Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2025;50(2):287-298
DOI: 10.17826/cumj.1621404

RESEARCH

Bone marrow-derived mesenchymal stem cells mitigate colistin-induced cochlear ototoxicity: an experimental study

Kemik iliği kaynaklı mezenkimal kök hücreler kolistin kaynaklı koklear ototoksisiteyi azaltır: deneysel bir çalışma

Özlem Öz Gergin[1], Sibel Seçkin Pehlivan[1], Adnan Bayram[1], Mustafa Birkin[1], Eda Köseoğlu[1], Demet Bolat[1], Esra Balcıoğlu[1]

[1]Erciyes University, Kayseri, Turkey

Abstract

Purpose: This study investigates the therapeutic potential of mesenchymal stem cells in colistin-induced hearing loss.
Materials and Methods: Forty Wistar albino rats were divided into four groups (n=10): Control (36 mg/kg/day intraperitoneal (ip) saline for 7 days), Colistin (36 mg/kg/day ip for 7 days), Mesenchymal stem cell group (MSC) (5×10^6 MSC via tail vein), and Colistin+Mesenchymal stem cell group (Col+MSC) (colistin for 7 days, then MSC 1 hour after final dose). After the experiment, cochlear tissues were extracted, fixed, decalcified, embedded in paraffin, sectioned (5µm), and stained with Masson's trichrome (MT) for histological evaluation under light microscopy.
Results: Colistin caused marked structural damage, especially in the basal and medial cochlear turns. In the apical and medial regions, stria vascularis thickness was significantly lower in the colistin group (10.64±1.90, 11.54±2.32) than in the control (14.12±2.03, 15.43±2.26) and MSC (13.91±1.49, 14.53±1.80) groups. Significant intergroup differences were also noted in the basal turn. Inner hair cell lengths were similar apically but significantly reduced in the colistin (30.73±4.28) and Col+MSC (31.06±6.96) groups medially. Outer hair cell lengths were significantly reduced in the colistin group in both apical (32.23±8.32) and basal (18.32±2.47) regions. Tectorial membrane thickness in the basal turn was significantly reduced in all treatment groups compared to the control (17.64±5.41). Based on Freitas et al.'s criteria, the colistin group showed significantly higher histopathological damage scores in all regions compared to the control and MSC groups. The Col+MSC group showed improved morphology and lower scores, though some differences lacked statistical significance.

Öz

Amaç: Bu çalışma, kolistin kaynaklı işitme kaybında mezenkimal kök hücrelerin (MSC) terapötik potansiyelini araştırmayı amaçlamaktadır.
Gereç ve Yöntem: Kırk Wistar albino sıçan dört gruba ayrıldı (n=10): Kontrol (7 gün boyunca 36 mg/kg/gün intraperitoneal (ip) serum fizyolojik), Kolistin (7 gün boyunca 36 mg/kg/gün ip kolistin), Mezenkimal kök hücre grubu (MSC) (kuyruk veninden 5×10^6 MSC), ve Kolistin+MSC grubu (Col+MSC) (7 gün boyunca kolistin, son dozdan 1 saat sonra 5×10^6 MSC). Deney sonunda, koklea dokuları çıkarıldı, fikse edildi, dekalcifiye edildi, parafin bloklara gömüldü, 5 µm kalınlığında kesitler alındı ve Masson's trikrom (MT) ile boyanarak ışık mikroskobu altında histolojik olarak değerlendirildi.
Bulgular: Kolistin grubu özellikle kokleanın bazal ve medial kıvrımlarında belirgin yapısal hasar gösterdi. Apikal ve medial bölgelerde, stria vasküläris kalınlığı kolistin grubunda (10.64±1.90, 11.54±2.32) kontrol (14.12±2.03, 15.43±2.26) ve MSC (13.91±1.49, 14.53±1.80) gruplarına göre anlamlı derecede azdı. Bazal kıvrımda da gruplar arasında anlamlı farklılıklar gözlendi. İç tüylü hücrelerin uzunlukları apikalde benzerken, medialde kolistin (30.73±4.28) ve Col+MSC (31.06±6.96) gruplarında anlamlı azalma görüldü. Dış tüylü hücre uzunlukları ise kolistin grubunda hem apikal (32.23±8.32) hem de bazal (18.32±2.47) bölgelerde anlamlı şekilde kısaydı. Bazal kıvrımda, tectorial membran kalınlığı tüm tedavi gruplarında kontrol grubuna (17.64±5.41) kıyasla anlamlı şekilde azaldı. Freitas ve ark. tarafından tanımlanan kriterlere göre, kolistin grubunun tüm bölgelerde histopatolojik hasar skorları kontrol ve MSC gruplarına göre anlamlı olarak daha yüksekti. Col+MSC grubu daha

Address for Correspondence: Özlem Öz Gergin, Department of Anesthesiology and Reanimation, Erciyes University, Faculty of Medicine, Kayseri, Türkiye E-mail: oozgergin@erciyes.edu.tr
Received: 16.01.2025 Accepted: 05.05.2025

Conclusion: MSCs significantly alleviated colistin-induced ototoxicity, indicating their protective and therapeutic potential in inner ear injury.

Keywords: Colistin, ototoxicity, mesenchymal stem cell, cochlea, organ of Corti

organize morfoloji ve düşük skorlar gösterse de bazı farklılıklar istatistiksel olarak anlamlı değildi.
Sonuç: MSC'ler, kolistin kaynaklı ototoksisiteyi anlamlı derecede hafifleterek iç kulak hasarında koruyucu ve terapötik potansiyele sahip olduklarını göstermektedir.
Anahtar kelimeler: Kolistin, ototoksisite, mezenkimal kök hücre, koklea, korti organı

INTRODUCTION

Hearing occurs due to the synergistic working of several tissue and cell types in the inner ear structure known as the cochlea. The primary cause of sensorineural hearing loss is harm to the hair cells that convert sound vibrations into electrical signals. These cells are quite sensitive to various factors, such as exposure to noise, ototoxic substances, and drugs. Literature indicates that as hearing loss advances, hair cells and supporting cells, along with neurons in the cochlear nerve, experience degeneration[1]. Colistin, an antibiotic commonly called polymyxin E, treats multidrug-resistant bacterial infections[2-6]. Nephrotoxicity and neurotoxicity are among the most frequently observed adverse effects of colistin treatment, which significantly restricts its use[7-9]. Neurotoxicity, which makes up about 0–7% of the harmful impacts of colistin, is usually marked by problems with nerves and muscles, like dizziness, walking impairment, and visual disturbances[10].

However, we have yet to thoroughly elucidate the specific effects of colistin on the auditory system, and the literature lacks documented data on colistin-induced ototoxicity. Mesenchymal stem cells (MSCs) are a therapeutic modality that is highly promising in a variety of regenerative medicine disciplines, including the treatment of ototoxic damage. By synthesizing neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), derived from bone marrow, adipose tissue, umbilical cord blood, and embryonic tissues, MSCs facilitate the regeneration and repair of nerve tissue. Reports suggest that these cells stimulate angiogenesis, synaptogenesis, axonal growth, and neurogenesis, leading to protective and reparative effects against nerve injury. Furthermore, the literature suggests these cells regulate inflammation by inhibiting apoptosis and cytotoxicity[11]. MSCs possess anti-inflammatory, anti-apoptotic, antifibrotic, and immunomodulatory characteristics attributable to the trophic substances they release[12]. Clinical and preclinical investigations frequently choose these multipotent stem cells for immune-mediated and degenerative diseases due to their immunomodulatory properties, differentiation potential, and self-renewal[13].

This study aims to investigate the ototoxic effects of colistin on the cochlea, particularly focusing on the organ of Corti, and to assess the potential protective role of mesenchymal stem cells (MSCs). Despite the well-documented sensitivity of inner and outer hair cells to various agents, data on colistin's specific impact remain limited. Addressing this gap, the study employs detailed histological and histomorphometric analyses to evaluate structural damage induced by colistin. Additionally, it explores the therapeutic potential of MSCs in mitigating such injury. By doing so, this research contributes novel insights into the application of stem cell therapy for drug-induced ototoxicity and supports the hypothesis that MSC administration can attenuate the morphological damage caused by colistin.

MATERIALS AND METHODS

Experiment procedure

The study complied with ethical guidelines following approval from the Erciyes Local Ethics Committee for Animal Experiments (Approval date 05.12.2024 and number 24/234). Erciyes Scientific Research Projects Coordination Unit supported this study (Grant number: TSA-2021-11331). The animals utilized in the experiment were procured from the Experimental Research Application and Research Center of Erciyes (DEKAM). The study comprised a total of 40 Wistar albino rats. The rats were maintained in a regulated environment featuring 12 hours of illumination (07:00-19:00) and 12 hours of darkness (19:00-07:00), with automated climate control set at 21±2 °C. The oral route provided standard rat feed and water. Throughout the trial duration, the rats were provided with commercial meals. All rats were maintained under these ambient settings for one week before the experiment.

Experimental design and grouping

Forty rats designated for the experiment were categorized into four groups: control, colistin, MSC, and colistin+MSC (Col+MSC). The procedures performed on the subjects are summarized in Table 1. The control group received 36 mg/kg/day saline for 7 days, and the colistin group received 36 mg/kg/day colistin intraperitoneally for 7 days. The MSC group was administered $5x10^6$ MSC from the tail vein, the Col+MSC group, intraperitoneal colistin administration at 36 mg/kg/day for 7 days, followed by the administration of $5x10^6$ MSC via the tail vein 1 hour after the last administration. All applications were carried out in DEKAM.

Table 1. Experimental setup for control and experimental groups.

Group Name	Application Method and Duration
Control Group	For 7 days, 36 mg/kg/day Intraperitoneal saline solution
Colistin Group	For 7 days, 36 mg/kg/day of Intraperitoneal colistin
MSC Group	$5X10^6$ MSC from the tail vein
Col+MSC Group	Intraperitoneal colistin administration at 36 mg/kg/day for 7 days, followed by the administration of $5X10^6$ MSC via the tail vein 1 hour after the last administration.

MSC: Mesenchymal Stem Cell; Col: Colistin; Col+MSC: Combined treatment with Colistin and Mesenchymal Stem Cells.

Tissue collection, processing, and histological evaluation

By ethical standards, the rodents were decapitated under anesthesia with 50 mg/kg Ketamine and 10 mg/kg Xylazine 72 hours following the procedures. The parietal bones were cut to their termination point, and the brain tissue was extracted by entering through the foramen magnum. To liberate the cochlea, the external auditory canal was also excised. The cochlea, which extends toward the middle cranial fossa and middle ear, was identified by focusing on the entry site of the cochlear nerve. The excess bone tissues surrounding the cochlea were removed using fine-tipped scissors. In this manner, the vestibule connection was unsealed, and the vestibule was also removed. The cochlea was fixed in a freshly prepared 10% formaldehyde solution after it was obtained. After the fixation, the bone tissue was softened in a solution of 80 mL distilled water, 10 mL formaldehyde, and 10 mL nitric acid. This process is known as decalcification, and the bone is kept in solution until it is soft enough to be examined[14-16]. The tissues were dehydrated by passing through increasing alcohol concentrations and washed in running tap water after the decalcification phase. It was embedded in paraffin after applying routine histological tissue tracking stages[14-16]. 5 μm-thick sections were taken until the cochlea tissue in the paraffin blocks was depleted, and one slide per 10 slides was deparaffinized and rehydrated. The cochlea tissue was stained with Masson Trichrome (MT) dye, considering that it would reflect the difference in staining between collagen fibers, bone, muscle tissue, and spinal ganglia. It was evaluated under a light microscope (Olympus BX51 light microscope) according to the specified criteria[14-16]. All tissue fixation, calcification, paraffin embedding, sectioning and staining steps were performed in the research laboratory of the Department of Histology and Embryology, Erciyes University Faculty of Medicine. In addition, histological evaluations were performed by two expert histologists at the Department of Histology and Embryology, Erciyes University Faculty of Medicine. After the evaluations were completed, paraffin blocks and MT-stained preparations were archived.

Morphometric measurements

The cochlea is a bony canal that rotates approximately 2.5 turns around the modiolus, forming its axis. It is known that the diameter of the basal fold is thicker than the apex. Based on this information in the literature, apical, medial, and basal regions were differentiated. Additionally, the stria vascularised thickness (X20), the inner and outer hair cells (X40), and the thickness of the tectorial membrane (X40) in the basal, medial, and apical turns of the cochlea were measured using the Image J software program (n=10) and statistically evaluated[14-17] (Figure 1).

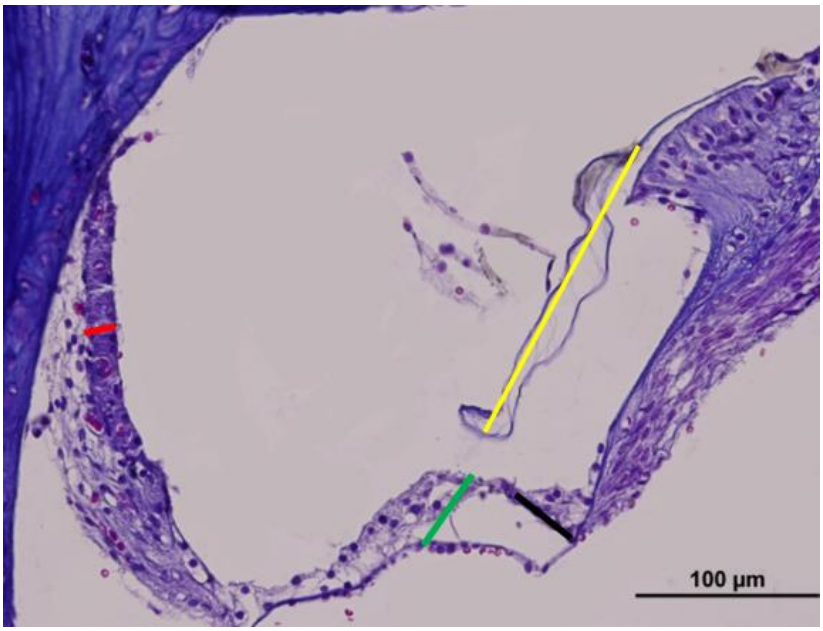

Figure 1. Representative micrographs showing where and how measurements were made (X40). red line; Stria vascularis thickness, black line; Inner hair cell length, green line; Outer hair cell length, yellow line; Tectorial membrane thickness.

Histopathological Evaluation

Our descriptive study by Freitas et al.[18] employed a histopathological scoring system to assess the stria vascularis, the organ of Corti, and the spiral ganglion. A four-point scale was utilized to evaluate ototoxicity (Table 2).

Table 2. Scoring system developed by Freitas et al.[18]

Histopathological Scoring System for the Stria Vascularis	
Score 0	No shrinkage
Score 1	Mild shrinkage
Score 2	Moderate shrinkage
Score 3	Severe shrinkage
Histopathological Scoring System for the Organ of Corti	
Score 0	Presence of 3 intact outer hair cells (OHCs)
Score 1	Presence of 2 intact OHCs
Score 2	Presence of 1 intact OHC
Score 3	Absence of OHCs
Histopathological Scoring System for the Spiral Ganglion	
Score 0	No pathological changes
Score 1	Mild pathological changes
Score 2	Moderate pathological changes
Score 3	Severe pathological changes

Statistical analysis

All statistical analyses were performed utilizing the Statistical Package for Social Sciences (SPSS) for Windows version 22.0. The conformity of the Morphometric Measurement and Histopathological Evaluation results to normal distribution was determined using the Shapiro-Wilk test. We compared the control and experimental groups using One-Way Analysis of Variance (ANOVA) for normally distributed variables. Once we identified a difference, we performed multiple comparisons using the Tukey test. The data were expressed as mean ± standard deviation, with p values less than 0.05 deemed statistically significant.

RESULTS

Using a light microscope, we examined the preparations from all groups and noted that the cochlea's external morphology preserved its cylindrical configuration. The cochlea, which completes 2.5 turns around the modiolus, clearly reveals the organ of Corti in the basal, medial, and apical regions. The basilar membrane, a fibrous connective tissue structure, and Reissner's membrane consistently delineated the scala media from the scala tympani compartment and the scala vestibuli compartment, respectively, upon examination of the light microscopic images from the control and MSC groups (Figure 2).

Light microscope pictures made it easy to see the stria vascularis, which comprises intermediate, marginal, and basal cells and has many blood vessels. The tectorial membrane, a gelatinous structure of parallel-arranged collagen fibers, was prominent and enveloped the hair cells. In the preparations of the control and MSC groups, the organ of Corti, located on the basilar membrane and housing sensory receptor hair cells, displayed three rows of outer hair cells and a single row of inner hair cells. The configuration of the nucleus of Deiter cells, which provide support by extending cytoplasmic projections to the hair cells, along with the pillar cells encircling the Corti tunnel, rendered them readily identifiable. The nuclei of the initial row of cells, which have a supporting role and are located on the basilar membrane, were positioned toward the base and belonged to Boettcher cells. The cells between the Boettcher cells' apical surface and the lumen constituted the Claudius cells (Figure 2).

Colistin's ototoxicity damages all inner ear structures. The basal membrane specifically showed deformation, accompanied by cytoplasmic and nuclear condensation. Furthermore, cell degeneration and vacuolization were significant in the marginal cells of the stria vascularis. At the same time, observing breaches in the Reissner membrane was common. Furthermore, the colistin group (Figure 2) noted anomalies and disruptions in the architecture of the tectorial membrane, which consists of collagen fibers organized in parallel. The light microscopic images of the colistin group revealed anomalies in the overall histological architecture of the organ of Corti, situated in the basal, apical, and medial turns. There was a lot of cell death and damage in the inner and outer hair cells, the outer and inner pillar cells that surround the Corti tunnel, and the Deiters, Boettcher, and Claudius cells that support the organ of Corti on the basilar membrane (Figure 2). The Col+MSC group had a more organized structure than the colistin group. There were fewer gaps between the cells that make up the stria vascularis, and the Reissner and tectorial membranes were more organized. While less pronounced than in the colistin group, the Col+MSC group exhibited losses of supporting cells, outer hair cells, and inner hair cells, constituting the organ of Corti. Furthermore, the intercellular gaps had diminished relative to the colistin group, enhancing cell distinctiveness. The inner and outer pillar cells encircling the Corti tunnel displayed a more uniform structure (Figure 2).

The spiral ganglion cells in the apical, basal, and medial regions of the cochlea in the control and MSC groups generally displayed a standard histological structure and a distinct nuclear structure, and no spaces were visible between the cells. However, the colistin group showed the presence of degenerated spiral ganglion cells, prominent intercellular spaces, and vacuolization in the ganglionic cells (Figure 2). In contrast to the colistin group, a reduction in the number of degenerated spiral ganglion cells was noted in the Col+MSC group, although vacuolization persisted in certain regions (Figure 2).

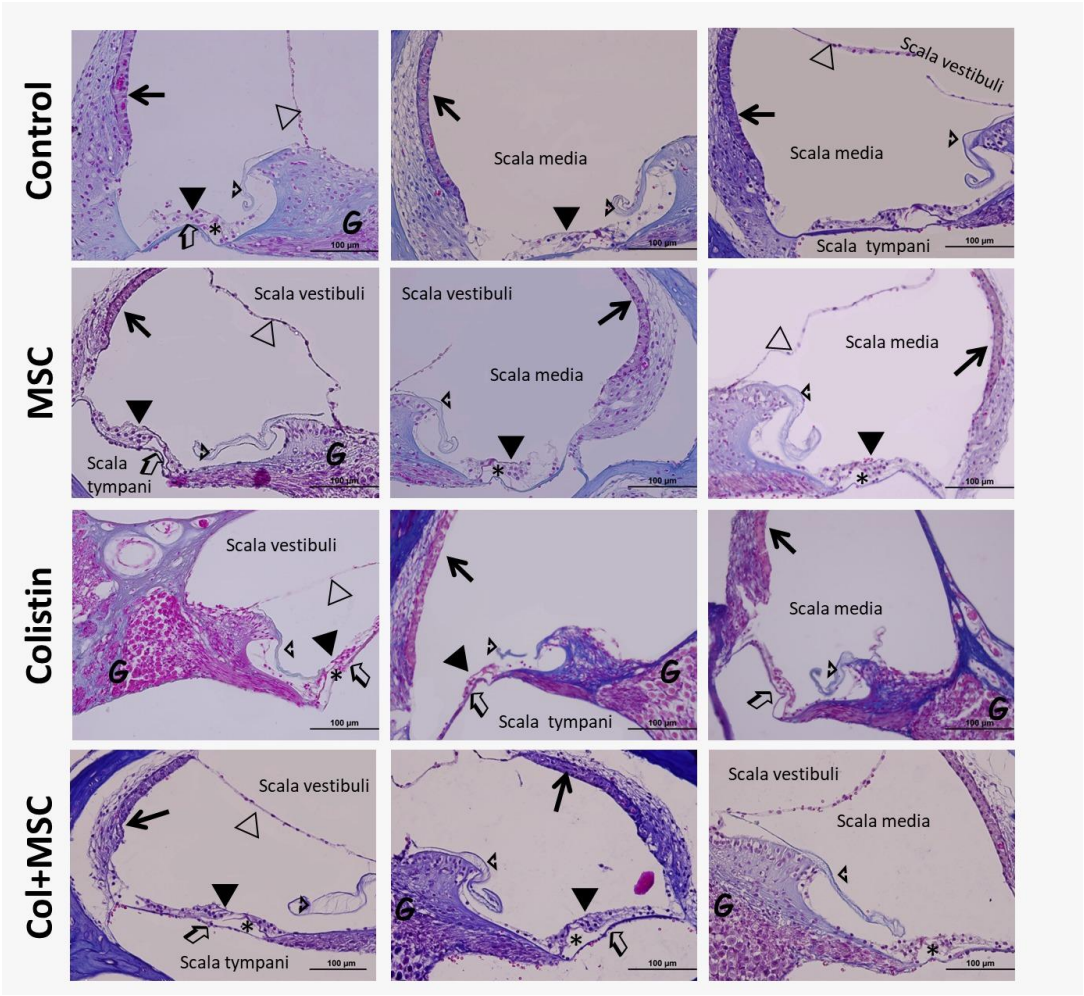

Figure 2. Light microscopic appearance of the corti organ from control and experimental groups (MTX40) (Bar=100 µm), Reissner membrane, Outer hair, *Corti tunnel, Tectorial membrane, Basilar membrane, G: Spiral Ganglion, Stria vascularis.

In the colistin group, the stria vascularis thickness at the apex and medial turn was much thinner than in the control and MSC groups (Table 3) ($p<0.05$). However, there was no significant difference between the Col+MSC group and the other groups, nor between the control group and the MSC group (There was a significant difference in the thickness of the stria vascularis in the basal turn between the control and MSC groups, as well as between the colistin and Col+MSC groups (Table 3) ($p<0.05$). This is thought to be the area most affected by colistin-induced ototoxicity. In the very top part of the cochlea, the lengths of the inner hair cells were about the same in the control, MSC, and colistin-treated groups (Table 3) ($p>0.05$). Conversely, in the medial turn, a statistically significant difference was noted between the control and colistin and Col+MSC groups ($p<0.05$). Evaluation of inner hair cell lengths at the basal turn revealed a significant difference solely between the colistin group and the MSC group (Table 3) ($p <0.05$).

We individually assessed the average lengths of the outer hair cells, which are crucial to the organ of Corti, for each turn. Comparison of average outer hair cell lengths in the apex region revealed a statistically significant difference solely between the

control and MSC groups, as well as the colistin group (Table 3) ($p<0.05$). Furthermore, we established that

the colistin group reduced the lengths of outer hair cells in the medial region, but this reduction was not statistically significant (Table 3) ($p>0.05$). Comparing the average lengths of the outer hair cells in the basal turn across groups revealed a significant difference between the MSC group and both the colistin and Col+MSC groups (Table 3) ($p<0.05$), whereas no significant differences were noted among the other groups (Table 3) ($p>0.05$).

No significant difference in tectorial membrane thickness was identified among the control, MSC, and colistin-treated groups in the apical turn of the cochlea (Table 3) ($p>0.05$). However, a statistically significant difference was noted between the control group and the other groups in the medial turn (Table 3) ($p<0.05$). A statistically significant difference was seen between the colistin and MSC groups in the medial region (Table 3) ($p<0.05$). In the basal return of the cochlea, the thickness of the tectorial membrane was significantly reduced in the Colistin, MSC, and Col+MSC groups compared to the control group (Table 3) ($p<0.05$).

Table 3. Measurement data derived from the cochlea's basal, medial, and apical regions for both the control and experimental groups

Measurement areas (n=10)		Control Group	Colistin Group	MSC Group	Col+MSC Group	p
Stria vascularis thickness	Apical Turn	14.12±2.03^{a}	10.64±1.90^{b}	13.91±1.49^{a}	12.09±1.74^{ab}	.000
	Medial Turn	15.43±2.26^{a}	11.54±2.32^{b}	14.53±1.80^{a}	13.63±2.33^{ab}	.003
	Basal Turn	18.68±4.38^{a}	12.39±4.17^{b}	18.13±3.45^{a}	13.51±1.23^{b}	.000
Inner hair cell length	Apical Turn	40.72±3.89^{a}	36.99±5.04^{a}	41.62±6.56^{a}	39.30±6.53^{a}	.293
	Medial Turn	37.84±4.59^{a}	30.73±4.28^{b}	36.14±2.54^{ab}	31.06±6.96^{b}	.003
	Basal Turn	34.19±0.32^{ab}	29.96±4.08^{b}	35.08±4.59^{a}	31.44±3.43^{ab}	.008
Outer hair cell length	Apical Turn	40.07±3.43^{a}	32.23±8.32^{b}	42.93±7.36^{a}	35.42±5.02^{ab}	.003
	Medial Turn	34.15±7.60^{a}	33.70±9.36^{a}	38.56±6.41^{a}	34.52±5.72^{a}	.442
	Basal Turn	21.13±1.79^{ab}	18.32±2.47^{a}	23.53±3.15^{b}	19.68±3.63^{a}	.002
Tectorial membrane thickness	Apical Turn	14.08±2.67^{a}	10.99±3.54^{a}	10.80±2.73^{a}	11.10±1.27^{a}	.028
	Medial Turn	16.12±3.07^{a}	8.93±2.32^{b}	12.03±2.14^{c}	9.88±1.54^{bc}	.000
	Basal Turn	17.64±5.41^{a}	11.46±3.60^{b}	13.34±1.63^{b}	12.20±2.03^{b}	.002

Identical superscript letters within the same row indicate no statistically significant difference between groups, whereas different letters denote significant differences (p <0.05). Abbreviations: MSC: Mesenchymal Stem Cell; Col: Colistin; Col+MSC: Combined treatment with Colistin and Mesenchymal Stem Cells.

According to the defined criteria, the stria vascularis was evaluated at the apical, medial, and basal turns, and the results are presented in Table 4. In the colistin group, moderate to severe shrinkage was observed, particularly in the basal and medial turns. Statistical analysis revealed a significant increase in shrinkage in the colistin group compared to the control and MSC groups ($p<0.05$). Additionally, in the Col+MSC group, mild to moderate shrinkage was observed. Although the stria vascularis scores were reduced in the Col+MSC group compared to the colistin group, this decrease did not reach statistical significance ($p>0.05$). Evaluation of the apical turns revealed a statistically significant difference between the colistin and control and MSC groups ($p<0.05$). Moreover, a considerable score reduction was observed in the Col+MSC group compared to the colistin group ($p<0.05$).

Based on the criteria established by Freitas et al.[18], the organ of Corti was assessed at the apical, medial, and basal turns, and the findings are also presented in Table 4. Within the colistin group, the highest scores were recorded in the basal turn, followed by the medial and apical turns. These scores were significantly elevated compared to those in the control and MSC groups ($p<0.05$). Although scores were decreased in the Col+MSC group, this reduction was not statistically significant compared to the colistin group ($p>0.05$). However, scores in the Col+MSC group remained significantly higher than those in the control and MSC groups ($p<0.05$). Similarly, the spiral ganglion was evaluated at the apical, medial, and basal turns according to the predefined criteria, and the outcomes are shown in Table 4. In the basal and medial turns, the lowest scores were observed in the control and MSC groups. Conversely, the colistin group exhibited significantly elevated scores, indicating more severe pathological changes ($p<0.05$). When the spiral ganglia at the apical turn were assessed using the Freitas et al. [18] criteria, a significant difference was observed between the colistin group and the control, MSC, and Col+MSC groups ($p<0.05$). Furthermore, a substantial reduction in the Col+MSC group scores was noted compared to the colistin group ($p<0.05$).

Table 4. Statistical results based on the scoring system developed by Freitas et al.[18]

		Control Group	Colistin Group	MSC Group	Col+MSC Group	p
Stria vascularis	Apical Turn	0.10 ± 0.31^{a}	2.00 ± 0.66^{b}	0.20 ± 0.42^{a}	0.90 ± 0.73^{c}	.001
	Medial Turn	0.40 ± 0.51^{a}	2.20 ± 0.78^{b}	0.30 ± 0.48^{a}	1.50 ± 0.52^{b}	.001
	Basal Turn	0.30 ± 0.48^{a}	2.30 ± 0.67^{b}	0.20 ± 0.42^{a}	1.70 ± 0.67^{b}	.001
Organ of Corti	Apical Turn	0.20 ± 0.42^{a}	1.60 ± 0.51^{b}	0.10 ± 0.31^{a}	1.00 ± 0.81^{b}	.001
	Medial Turn	0.10 ± 0.31^{a}	2.00 ± 0.81^{b}	0.10 ± 0.31^{a}	1.40 ± 0.69^{b}	.001
	Basal Turn	0.20 ± 0.42^{a}	2.10 ± 0.56^{b}	0.20 ± 0.42^{a}	1.50 ± 0.85^{b}	.001
Spiral ganglion	Apical Turn	0.30 ± 0.48^{ac}	1.80 ± 0.63^{b}	0.20 ± 0.42^{c}	1.00 ± 0.81^{a}	.001
	Medial Turn	0.20 ± 0.42^{a}	2.10 ± 0.56^{b}	0.10 ± 0.31^{a}	1.70 ± 0.48^{b}	.001
	Basal Turn	0.30 ± 0.48^{a}	2.30 ± 0.67^{b}	0.20 ± 0.42^{a}	1.80 ± 0.63^{b}	.001

Identical letters on the same line signify similarity among groupings. Diverse letters convey distinction. Abbreviations: MSC: Mesenchymal Stem Cell; Col: Colistin; Col+MSC: Combined treatment with Colistin and Mesenchymal Stem Cells.

DISCUSSION

This study examined the detrimental effects of the antibiotic colistin on nerve cells within the cochlear structure and the potential mitigating role of MSCs. Investigators employing histology and histomorphometry have determined that colistin administration adversely affects the cochlea's inner and outer hair cells, stria vascularis, and spiral ganglion. This is particularly evident in the stria vascularis region, which supplies blood to the inner ear. There are issues with hemorrhaging and cellular

function in that area. The cohort administered MSCs and colistin exhibited significantly reduced cochlear damage and enhanced cellular structure preservation. The studies demonstrate that MSCs confer protection against colistin-induced ototoxicity. Ototoxic chemicals can lead to hearing loss and balance disorders, presenting significant clinical challenges due to the potential for irreparable harm[19].

MSCs play a crucial role in immune regulation and inflammation suppression, primarily through the secretion of cytokines and growth factors. A key component of this mechanism is the secretion of interleukin-10 (IL-10), an anti-inflammatory cytokine that significantly contributes to the immunomodulatory effects of MSCs. IL-10 is essential in various contexts, including autoimmune diseases and tissue repair, where it helps maintain immune homeostasis and reduce inflammation. The following sections elaborate on the specific roles and mechanisms of IL-10 in MSC-mediated immune regulation[20]. MSCs have been shown to enhance the secretion of neurotrophic factors, including brain-derived neurotrophic factor (BDNF), which are crucial for the survival, growth, and regeneration of nerve cells. The therapeutic potential of MSCs in neurological contexts is supported by various studies highlighting their paracrine effects and ability to modulate the neuroprotective environment[21]. Colistin, an antibiotic primarily used against multidrug-resistant gram-negative bacteria, exerts its effects by targeting the outer membrane of these cells. This mechanism involves displacing calcium and magnesium ions, leading to membrane destabilization and increased permeability, which can result in cell death. In the stria vascularis of the inner ear, where ionic balance is crucial for auditory function, such disruptions can lead to ototoxicity. The following sections elaborate on the implications of colistin's action[22].

The World Health Organization (WHO) projects that by 2050, roughly one-quarter of the world's population will have hearing loss[1]. The current situation has sped up research into ototoxicity, chemicals' harm to the inner ear, and environmental factors. It has been found that ototoxic agents like amikacin, neomycin, kanamycin, and sisomicin hurt the cochleae of test animals, just as they hurt humans[23]. In the literature, gentamicin has been reported to cause significant structural damage by targeting the outer hair cells in the cochlea's basal turn. This effect extends to the inner hair cells with increased dosage, leading to distinct histopathological alterations. Moreover, it has been emphasized that nerve fibers are also affected independently of the condition of the sensory cells[24]. In the literature, streptomycin has been reported to induce the most pronounced cochleotoxic effect by causing significant damage to the sensory hair cells of the cochlear epithelium, followed by gentamicin and netilmicin. Additionally, surface macrophages associated with auditory hair cells have been suggested to contribute to immune surveillance within the organ of Corti. These findings indicate that aminoglycoside-induced hearing loss may be associated with cellular damage and immunological mechanisms[25].

Histopathological examination of temporal bones from patients treated with cisplatin reveals degenerative changes primarily in the cochlea, especially affecting the outer hair cells in the basal turns (base-hook region) of the organ of Corti. This correlates with the clinical pattern of high-frequency hearing loss[26]. Clinical reports and reviews have indicated that ciprofloxacin, combined with dexamethasone and during long-term use, does not lead to significant ototoxicity in patients. Auditory function tests conducted before and after treatment revealed no meaningful hearing loss attributable to ciprofloxacin. These findings support the safety of ciprofloxacin as a topical agent in otological applications [27]. Outer hair cells (OHCs) are primarily affected first, followed by inner hair cells (IHCs). The degeneration starts at the basal turn of the cochlea, which corresponds to the region sensitive to high-frequency sounds, and progresses toward the apex, which processes lower frequencies. This pattern explains why hearing loss initially affects high-frequency sounds before involving lower frequencies [28]. Aminoglycoside drugs have been demonstrated to induce damage to both hair cells and spiral ganglion cells[29]. Smith et al.[30] demonstrated that gentamicin and tobramycin induced cochlear impairment in 16% of individuals and vestibular damage in 15% of patients. The results of our investigation align with the existing literature. It has been shown that giving colistin to animals does much damage to the outer and inner hair cells in the basal turn of the cochlea, which leads to the death of supporting cells. Along with this, there was deformation of the basilar membrane, vacuolization, cell death in the stria vascularis, rupture of the Reissner membrane, and problems with the tectorial membrane. In the Col+MSC group, Corti's cochlea and organ had a

more organized structure, albeit to a lesser extent than the control group.

Research assessing the thickness of the stria vascularis[31] indicated that a single administration of 8 mg/kg cisplatin, especially in the basal turn of the cochlea, resulted in an increased thickness of the stria vascularis relative to the control group. Recent studies on the causes of cochlear damage caused by aminoglycosides have concentrated on inflammatory responses[32]. Simultaneously, research by Koo et al.[33] showed that dilatation in the capillaries of the stria vascularis was detected in instances of cochlear injury induced by aminoglycosides.

Our investigation found that colistin-induced damage to the stria vascularis leads to vacuolization and cell atrophy. The Col+MSC-treated mice, on the other hand, had less vacuolization and atrophy in the stria vascularis. In contrast to previous studies, this one measured the thickness of the stria vascularis in the apical, medial, and basal areas. The group that was treated with colistin had a loss, while the group that was treated with MSC as a therapeutic drug had an increase. This study differs from others because it found that colistin therapy hurt the inner and outer hair cells and the thickness of the tectorial membrane. Remarkably, the colistin therapy with MSC led to improvements in these parameters. These data imply that stem cells may protect against colistin-induced ototoxicity. The deterioration of hair cells in the organ of Corti is typically an irreversible phenomenon, underscoring the need to avoid exposure to ototoxic agents and the early diagnosis of cochlear impairment[34].

Despite the complexity of utilizing stem cell therapy to address hearing loss, research in this domain produces encouraging outcomes. There are many different ways stem cell treatments for the inner ear work, such as differentiating endogenous stem cells and implanting foreign stem cells. To do this, many types of stem cells have been tested in living things (in vivo)[35-37]. These include embryonic stem cells, neural stem cells, mesenchymal stem cells, and induced pluripotent stem cells. Corrales et al. reported the formation of in vitro inner ear progenitor cells derived from mouse embryonic stem cells and their incorporation into the epithelial-damaged hair cell areas of the developing inner ear[38].

Further research in the literature indicated that neural stem cells derived from the olfactory epithelium of rats subjected to noise moved to spiral ganglion neurons, resulting in better hearing loss[39]. Elbana et al. have demonstrated the protective effect of bone marrow-derived stem cells in amikacin-induced ototoxicity[40]. These results endorse the notion that stem cells represent a potential strategy for addressing hearing loss. The histology findings from our investigation align with the existing literature. According to the histological study, colistin's ototoxic effects led to changes in the structure of the organ of Corti, specifically the loss of outer and inner hair cells. As part of our study, $5X10^6$ MSC was given through the tail vein one hour after 36 mg/kg/day of intraperitoneal colistin for 7 days. The colistin caused damage to the cochlea, which was most noticeable in the basal turn. The MSC application significantly reduced this damage, even though the complicated cochlea and stem cell transplantation studies are hard to access. The acquired histopathological results imply that MSCs could help cochlear cells regenerate and heal a stria vascularis injury. Lastly, the cochlea and organ of Corti were looked at histopathologically, and MSC might be able to fix damage to the cochlea caused by colistin. Though ototoxic chemicals mainly target hair cells, the molecular pathways causing their death remain primarily unknown.

This study has certain limitations. The study looked at how colistin and MSCs work in rodents, but because rodents and humans are different in body structure and function, the results may not directly apply to humans. Additionally, the study focused solely on histological and histomorphometric analyses without assessments at the molecular and functional levels. Immunohistochemistry, gene expression analyses, and otoacoustic emissions could contribute to a more comprehensive understanding of the underlying mechanisms. Since the observation period was limited to the acute phase of ototoxicity, further studies encompassing longer timeframes are necessary to evaluate the long-term effects of MSC administration.

Future studies should focus on understanding how MSCs help protect the cochlea, especially regarding oxidative stress, inflammation, and apoptosis. It is also essential to evaluate how well MSCs work at different doses, times, and delivery methods to find the best treatment approach. It is also necessary to assess the efficacy of MSCs across varying dosages, timing, and routes of administration to determine the most effective therapeutic protocol. Additionally, advanced methods like transcriptomics, proteomics, and imaging tests could provide a deeper

understanding of how these processes work. To guarantee translational relevance, these results must ultimately be confirmed in sizable animal models and, eventually, in carefully planned human clinical trials. Such comprehensive investigations will pave the way for developing innovative, cell-based therapeutic strategies for preventing and treating drug-induced ototoxicity.

Author Contributions: Concept/Design: ÖÖG; Data acquisition: EB, EK, DB; Data analysis and interpretation: EB, DB, ÖÖG, Drafting manuscript: ÖÖG, EB; Critical revision of manuscript: EB, DB, AB, SSP, ÖÖG; Final approval and accountability: ÖÖG, SSP, AB, MB, EK, DB, EB; Technical or material support: -; Supervision: ÖÖG; Securing funding (if available): n/a.
Ethical Approval: Ethical Approval: All animal procedures and experimental protocols were approved by the Experimental Animals Ethics Committee of Erciyes, Turkey (Number 24/234- Approval date 05/12/2024).
Peer-review: Externally peer-reviewed.
Conflict of Interest: The Authors declared no conflict of interest.
Financial Disclosure: This study was supported by the Erciyes Scientific Research Projects (TSA-2021-11331).

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