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Original Article

Effect of erdosteine on radiation-induced cochlear damage in rats

Alparslan Serarslan[1], Nilgun Ozbek Okumus[1], Yakup Sancar Baris[2], Figen Basar[3], Bilge Gursel[1], Deniz Meydan[1], Yusuf Tufenkci[4]

Departments of [1]Radiation Oncology, [2]Pathology, [3]Ear-Nose-Throat Audiology, [4]Ear-Nose-Throat Surgery, Faculty of Medicine, Ondokuz Mayis University, Atakum/Samsun 55139, Turkey

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Abstract: Oxidative stress has an important role in the pathogenesis of radiation-induced cochlear damage. We examined the effects of the antioxidant erdosteine (ERD) on this damage. Healthy rats (n = 92) were divided into four groups: control (C-g), erdosteine alone (ERD-g), radiotherapy alone (RT-g), and erdosteine + radiotherapy ((ERD+RT)-g). Except for the C-g, all groups were further divided into the 1st day, 8th day, and 8th week subgroups for evaluating acute, subacute, and chronic radiation effects, respectively, on the cochlea. All rats underwent distortion product otoacoustic emission (DPOAE) testing before irradiation. The C-g received neither ERD nor radiation. The ERD-g and (ERD+RT)-g received 10 mg kg^{-1} day^{-1} ERD orally 2 days prior to irradiation, and ERD was continued for 5 consecutive days during irradiation. RT-g and (ERD+RT)-g received whole cranial radiation of 33 Gray (Gy) total in the form of 6.6 Gy/day on 5 consecutive days. After the last dose of radiation, rats were evaluated by DPOAE and then sacrificed at the relevant time point. DPOAE responses before and after irradiation were compared. Cochleas from the experimental groups were examined by light microscopy and were compared with those of the C-g. Both the DPOAE responses and the microscopic examination results were better in the (ERD+RT)-g than RT-g ($P < 0.05$). However, progressive decreases in DPOAE responses at all studied frequencies were detected despite the use of ERD in the (ERD+RT)-g. In conclusion, ERD reduced the degree of radiation-induced cochlear damage but did not prevent progression of the damage.

Keywords: Cochlea, DPOAE, erdosteine, hearing loss, radiation

Introduction

Hearing is one of our most important senses and occurs as a multistep progressive process. Ears detect the sound waves coming from the outside environment and transform them first into mechanical, then into electrophysiological signals, which are then transmitted to the central nervous system via the eighth cranial nerve [1]. The formation of electrophysiological signals is carried out by the organ of Corti in the cochlea via electro-mechanical sensitive hair cells and endolymph produced by the stria vascularis (SV). There are two types of hair cells: the inner hair cells (IHC) and outer hair cells (OHC). The cochlear spiral ganglion (SG) includes bipolar neurons providing the link between the hair cells and eighth cranial nerve [2, 3]. Damage that occurs in any of the cochlear structures and/or the eighth cranial nerve can cause permanent sensorineural hearing loss (SNHL) [4-6].

Radiotherapy (RT) is commonly used in patients with head, neck, and brain tumors, and the cochlea is often exposed to radiation in this region [7]. Radiation often causes cochlear damage via direct or indirect mechanisms. The indirect mechanism is mediated by reactive oxygen species and is responsible for two-thirds of the radiation damage leading to radiation-induced sensorineural hearing loss (RISNHL) [4]. RISNHL is observed in 20-40% of patients whose inner ear is included within the radiation field [5]. RISNHL is a dose-dependent, progressive, late-occurring permanent morbidity and affects the patient's quality of life adversely [6].

SNHL is determined using two objective tests; these are the distortion product otoacoustic

emission (DPOAE) and auditory brainstem response (ABR) tests. They are used to assess structures affected in the cochlea and eighth cranial nerve, respectively [5]. DPOAE is generated by the electromotor activity of the OHC of the organ of Corti [8].

Erdosteine (ERD) was first discovered by Gobetti et al. in 1986 [9]. Originally, it was developed as a mucolytic agent. ERD has two blocked sulfur atoms. One of these is located in a thiolactone ring, and the other is in the aliphatic side chain. ERD does not contain a free thiol (-SH) group. The molecule is stable in the acidic environment of the stomach or in a dry state. The thiolactone ring opens slowly in an alkaline environment and enters the bloodstream. Then, ERD is metabolized in the liver. As a result of metabolism, an active metabolite of ERD, called metabolite-1 (Met-1), containing a free thiol group is formed. The free thiol group provides antioxidant properties [10].

The antioxidant effects of ERD were demonstrated in various animal studies. Some of these showed a protective effect against bleomycin-induced lung fibrosis, vancomycin-induced pancreatic damage, and cisplatin-induced ototoxicty [11-15].

Because a major cause of RISNHL is radiation-induced free radical injury, anti-oxidants are potential agents for its prevention. To our knowledge, there are no previous reports on the effects of ERD on radiation-induced cochlear damage in humans or animal models.

Materials and methods

Ethics statement

This study was approved by the local ethics committee of the Faculty of Medicine of Ondokuz Mayis University, Samsun, Turkey (acceptance date and number: 21/06/2010; 2010/29). In this study, we followed the proper guiding principles for the care and use of laboratory animals in accordance with the recommendations of the Declaration of Helsinki.

Animals

Rats were supplied from the Ondokuz Mayis University Animal Laboratory. In total, 92 male Wistar albino rats (mean age = 8 weeks old, weight = 200-250 g) with normal otologic examinations and DPOAE tests were used. The rats were fed standard rat food and tap water *ad libitum* and were housed in individual cages in a quiet room with a background noise level below 50 decibel (dB), under a 12/12-h light/dark cycle (7 a.m. to 7 p.m.) and a constant environment (21°C, 75% humidity), starting from 1 week before the experiment.

Anesthesia

The DPOAE test, simulation, RT, and sacrificing were performed under general anesthesia with 50 mg kg^{-1} intraperitoneal ketamine hydrochloride (Ketasol 10%, Richter Pharma, Wels, Austria).

Groups

Rats were randomized into four groups: 1) ERD-alone group (ERD-g, n = 28), 2) RT-alone group (RT-g, n = 28), 3) ERD+RT group ((ERD+RT)-g, n = 28), and 4) control group (C-g, n = 8). Except for the C-g, all groups were further divided into the 1st day (n = 8), 8th day (n = 8) and the 8th week (n = 12) subgroups for evaluating acute, subacute, and chronic radiation effects, respectively, on the cochlea. The periods evaluated were chosen based on previous studies related to RT in experimental rat models [16, 17].

Experimental design

The ERD-g received 10 mg kg^{-1} day^{-1} ERD (Erdostin 300 mg, 20 capsules; Sandoz, Istanbul, Turkey) 2 days prior to sham irradiation, and this continued for 5 consecutive days (administration time = 7 days) during sham irradiation, same as the irradiation time. ERD was dissolved in distilled water and administered by oral gavage. The RT-g was exposed to a total of 33 Gy total cranial radiation in five fractions (5 × 6.6 Gy; irradiation time = 5 days) with a calculated (α/β = 3.5) biological effective dose of fractionated irradiation equal to 60 Gy conventional fractionation [17], and these rats received oral distilled water at a volume equal to the ERD dose. The (ERD+RT)-g received ERD and total cranial radiation in the same manners as the previous groups. The C-g received oral distilled water at a volume equal to the ERD dose and then received sham irradiation for the same amount of time as that of RT. After the completion of irradiation, the subgroups were evaluated by DPOAE tests and then sacrificed

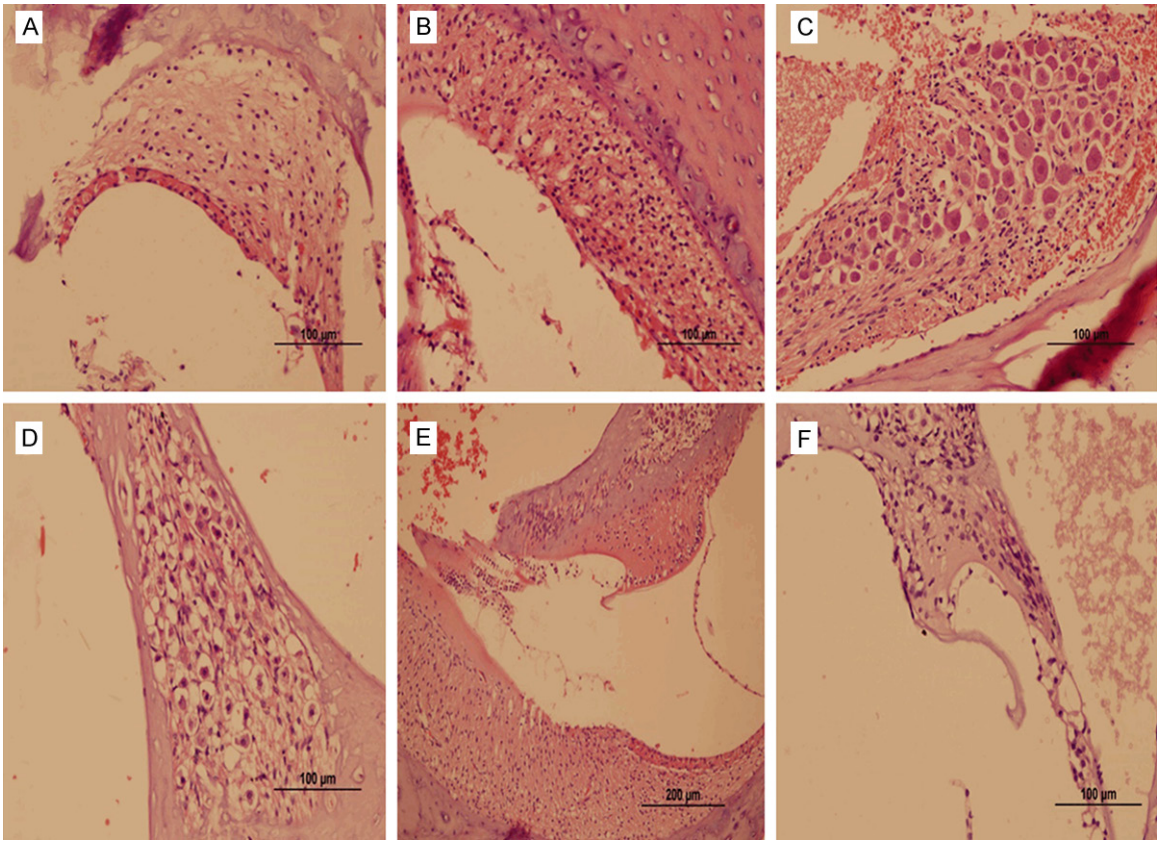

Figure 1. A-F. Hematoxylin and eosin-stained cross sections of the stria vascularis (A = score 0; B = score 2), spiral ganglion (C = score 0; D = score 2), and outer hair cells (E = score 0; F = score 2).

at the relevant time points under anesthesia. Their cochleas were enucleated for histopathological examination by light microscopy. The drug and radiation doses were chosen based on previous studies evaluating the antioxidant effects of ERD and radiation in experimental rat models, respectively [11, 12, 17].

Distortion product otoacoustic emission recordings

The DPOAE responses were recorded in a sound-proof room using the computer-based ILO V6 (ILO Echoport ILO292-II; Otodynamics Ltd; UK) equipment and a newborn probe. The room's ambient noise level was below 50 dB. DPOAE responses were measured prior to irradiation and at the 1st day, 8th day, and 8th week after the completion of irradiation. After anesthesia, all rats were placed in the prone position, and the probe was placed in the right and left external ear canals. The probe's placement control and calibration were performed automatically by the measurement system before the DPOAE test. During the DPOAE measurements, the impedance frequency amplitudes for frequencies f1 and f2 were L1 (82 dB SPL) and L2 (80 dB SPL), respectively. DPOAE results at 2, 3, 4, 6, and 8 kHz frequencies were recorded for 30 s. From DPOAE results, signal-to-noise ratio (SNR) values were calculated automatically by the system at all frequencies.

Simulation and irradiation

Three-dimensional (3-D) conformal RT planning was performed using a computed tomography (CT) simulator (Asteion Super 4; Toshiba Medical Systems, Otawara, Japan). The front and hind legs of rats lying in the prone position were fixed with adhesive tape on a Plexiglas tray, and whole-body CT images were obtained. CT slices were transferred to the planning system (Eclipse 8.6; Varian Medical Systems, Palo Alto, CA, USA) using a Digital Imaging and Communications in Medicine network. The brain and bilateral cochleas were delineated, and 3-D reconstructions were performed. The brain

Table 1. The scores of cochlear damage by microscopic evaluation

	C-g	ERD-g			RT-g			(ERD+RT)-g		
Period after last irradiation	8th week	1st day	8th day	8th week	1st day	8th day	8th week	1st day	8th day	8th week
Number of rats (*n*)	8	8	8	11	8	8	12	8	8	10
Scores of the stria vascularis										
Absent (0)	1	0	0	2	1	1	1	2	0	1
Mild (1)	7	7	8	9	7	7	2	6	7	7
Moderate (2)	0	1	0	0	0	0	9	0	1	2
Severe (3)	0	0	0	0	0	0	0	0	0	0
Scores of the spiral ganglion										
Absent (0)	2	6	2	6	3	0	0	6	3	3
Mild (1)	6	2	5	5	3	2	4	2	4	6
Moderate (2)	0	0	1	0	2	6	8	0	1	1
Severe (3)	0	0	0	0	0	0	0	0	0	0
Scores of outer hair cells										
Absent (0)	1	0	1	2	1	0	0	3	2	3
Mild (1)	7	7	7	9	4	1	2	5	6	6
Moderate (2)	0	1	0	0	3	5	9	0	0	1
Severe (3)	0	0	0	0	0	2	1	0	0	0
Scores of inner hair cells										
Absent (0)	4	2	3	5	0	0	0	3	2	3
Mild (1)	4	6	5	6	6	4	4	5	6	7
Moderate (2)	0	0	0	0	2	4	7	0	0	0
Severe (3)	0	0	0	0	0	0	1	0	0	0

Abbreviations: C-g = control group; ERD-g = erdosteine alone group; RT-g = radiotherapy alone group; (ERD+RT)-g = erdosteine and radiotherapy group.

and cochleas were defined as the clinical target volume (CTV). The planning target volume (PTV) was defined as the volume within a 1-cm margin radius around the CTV. After defining the PTV, a 0.5-cm margin in all directions was set for the PTV using a multi-leaf collimator. The plan was performed from a single posterior field using a 6 MV X-ray at a skin-source distance of 100 cm. Because of the build-up of high-energy photons, a 1-cm-thick bolus material (MT-CB-410S, Transparent Bolus; CIVCO Medical Solutions, Orange City, IA, USA) was placed on the skin. The reference isodose was defined as the isodose covering 95% of the target volume. The same standard planning was used for all rats receiving radiation. Radiation was applied at a 3-Gy/min dose rate using a linear accelerator teletherapy machine (Clinac DHX; Varian Medical Systems).

Morphological analysis

Tympanic bullae were fixed in 10% neutral buffered formaldehyde solution at 4°C for 24 h. For decalcification, specimens were stored in 10% ethylenediamine tetraacetic acid solution at 4°C for 20 days. All specimens were dehydrated, embedded in paraffin wax, and cut serially into 5-μm slices. Sections were stained with hematoxylin and eosin and examined by light microscopy (BX50; Olympus Optical Co., Tokyo, Japan) by a pathologist blinded to the groups. Changes in the organ of Corti (hydropic and vacuolar degeneration and loss of hair cells), in the SG (cytoplasmic and nuclear condensation, nucleolus and neuron loss), and in the SV (edema, vacuolization and cell loss) were noted. These changes were scored as absent (0), mild (1), moderate (2), or severe (3), according to Altas et al. [17].

Statistical analysis

Statistical analyses were performed using the SPSS software (ver. 16.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the normality of the distributions, and normality was rejected for all vari-

Table 2. Comparison of cochlear damage by microscopic evaluation between the control and experimental groups at the 1st day, 8th day, and 8th week

	p-values (compared with the control group)		
	Control-1st day	Control-8th day	Control-8th week
Stria vascularis			
ERD-g	0.721	0.721	0.571
RT-g	0.721	0.721	0.012
(ERD+RT)-g	0.694	1	1
Spiral ganglion			
ERD-g	0.105	0.959	0.238
RT-g	0.878	0.005	0.004
(ERD+RT)-g	0.105	0.867	0.717
Outer hair cell			
ERD-g	0.442	0.721	0.910
RT-g	0.279	0.001	0.001
(ERD+RT)-g	0.442	0.613	0.840
Inner hair cell			
ERD-g	0.442	1	0.678
RT-g	0.105	0.01	0.001
(ERD+RT)-g	0.721	0.536	0.272

Abbreviations: C-g = control group; ERD-g = erdosteine alone group; RT-g = radiotherapy alone group; (ERD+RT)-g = erdosteine and radiotherapy group.

ables. The DPOAE recordings were analyzed in terms of SNR, which reflected the hearing levels. The post-irradiation SNR values and all histopathological specimen data were analyzed within groups by Kruskal-Wallis variance analysis. When a difference was found, the Mann-Whitney U-test was used for pair-wise comparisons. The Wilcoxon signed rank test was used to compare the post-irradiation SNR values with baseline values. A value of $P < 0.05$ was considered to indicate statistical significance.

Results

Histopathological analysis

The cochleas of three rats could not be examined due to damage. The scoring of the cochlear structures and the scores are shown in **Figure 1A-F** and **Table 1**, respectively. The scores in the SV of the RT-g compared with the C-g showed statistically significant differences at the 8th week (P = 0.012). Also, the scores in the SG, OHC, and IHC of the RT-g, compared with the C-g, were statistically significant at the 8th day and 8th week (ranges of P = 0.005-0.01). However, the scores in the SV, SG, OHC, and IHC of both the ERD-g and (ERD+RT)-g compared with the C-g were not statistically significant at the 1st day, 8th day, or the 8th week (P > 0.05). That is, histological appearances were similar between the ERD-g and (ERD+RT)-g (**Table 2**).

Electrophysiological analysis

Post-irradiation DPOAE measurements could not be made due to external ear fibrosis and otitis media in 2 and 24 ears, respectively. There was no statistically significant difference in baseline SNR values among the groups (P > 0.05). The SNR values in the ERD-g at all frequencies compared with baseline values were not statistically significant at any period (P > 0.05). The effects of radiation at all frequencies compared with baseline values in both the RT-g and (ERD+RT)-g showed statistically significant differences at the 1st day, and this radiation effect continued until the 8th week (ranges of p = 0.008 to ≤ 0.001; **Table 3**). SNR values at all frequencies, except 2 kHz, in the ERD-g compared with the (ERD+RT)-g showed statistically significant differences at the 1st day, 8th day, and 8th week (ranges of P = 0.012 to ≤ 0.001). SNR values at 2, 3, and 6 kHz in the (ERD+RT)-g compared with the RT-g were statistically significantly different at the 8th day and 8th week (ranges of P = 0.02 to < 0.001). SNR values at 4 and 8 kHz in the (ERD+RT)-g compared with the RT-g were statistically significant at the 1st day, 8th day, and 8th week (ranges of P = 0.047 to ≤ 0.001; **Table 4** and **Figure 2A-E**).

Discussion

The otological effects of radiation on animals in the literature were first reported by Ewald in 1905. Ewald placed radium sources in the middle ear and reported labyrinthine symptoms [18]. However, radiation-induced cochlear damage was first reported by Marx in 1909 [19].

Light microscopy findings of radiation-induced cochlear damage in the literature include hydropic and vacuolar degeneration in both OHC and IHC; loss of OHC, IHC, and pillar cells; edema, neuronal loss, cytoplasmic and nuclear condensation in the SG; edema, epithelial

Table 3. Comparison of SNR values with baseline values

	SNR Mean ± standard deviation			
	Baseline [0]	1st day [0-1st day, *p*-value]	8th day [0-8th day, *p*-value]	8th week [0-8th week, *p*-value]
2 kHz				
ERD-g	17.13 ± 7.67	18.77 ± 8.81 [P = 0.2]	18.29 ± 5.27 [P = 0.19]	15.55 ± 6.52 [P = 0.095]
RT-g	15.37 ± 8.14	15.00 ± 4.94 [P = 0.000]	5.34 ± 5.54 [P = 0.001]	2.24 ± 4.29 [P = 0.005]
(ERD+RT)-g	15.07 ± 6.66	15.25 ± 3.25 [P = 0.002]	11.87 ± 4.91 [P = 0.001]	6.13 ± 4.06 [P = 0.001]
3 kHz				
ERD-g	30.02 ± 6.01	30.75 ± 5.74 [P = 0.79]	29.75 ± 5.87 [P = 0.19]	26.44 ± 4.99 [P = 0.4]
RT-g	25.98 ± 7.64	23.38 ± 2.89 [P = 0.000]	10.45 ± 8.45 [P = 0.000]	6.60 ± 6.48 [P = 0.001]
(ERD+RT)-g	25.47 ± 7.39	25.31 ± 4.78 [P = 0.000]	23.01 ± 5.38 [P = 0.001]	13.07 ± 3.57 [P = 0.000]
4 kHz				
ERD-g	33.08 ± 5.7	34.67 ± 6.61 [P = 0.1]	34.03 ± 5.11 [P = 0.71]	28.30 ± 5.22 [P = 0.25]
RT-g	32.44 ± 4.58	26.44 ± 3.30 [P = 0.000]	11.07 ± 8.26 [P = 0.000]	6.38 ± 5.19 [P = 0.001]
(ERD+RT)-g	32.21 ± 4.46	29.47 ± 3.78 [P = 0.008]	25.37 ± 4.30 [P = 0.001]	16.06 ± 4.06 [P = 0.000]
6 kHz				
ERD-g	37.53 ± 6.10	37.05 ± 7.58 [P = 0.179]	37.64 ± 6.39 [P = 0.79]	35.77 ± 4.30 [P = 0.093]
RT-g	36.31 ± 8.07	29.90 ± 2.82 [P = 0.000]	15.09 ± 9.53 [P = 0.000]	9.13 ± 6.39 [P = 0.001]
(ERD+RT)-g	36.43 ± 4.04	32.35 ± 4.20 [P = 0.008]	29.76 ± 4.75 [P = 0.001]	20.44 ± 4.59 [P = 0.000]
8 kHz				
ERD-g	42.88 ± 8.18	42.81 ± 11.99 [P = 0.67]	44.65 ± 5.07 [P = 0.48]	40.36 ± 4.90 [P = 0.15]
RT-g	42.13 ± 3.84	33.43 ± 3.50 [P = 0.000]	16.49 ± 10.28 [P = 0.000]	9.10 ± 5.76 [P = 0.001]
(ERD+RT)-g	42.59 ± 3.55	37.78 ± 4.01 [P = 0.000]	33.85 ± 2.60 [P = 0.001]	26.22 ± 3.86 [P = 0.000]

Abbreviations: C-g = control group; ERD-g = erdosteine alone group; RT-g = radiotherapy alone group; (ERD+RT)-g = erdosteine and radiotherapy group.

degeneration, vacuolization, loss of cells, and atrophy in the SV; and finally, deformation of the basilar membrane in the organ of Corti and fibrinoid material deposition in the scala tympani and scala media [5, 8, 17, 20]. As expected, our microscopic findings in the RT-g were consistent with these reports.

Gamble et al. exposed rats to a single dose of radiation of 5-60 Gy. They found evidence of radiation-induced cochlear damage evident at 30 Gy and above and found that it increased with the radiation dose [21]. In the study by Winther et al., OHC damage started at 40 Gy [8, 22, 23]. Also, Nagel and Schafer reported that radiation-induced OHC and IHC damage started at 40 and 50 Gy, respectively [8, 24]. In subsequent studies, cochlear structures were reportedly affected at doses of 40 Gy or higher [25, 26]. In our study, the equivalent of 60 Gy radiation was applied, because this biologically effective dose exceeds 40 Gy and is commonly used in patients with head, neck and brain tumors in daily practice.

Radiation causes structural changes, especially in the basal turn of the cochlea [20]. The basal turn of the cochlea, which is responsible for detection of high frequency (> 2 kHz) sounds, is more sensitive to radiation than are other cochlear regions [7, 20]. Thus, RT usually affects the higher frequencies of the hearing range and progresses toward lower frequencies, at which sounds become perceptible to the patients [4]. In our study, 2 kHz and higher frequencies were evaluated.

Only a few studies investigating the effects of antioxidant drugs on radiation-induced cochlear damage in rats have been reported. These antioxidants included radix salvia miltiorrhizae (RSM), N-acetylcysteine (NAC), L-carnitine (LC), piracetam (PIR), amifostine (AMI), and melatonin (MEL) [8, 17, 27-30]. In RSM and NAC studies, 1 × 60 Gy and 1 × 70 Gy radiation doses were applied, respectively. Rats were sacrificed 14 days after irradiation in both studies. The loss of hair cells was less in the (RSM+RT) and (NAC+RT) groups than the RT-alone group.

Table 4. *p*-values for between-group comparisons according to the period

	p Values		
	1st day	8th day	8th week
2 kHz			
ERD-g & RT-g	0.11	0.00002	0.00006
ERD-g & (ERD+RT)-g	0.17	0.003	0.0001
RT-g & (ERD+RT)-g	0.92	0.005	0.02
3 kHz			
ERD-g & RT-g	0.0002	0.00000009	0.0000001
ERD-g & (ERD+RT)-g	0.007	0.001	0.000000003
RT-g & (ERD+RT)-g	0.2	0.0001	0.005
4 kHz			
ERD-g & RT-g	0.00002	0.000000003	0.00000002
ERD-g & (ERD+RT)-g	0.0002	0.0001	0.00000006
RT-g & (ERD+RT)-g	0.047	0.00000005	0.00002
6 kHz			
ERD-g & RT-g	0.001	0.00000001	0.00000002
ERD-g & (ERD+RT)-g	0.012	0.001	0.000000003
RT-g & (ERD+RT)-g	0.08	0.0000005	0.000000001
8 kHz			
ERD-g & RT-g	0.000004	0.000000003	0.00000002
ERD-g & (ERD+RT)-g	0.00005	0.000002	0.000003
RT-g & (ERD+RT)-g	0.001	0.00000003	0.00000003

Abbreviations: C-g = control group; ERD-g = erdosteine alone group; RT-g = radiotherapy alone group; (ERD+RT)-g = erdosteine and radiotherapy group.

Additionally, in the NAC study, it was reported that malondialdehyde (a lipid peroxidation product) and superoxide dismutase (antioxidant enzyme) might be involved in the pathogenesis of cochlear damage [27, 28]. In both the LC and PIR studies, irradiation was applied at 5 × 6.6 Gy, as in our study. Cochleas were evaluated 4, 24, and 96 h after the completion of irradiation by light microscopy. Radiation-induced damage in the SV, SG, OHC, and IHC started 4 h after irradiation completion, and the extent of damage was less in the (LC+RT) and (PIR+RT) groups than the RT-alone group [17, 29]. In the AMI study, the applied radiation dose was 1 × 3.5 Gy, and the cochleas were evaluated 30 days after the completion of irradiation using electron microscopy. It was found that the extent of the outer hair cells injury was less in the (AMI+RT) group than the RT-alone group [30]. Karaer et al. investigated the effects of melatonin (MEL) on radiation-induced cochlear damage using the DPOAE test and light microscopy [8]. In this MEL study, irradiation was applied at 5 × 6.6 Gy, as in our study. Rats underwent the DPOAE test before and 10 days after the experiment. After the last DPOAE measurements, rats were sacrificed, and the cochleas were examined by light microscopy. It was found that radiation-induced cochlear damage was less in the (MEL+RT) group than in the RT-alone group. Also, DPOAE responses were significantly higher in the (MEL+RT) group than the RT-alone group at the end of the study [8].

However, these studies had some limitations, as follows: 1) applying high radiation doses in a single fraction in the RSM and NAC studies, 2) evaluation of cochleas using only microscopic examination, with no electrophysiological tests, with the exception of the MEL study, 3) differences in fraction size compared with human protocols in all studies, 4) insufficient radiation dose in the AMI study, and 5) short follow-up periods to detect the chronic effects of antioxidant drugs + RT on the cochlea. Also, a cochlea-protective effect of antioxidants against radiation would be expected to some degree. Moreover, no information could be obtained from these previous studies regarding the continuity of the antioxidant protective effects.

ERD has been shown to possess protective effects against oxidative stress-induced ototoxicity [13, 14]. However, there are only three reported studies examining the relationship between ERD and ototoxicity in the literature, and all examined the effects of ERD against cisplatin-induced ototoxicity. The first study reported the protective effect of orally administered ERD on cisplatin-induced ototoxicity in rats using DPOAE tests and biochemical markers [13]. The second reported the ineffectiveness of intratympanic administration of ERD against cisplatin-induced ototoxicity in rats using ABR and electron microscope [15]. The last study reported the protective effect of intraperitoneally administered ERD on cisplatin-induced ototoxicity in rats using ABR tests and electron microscopy [14].

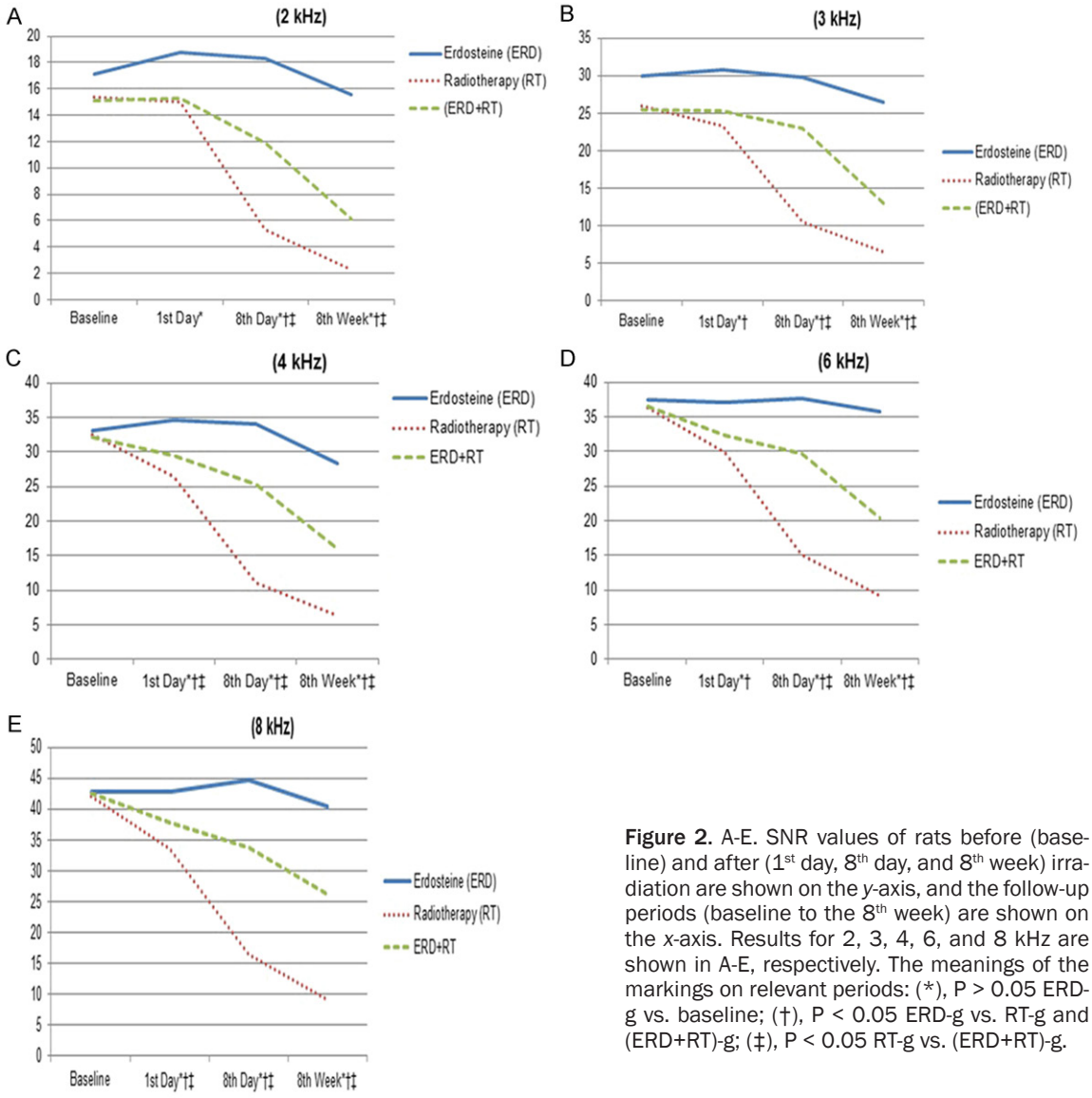

Figure 2. A-E. SNR values of rats before (baseline) and after (1st day, 8th day, and 8th week) irradiation are shown on the *y*-axis, and the follow-up periods (baseline to the 8th week) are shown on the *x*-axis. Results for 2, 3, 4, 6, and 8 kHz are shown in A-E, respectively. The meanings of the markings on relevant periods: (*), $P > 0.05$ ERD-g vs. baseline; (†), $P < 0.05$ ERD-g vs. RT-g and (ERD+RT)-g; (‡), $P < 0.05$ RT-g vs. (ERD+RT)-g.

Our animal experiments are the first to examine the effects of ERD on radiation-induced cochlear damage and also provide the longest follow-up, using DPOAE tests and microscopic examinations. In our study, we found that ERD did not cause microscopic or electrophysiological cochlear damage. Microscopic examination showed that the cochlear structures were affected negatively by radiation. In contrast to both LC [17] and PIR [29] studies, the latest affected cochlear structure in the RT-g was the SV. We found using microscopic evaluation that radiation-induced cochlear damage might be prevented by ERD ((C-g vs. (ERD+RT)-g; $P > 0.05$)). Electrophysiological analysis further revealed that SNR values were better in the (ERD+RT)-g than in the RT-g ((RT-g vs. (ERD+RT)-g; $P < 0.05$)). However, although better SNR values were found in the (ERD+RT)-g than RT-g using DPOAE tests, the SNR values of irradiated rats decreased progressively over time at all frequencies evaluated, despite the use of ERD. As in our and the previously mentioned MEL study, the protective effects of a drug can be detected using microscopic examination and post-irradiation single-time electrophysiological tests, but the lack of continuity of the protective effect can only be detected by the addition of multiple electrophysiological tests with a longer follow-up period, because radiation causes progressive deterioration of hearing function over time [5]. If researchers compare post-irradiation

single-time electrophysiological tests with pre-irradiation baseline tests, the results would likely be better in any (antioxidant + RT) group than the RT-alone group. As a result, the supposedly protective effects of antioxidant medications will continue to be reported.

Potential limitations of our study are the use of tumor-free rats, difference in fraction size compared with human protocols, and the lack of ABR tests.

In conclusion, ERD reduced the degree of radiation-induced cochlear damage but could not prevent damage progression. Thus, the ERD should not be used as the only method to decrease the adverse effects of radiation on the cochlear structures. The main method for decreasing the adverse effects of radiation on the cochlear structures is suggested to be irradiation applied at lower than ototoxic doses, rather than ERD. The antioxidant ERD may be used as an adjunct. In addition, we recommend a combination of at least 8 weeks of follow-up + microscopic examination + multiple post-irradiation electrophysiological tests to determine the long-term and preventative effects (or continuity of protection) of antioxidants on radiation-induced cochlear damages. Further studies regarding the preventative effects of antioxidants against radiation-induced cochlear damage are needed.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Alparslan Serarslan, Department of Radiation Oncology, Faculty of Medicine, Ondokuz Mayis University, Kurupelit, Atakum/Samsun 55139, Turkey. Tel: +90.362.312.19.19-2233 (Office); +90.533.488.49.49 (Mobile); Fax: +90.362.457.60.41; E-mail: alparslanserarslan@hotmail.com

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