USA) (250 mg/kg) was dissolved in saline and administered orally via gavage, while cisplatin (CP-Koçak, Türkiye, 50 mg/100 mL IV infusion concentrate) (8 mg/kg) was administered intraperitoneally. The Sham group received saline for 30 days, while the betaine and cisplatin+betaine groups received betaine dissolved in saline via gavage. Cisplatin was administered once a week for 4 weeks to the Cisplatin and Cisplatin+Betaine groups.

To complete the experiment, 2 weeks after the final betaine administration, the rats were anesthetized with ketamine (Pfizer, USA) (50 mg/kg) and xylazine (Bayer, Germany) (10 mg/kg). Following anesthesia, euthanasia was performed.[19]

HISTOPATHOLOGICAL STUDIES

For histological examination, the cochlear tissues from the rats were fixed in a 10% formaldehyde solution for 72 h. After fixation, the tissues underwent decalcification in a solution prepared with 10% formaldehyde+80% distilled water and 10% nitric acid. Following decalcification, routine histological tissue processing procedures were applied. Briefly, tissues were washed in running tap water, passed through increasing concentrations of alcohol, cleared with xylene, embedded in paraffin blocks, and sectioned at a thickness of 5 µm. Sections were stained with Masson's Trichrome (MT) to evaluate the connective tissue structure and hematoxylin and eosin (H&E) to assess the overall histological structure. The structure of the cochlea and the organ of Corti were histopathologically evaluated using an Olympus BX51 light microscope (Olympus Corp., Tokyo, Japan). The evaluated structures were as follows: stria vascularis, basilar membrane, vestibular membrane, tectorial membrane, sensory hair cells, tunnel of Corti, and spiral ganglion cells. In addition, the thickness of the stria vascularis and basilar membrane, the length of the tectorial membrane, and the outer and inner hair cells were measured using the ImageJ Software program (ImageJ Rasband, USA) the number of spiral ganglion cells was counted. The results were statistically analyzed.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for Social Sciences (IBM Corporation, USA) for Windows 22.0 software. One-way Analysis of Variance (ANOVA) was used for intergroup comparisons of normally distributed variables, and post-hoc multiple comparisons were performed using the Tukey test if differences were found. p values 0.05 were considered significant.

RESULTS

HISTOPATHOLOGICAL FINDINGS

When light microscope images were evaluated for all groups, it was seen that the cochlea preserved its cylindrical structure in its external form. Additionally, the structure of the cochlea, which completes 2.5 turns around the modiolus located at the center with a vertical axis, and the presence of the organ of Corti in the basal, medial, and apical turns of the cochlea were identifiable in the light microscopic images (Figure 1).

When evaluating the light microscopic images obtained from the Sham and Betaine groups, it was observed that the scala media was regularly separated from the scala tympani by the basilar membrane and from the scala vestibule by the vestibular membrane. The fibrous connective tissue structure of the basilar membrane extending toward the stria vascularis displayed a regular pattern (Figure 2). In both groups, the stria vascularis, with its marginal, intermediate, and basal cells and many blood vessels, was easily distinguishable in the light microscopic images and extended up to the vestibüler membrane. The tectorial membrane, a gelatinous structure composed of parallel-aligned collagen fibers, covered the organ of Corti, starting from the spiral lamina (Figure 2). Additionally, in both groups, the organ of Corti, which sits on the basilar membrane separating the scala tympani and scala media regions and serves as a specialized auditory receptor, exhibited a regular structure (Figure 1). At 40x magnification under light microscopy, when examining the organ of Corti, it was observed that the sensory receptor hair cells were arranged in 3 rows for the outer hair cells and a single row for the inner hair cells. Deiter cells, at the base of the hair cells and providing support through their cytoplasmic extensions, were distinguished by their nuclei on the basilar membrane. The organ of Corti was

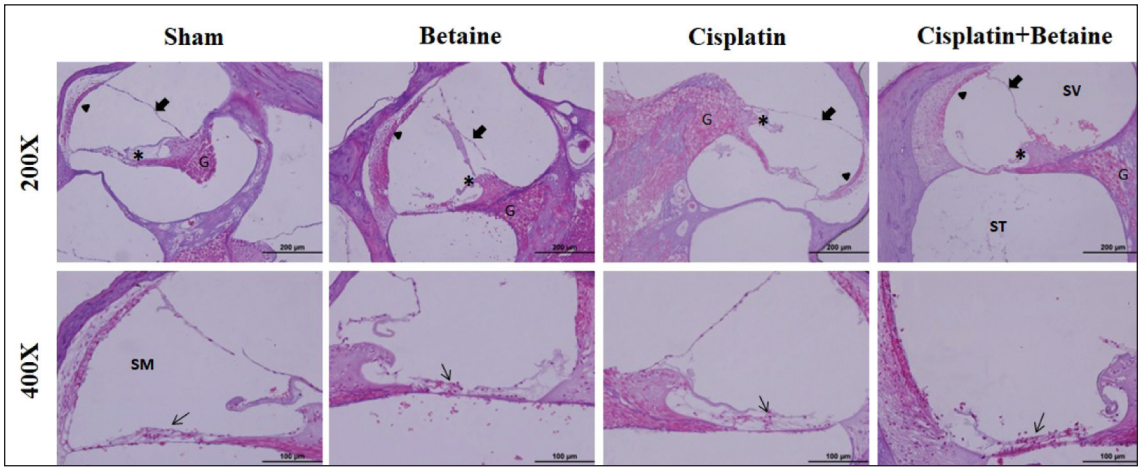

FIGURE 1: Images of groups with H&E stainings
Arrowhead: Stria vascularis; Thick arrow: Vestibüler membran; *: Tectorial membrane; Thin arrow: Outer hair cell; G: Spiral ganglion; SV: Scala vestibuli; SM: Scala media; ST: Scala tympani. (Respectively; Original magnification=200x, 400x; scale bar=200 µm, 100 µm)

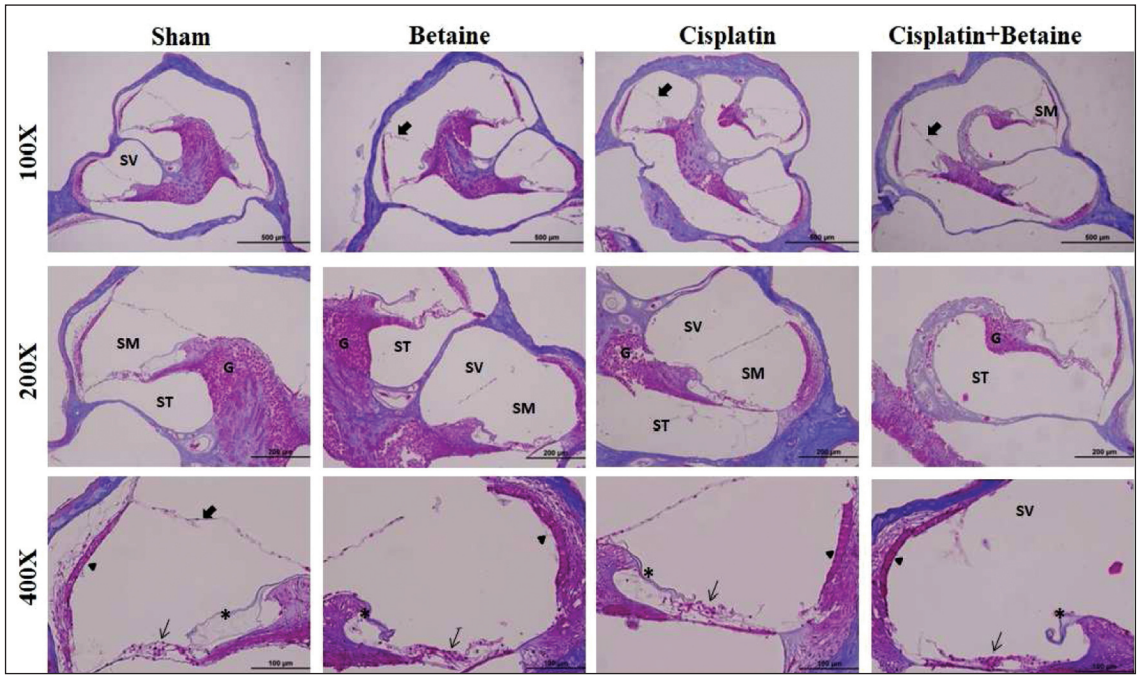

FIGURE 2: Images of groups with MT staining
Arrowhead: Stria vascularis; Thick arrow: Vestibüler membran; *: Tectorial membrane; Thin arrow: Outer hair cell; G: Spiral ganglion; SV: Scala vestibuli; SM: Scala media; ST: Scala tympani. (Respectively; Original magnification=200x, 400x; scale bar=200 µm, 100 µm)

observed to have a triangular-shaped structure, with pillar cell nuclei positioned at the corners of the triangle on either side of the Corti tunnel in the center. Following the three rows of outer hair cells, the supporting Hensen, Böttcher, and Claudius cells exhibited a regular arrangement. In the groups not treated with cisplatin, the spiral ganglion cells generally displayed a standard histological structure with distinct nuclear features, and there were no gaps between the cells (Figure 2).

Cisplatin-induced ototoxicity affected all inner ear structures, with more severe damage observed, particularly in the basal turn of the cochlea. Deformation of the basilar membrane and cytoplasmic and nuclear condensation were noted. Additionally, in the lateral region of the scala media, at the stria vascu-

laris, a loss of marginal cells was observed (Figure 1). Tears were observed in the vestibular membrane, located between the scala vestibule and scala media, and irregularities were detected in the tectorial membrane, which exhibits a parallel arrangement of collagen fibers. Examination of light microscopic images at 40x magnification revealed that ototoxicity caused disturbances in the overall histological structure of the Corti organ, with the most severe damage occurring in the basal turn. The most striking finding was the presence of hydropic and vacuolar degeneration and cell loss in the Corti organ. The cells exhibited swollen cytoplasm, shrunken nuclei, and increased intercellular spaces. In the cisplatin group, degenerative spiral ganglion cells were observed, stained eosinophilically, with prominent intercellular spaces (Figure 1, Figure 2). Compared to the Cisplatin group, the cisplatin+betaine group exhibited a more organized structure in the organ of Corti, the intercellular spaces between the marginal cells forming the stria vascularis were reduced, and the integrity of the vestibular membrane between the scala media and scala vestibuli was preserved (Figure 1). The cytoplasmic and nuclear condensation observed in the basilar membrane in the cisplatin group was also minimized in the cisplatin+betaine group. While the gelatinous tectorial membrane, composed of parallel-arranged collagen fibers, did not show as much regularity as in the Sham group, it still exhibited a more organized structure than the cisplatin group (Figure 2). Additionally, the degeneration of supporting cells in the Corti organ and the shedding of outer and inner hair cells was less severe in the Cisplatin+Betaine group than in the Cisplatin group. Furthermore, the spaces between the cells forming the Corti organ were reduced, and the nuclei became more distinct. Along with these findings, the corti tunnel and the pillar cells surrounding it also showed a more regular structure in the Cisplatin+Betaine group compared with the cisplatin group (Figure 2). In the group exposed to Betaine treatment, the number of degenerated spiral ganglion cells decreased although vacuolization continued in some areas (Figure 1).

HISTOPATHOLOGICAL MEASUREMENT STATISTICAL RESULTS

The results are as follows, with all details presented in Table 1. The thickness of the stria vascularis in the basal, medial, and apical regions of the cochlea, the length of the tectorial membrane, the length of the

TABLE 1: Statistical results of differences in basal, medial, and apical regions between the Sham and experimental groups

Evaluation criteria		Sham	Cisplatin	Betaine	Cisplatin+Betaine	p value
SV thickness	B	13.89±1.10^{a}	10.95±1.55^{b}	13.46±1.56^{a}	12.34±1.06^{ab}	0.001
	M	14.40±1.02^{a}	11.67±0.89^{b}	13.59±1.66^{a}	12.72±2.01^{ab}	0.001
	A	15.25±3.56^{a}	12.84±2.04^{a}	13.51±1.92^{a}	13.21±1.11^{a}	0.119
TM length	B	114.29±8.70^{a}	91.09±9.27^{b}	114.56±7.82^{a}	101.30±6.29^{c}	0.001
	M	126.53±9.03^{a}	107.41±9.00^{b}	127.24±7.58^{a}	120.70±6.80^{a}	0.001
	A	172.84±84^{a}	127.06±3.43^{b}	174.14±6.51^{a}	158.09±8.43^{c}	0.001
OHC length	B	32.01±3.36^{a}	24.68±3.53^{b}	31.76±7.51^{a}	29.33±2.05^{ab}	0.003
	M	34.82±5.10^{a}	28.27±2.74^{b}	34.95±7.96^{a}	31.94±4.38^{ab}	0.028
	A	40.28±1.58^{a}	27.49±2.30^{b}	40.47±3.01^{a}	39.17±5.69^{a}	0.001
IHC length	B	30.54±7.10^{a}	24.89±1.80^{b}	31.72±0.44^{a}	27.77±3.40^{ab}	0.003
	M	36.75±6.09^{a}	30.71±5.65^{b}	37.51±2.98^{a}	34.11±3.79^{ab}	0.014
	A	38.98±1.76^{a}	28.61±5.33^{b}	37.91±1.90^{a}	31.66±3.57^{b}	0.001
BM thickness	B	5.54±1.78^{a}	4.20±0.15^{b}	5.30±0.67^{a}	5.02±0.96^{ab}	0.044
	M	4.84±1.87^{a}	2.97±0.48^{b}	4.69±1.04^{a}	3.67±1.96^{ab}	0.023
	A	3.94±2.02^{a}	2.31±0.18^{b}	3.86±0.71^{a}	3.60±1.28^{ab}	0.022
Ganglion count	B	29.30±1.16^{a}	23.10±2.13^{b}	30.00±1.24^{a}	25.10±2.23^{b}	0.001
	M	30.90±1.79^{a}	25.20±1.75^{b}	31.50±1.17^{a}	27.40±1.57^{c}	0.001
	A	31.70±1.41^{a}	26.40±1.57^{b}	32.30±1.56^{a}	28.30±1.16^{c}	0.001

*p≤0.05 indicates statistically significant results. Identical letters (a, b, c) within the same row indicate similarity between groups, while different letters (a, b, c) indicate a difference between groups. SV: Stria vascularis; TM: Tectorial Membrane; OHC: Outer Hair Cell; IHC: Inner Hair Cell; BM: Basilar Membrane; B: Basal turn; M: Medial turn; A: Apical turn

outer and inner hair cells, and the thickness of the basilar membrane were measured using ImageJ Software from the obtained images. The collected data were then statistically analyzed. It was observed that the thickness of the stria vascularis in the basal and medial turns of the Cisplatin group was significantly reduced compared to the Sham and Betain groups (<0.001). An increase in stria vascularis thickness was observed in the cisplatin+betaine group; however, this increase was not statistically significant compared with the sham and cisplatin groups (>0.05). In contrast, in the apical turn, no statistically significant difference was observed between the Sham, Cisplatin, Betain, and Cisplatin+Betain groups (>0.05). When the tectorial membrane lengths in the basal and apical regions were compared between the groups, no statistically significant difference was found between the Sham and Betain groups (>0.05). However, statistically significant differences were observed between these 2 groups and the Cisplatin and Cisplatin+Betain groups (<0.05). In the medial region, the tectorial membrane length in the Cisplatin+Betain group was increased compared with the Cisplatin group, which was statistically significant (<0.05). As a result, the tectorial membrane length was more significant in all groups than in the Cisplatin group, and the observed increase was statistically significant (<0.05). Although the length of the outer hair cells in the Cisplatin group was significantly lower than that in the sham and betain groups in the basal and medial turns (<0.001), there was no significant difference between the Cisplatin and Cisplatin+Betain groups in these turns (>0.05). However, in the apical turn, the length of the outer hair cells in the Cisplatin group was significantly decreased compared to the Sham, Betain, and Cisplatin+Betain groups (<0.001). In the basal and medial turns, the length of the inner hair cells in the Cisplatin group was significantly decreased compared to the Sham and Betain groups (<0.001). However, there was no significant difference between the Cisplatin and Cisplatin+Betain groups (>0.05). In the apical turn, there was no difference in the inner hair cell length between the Sham and the group treated only with Betain (>0.05). However, a statistically significant difference was observed between the Cisplatin and Cisplatin+Betain groups and the other groups (<0.001).

In the basal, medial, and apical turns, a statistically significant difference was found between the Sham and Betain groups compared to the Cisplatin group (<0.05). However, no significant difference was observed between the Cisplatin and Cisplatin+Betain groups (>0.05). Furthermore, no differences were found between the Cisplatin+Betain and the other groups (>0.05). Additionally, the number of spiral ganglion cells in each group was counted using the ImageJ software, and intergroup comparisons were made. Accordingly, when the number of spiral ganglion cells in the basal turn was compared between the Cisplatin group and all other groups, the number of ganglion cells in the Cisplatin group was found to be significantly lower than in the Control and Betaine groups (<0.05). However, while an increase was observed in the Cisplatin+Betaine group compared with the Cisplatin group, this increase was not statistically significant (>0.05). Furthermore, the number of spiral ganglion cells in the apical and medial turns of the cochlea was significantly lower in the Cisplatin group than in the Control and Betaine groups (<0.05). Additionally, in the Cisplatin+Betaine group, the number of spiral ganglion cells showed a significant increase compared with the Cisplatin group (<0.05).

DISCUSSION

Cisplatin is an antineoplastic drug synthesized in 1844 by M. Peyrone and became the subject of scientific research after its ability to inhibit cell division was discovered.[20] Cisplatin is used for treating various cancers, including lung carcinoma, ovarian carcinoma, head and neck carcinomas, and breast carcinoma.[21] Considering that there is currently no proven drug to treat cisplatin-induced ototoxicity in clinical practice, developing protective strategies during cisplatin treatment is essential.[22] Various agents have been investigated for this purpose, but new searches continue because the results are still insufficient. In this context, the present study investigated the protective role of betaine, which is gaining attention in the literature for its antioxidant and anti-inflammatory effects against cisplatin-induced

ototoxicity. Various structures in the cochlea were histopathologically evaluated for this purpose. The results showed a reduction in the stria vascularis thickness due to cisplatin. Betaine's protective role against cisplatin-induced damage was observed by increasing the stria vascularis thickness, bringing it closer to the Sham group's. However, this was not always statistically significant. Other parameters assessed in the current study, such as tectorial membrane length, outer and inner hair cell length, and basilar membrane thickness, were shortened due to cisplatin. It was observed that betaine reduced the side effects caused by cisplatin by increasing the tectorial membrane length, outer and inner hair cell length, and basilar membrane thickness compared with the cisplatin group. However, this increase was not always statistically significant. Additionally, the obtained results demonstrated that cisplatin administration led to a decrease in the number of spiral ganglion cells in the cochlea, whereas betaine treatment was effective in preserving the number of spiral ganglion cells.

The mechanism of action of cisplatin relies on oxidative stress, reactive oxygen species formation, and DNA damage. In normal cells, reactive oxygen species levels are attempted to be balanced by antioxidant systems, while cancer cells experience higher oxidative stress due to increased metabolic activity and mitochondrial dysfunction. Reactive oxygen species are produced in increased amounts under oxidative stress conditions and can damage cellular proteins, lipids, and DNA, leading to lethal lesions in cells.[23] However, the effects of cisplatin are not limited to cancer cells, as it similarly affects normal cells.[24] This can lead to damage and loss of function in healthy cells. Because of the histopathological evaluations in this study, significant damage, particularly in the basal turn of the cochlea, was caused by cisplatin. Deformation was observed in the basilar membrane, marginal cell loss in the stria vascularis, and ruptures in the vestïbüler membrane. The arrangement of collagen fibers in the tectorial membrane was disrupted, and cell loss, along with hydropic and vacuolar degeneration, was observed in the organ of Corti. Degeneration and increased intercellular spaces in the spiral ganglion cells were also prominent. These findings demonstrate that cisplatin causes severe structural damage to the inner ear structures. Therefore, supporting antioxidant systems in healthy cells is crucial to avoiding the side effects associated with cisplatin treatment. In this context, various agents have been investigated. In Cingi et al., sodium thiosulfate reduced the cochlear cell damage and degeneration caused by cisplatin.[12] Similarly, it has been reported that when N-acetylcysteine is added to the cisplatin treatment protocol, cisplatin-induced ototoxic damage can be reduced.[13] Cisplatin primarily affects the inner ear, disrupting the patients' hearing and balance functions.[25] High-dose coenzyme Q10 use is recommended as a protection against cisplatin-induced hearing loss.[14] The ototoxic effect of cisplatin, as observed in the present study, predominantly manifests as cochlear toxicity.[26] The cochlea tends to retain cisplatin for a longer duration compared with other organs.[4] Cisplatin enters the cochlea from the bloodstream through the capillaries in the stria vascularis. From there, it is transported into the endolymph of the scala media and subsequently reaches the hair cells.[27] Cisplatin accumulates in the cochlea and remains there for an extended period, leading to ototoxicity. It has toxic effects by affecting the cochlea's sensory hair cells, spiral ganglion, stria vascularis, spiral ligament, and secretory cells.[28]

Cisplatin-induced ototoxicity is bilateral, with a particular loss of outer hair cells in the basal turn of the cochlea.[29] In this study, a loss of outer hair cells due to cisplatin was observed, and this effect was reduced by betaine. Although cisplatin toxicity primarily affects auditory functions, it can also manifest as tinnitus and, less commonly, as ear pain or loss of balance due to vestibulotoxicity.[30]

Various agents have been investigated as protectants against cisplatin ototoxicity.[31] Antioxidant drugs are protective against cisplatin-induced ototoxicity.[32] Sodium thiosulfate has reduced ototoxicity when applied systemically or locally; however, it has been observed that systemic administration may affect survival in metastatic cancer cases.[33,34] Compounds such as N-acetylcysteine and D-methionine have shown cytoprotective effects in animal models.[35,36] Oral administration of β-lapachone reduces ototoxicity without compromising the therapeutic ef-

fects of cisplatin.[37,38] Protective strategies aimed at reducing cytotoxicity through these mechanisms are essential, and research is ongoing.

Betaine donates its methyl group to homocysteine via betaine-homocysteine methyltransferase, facilitating the conversion of homocysteine to methionine.[16] Hyperhomocysteinemia triggers oxidative stress and apoptosis.[39] Methionine plays a crucial role in antioxidation by reducing oxidative stress through chelation and serving as a substrate for glutathione synthesis in hepatocytes.[40] Another known role of betaine is its function as an osmoprotectant, accumulating in various organs without disrupting cell function. This helps protect cells, proteins, and enzymes under osmotic stress.[17] In addition to being obtained from dietary sources, it can also be endogenously synthesized by the kidneys and liver.[41]

Nuclear factor-kappa B regulates pro-inflammatory cytokines involved in inflammation, such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-23.[42] Betaine alleviates inflammation by inhibiting the Nuclear factor-kappa B signaling pathway.[43] Additionally, betaine acts as an effective antioxidant and anti-inflammatory agent in conditions such as obesity, cancer, and Alzheimer's disease.[44-46] The findings of this study suggest that betaine could be a potential prophylactic agent against cisplatin-induced ototoxicity. As supported by previous research, its protective effects may be linked to its antioxidant and anti-inflammatory properties.

A limitation of the study is that it did not investigate whether betaine affects the chemotherapeutic effects of cisplatin while examining its protective role against cisplatin-induced ototoxicity. This study's findings on betaine's protective efficacy against cisplatin-induced ototoxicity offer essential insights for future research.

CONCLUSION

This study highlights the potential protective role of betaine against cisplatin-induced ototoxicity. Histopathological findings revealed that betaine reduced the damage in the cochlear structures caused by cisplatin, particularly in the stria vascularis, tectorial membrane, and hair cells. It can be concluded that this effect of betaine may be attributed to its antioxidant and anti-inflammatory properties. The results are promising for ototoxicity prophylaxis, but further research is still needed.

In this study, the authors carefully used the ChatGPT 3.5 program to improve language and readability and took full responsibility for the content by reviewing and editing it as necessary.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Menekşe Ülger;* ***Design:*** *Menekşe Ülger;* ***Control/Supervision:*** *Menekşe Ülger;* ***Data Collection and/or Processing:*** *Esra Balcıoğlu;* ***Analysis and/or Interpretation:*** *Esra Balcıoğlu;* ***Literature Review:*** *Menekşe Ülger;* ***Writing the Article:*** *Menekşe Ülger, Esra Balcıoğlu;* ***Critical Review:*** *Esra Balcıoğlu;* ***References and Fundings:*** *Menekşe Ülger;* ***Materials:*** *Menekşe Ülger.*

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