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Potential protective effect of resveratrol on acoustic trauma: electron microscopy study

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Abstract. – OBJECTIVE: To investigate the potential preventive effect of resveratrol in rats exposed to acoustic trauma (AT).

MATERIALS AND METHODS: In this experimental study, Wistar albino rats were divided into three groups: Group 1 (Control, n = 6), Group 2 (AT, n = 6), and Group 3 (resveratrol + AT). The rats in Group 2 were exposed to AT. The rats in Group 3 received resveratrol (300 mg/kg/day) via gavage for 7 days. On day 7, the rats were exposed to AT 10 min following resveratrol treatment. Histological sections of the cochleae were examined using light microscopy, transmission (TEM), and scanning electron microscopy (SEM).

RESULTS: The cochlear hair cells, stereocilia, and Deiters' cells of the control group appeared normal in all microscopic evaluations. In Group 2, light microscopy revealed predominantly inner hair cell loss, although the outer hair cells were affected. TEM and SEM examination showed severe loss of stereocilia and SEM revealed stereocilia arranged in an asymmetric array. The cochlear structure in Group 3 appeared well preserved under the light microscope, and although TEM and SEM revealed stereocilia loss, the hair cells and stereocilia appeared near normal compared with those of Group 2.

CONCLUSIONS: Resveratrol may have a protective effect against AT damage in the cochlea, most likely through its antioxidant activity. Our results may be useful for studies in humans exposed to AT and noise-induced hearing loss related to chronic exposure to occupational noise.

Key Words:
Resveratrol, Acoustic trauma, Light microscopy, Transmission electron microscopy, Scanning electron microscopy.

Introduction

Resveratrol (3,4′,5-trihydroxy-*trans* stilbene), a non-flavonoid polyphenolic antioxidant, is an extensively investigated phytochemical with antioxidant, anticancer, and anti-inflammatory properties[1-3]. Resveratrol belongs to the stilbene class of aromatic phytochemicals and exists in *cis* and *trans* forms. The phytochemical is predominantly found in peanuts (*Arachis hypogaea*)[4] and grapes (*Vitis vinifera*)[5-7]. Crowell et al[8]) found no adverse effects in rats administered resveratrol (300 mg/day) for 4 weeks, suggesting that the phytochemical may be a chemopreventive agent with no adverse effects[9].

Given its antioxidant capacity, we investigated the preventive effect of resveratrol in rats exposed to acoustic trauma (AT). Histological sections of the cochlea were examined using light microscopy, transmission (TEM) and scanning electron microscopy (SEM).

Materials and Methods

Our study was conducted in the Eski ehir Osmangazi University Faculty of Medicine. The animals were acclimated and housed in the Experimental Animal Breeding and Experimental Studies Center of Eski ehir Osmangazi University. The experiments were conducted in this facility in compliance with the principles of the Declaration of Helsinki[10]. The study was approved by the Ethics Committee of Eski ehir Osmangazi University.

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Animals

The study was performed on 18 healthy Wistar albino rats (190-220 g) individually housed in an acclimatized room maintained at 20°C, and they were fed *ad lib* for the duration of the study.

The animals were randomly divided into three groups: Group 1 received no drug treatment or AT (Control, $n = 6$); Group 2 underwent AT but received no drugs (AT, $n = 6$); and Group 3 received resveratrol solved in eudistilated water (300 mg/kg/day) via gavage for 7 days. On day 7, the rats were exposed to AT 10 min following resveratrol administration (RS+AT, $n = 6$).

Anesthesia

Rats were anesthetized via intramuscular injection of 40 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Michigan, USA) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany). Eye-blink reflexes and respiratory rhythms were monitored during the experiments and deep anesthesia was maintained by repeated doses.

Acoustic Trauma

A MATLAB script that produced a single unit of variance sound was used to create a 1-12-kHz band of white noise to produce AT. The sound was then filtered from 1-12 kHz using a finite impulse response (FIR) type digital filter and an additional filter with a frequency of 200 Hz. The filtered noise was recorded as computer-based WAV files. The animals were exposed to continuous noise at a level of 110 dB for 6 h to cause AT, monitored using a decibel meter.

Electron Microscopy Analysis

The rats were sacrificed 30 min following exposure to AT using 80 mg/kg Pentothal (Abbott, Washington, DC, USA). The temporal bones were removed immediately and the otic bullas were excised (11). The bony capsule of the cochlea was carefully removed under a dissecting microscope, and the lateral wall was cut away to reveal the organ of Corti. We assessed hair cell damage in two different areas of the middle and basal turns.

Transmission Electron Microscopy Analysis

The samples for TEM were fixed in 2.5% glutaraldehyde and 0.1% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) then post-fixed in 2% osmium tetroxide (OsO_4), dehydrated in an ethanol series, and embedded in epoxy resin. After polymerization, samples were cut using a Leica EMU6 ultra microtome (Leica Microsystems, Wetzlar, Germany) and collected on a copper grid. Semi-thin sections of approximately 800-700 nm were stained with toluidine blue. Thin sections of approximately 70-80 nm were double stained with uranyl acetate and lead citrate[12] and images were acquired using a FEI (Bio Twin, Uitgeest, The Netherlands) transmission electron microscope.

Scanning Electron Microscopy Analysis

The cochlea and organ of Corti were dissected and fixed and post-fixed for SEM using the procedure described for TEM samples. Then the cochlea was dehydrated through a graded ethanol series ranging from 50%, 70%, and 90% to absolute ethanol prior to desiccation using the critical point drying method of Lovell et al[13]. Fully desiccated samples were mounted on a specimen stub using a carbon tab. Following critical point drying using CO_2, the specimens were sputter-coated with gold according to standard procedures and investigated using a Zeiss Ultra 50 SEM at operated at a 5-kV accelerating tension[13].

Results

The TEM and SEM images revealed no cochlear damage in the control group. In contrast, considerable cochlear damage was detected in the AT group; inner hair cell stereocilia loss was detected in some regions and the outer hair cell stereocilia were severely damaged with a considerable number lying flat or missing. The damage was most evident in the outer hair cells in the basal turns of the cochlea. Although cochlear damage was detected in the RS+AT group, it was less severe than that observed in the AT group. The RS+AT group cochleae showed inner and outer hair cell loss and minor damage to the outer hair cell stereocilia.

Light Microscopy Analysis

Examination under the light microscope revealed a normal-appearing cochlear structure in the control animals (Figure 1). We observed significant cochlear damage in the AT group with predominantly inner hair cell loss, although the outer hair cells were affected (Figure 2). In contrast, the cochlea structure of the RS+AT group was preserved and appeared near normal compared with that of the AT group (Figure 3).

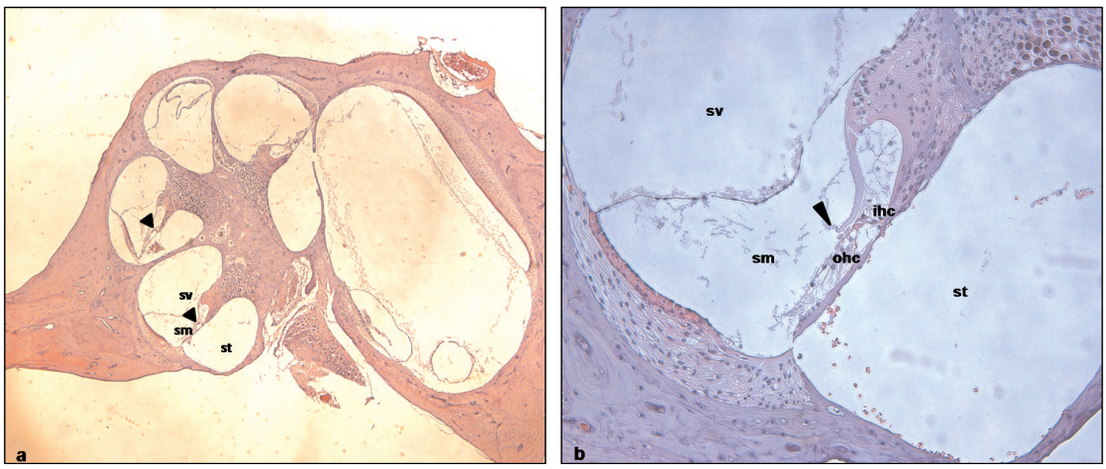

Figure 1. Control group. Light microscopic examination of the cochlea revealed normal-appearing cochlear structures. sv, scala vestibuli; sm, scala media; st, scala tympani; arrow head, organ of Corti; ohc, outer hair cell; ihc, inner hair cell; HE, hematoxylin-eosin; scale bar: 500 μm (a), 100 μm (b),

Transmission Electron Microscopy Analysis

The TEM analysis revealed normal outer hair cells and stereocilia structure in the control group (Figure 4a) and severe stereocilia loss in the AT group (Figure 4b). Although stereocilia loss was detected in the RS+AT group, the hair cells and stereocilia structure appeared to be near normal compared with those of the AT group (Figure 4c).

Scanning Electron Microscopy Analysis

SEM revealed normal inner and outer hair cells and vertically arranged stereocilia in the control group (Figures 5a and 6a). The AT group showed significant stereocilia loss, and the remaining stereocilia were arranged in an asymmetrical array (Figures 5b and 6b). Although we observed stereocilia loss in the RS+AT rats, the hair cells and stereocilia structures were near normal compared with those of the AT group (Figures 5c and 6c).

Discussion

Noise-induced hearing loss (NIHL) causes permanent cochlear damage as the result of a

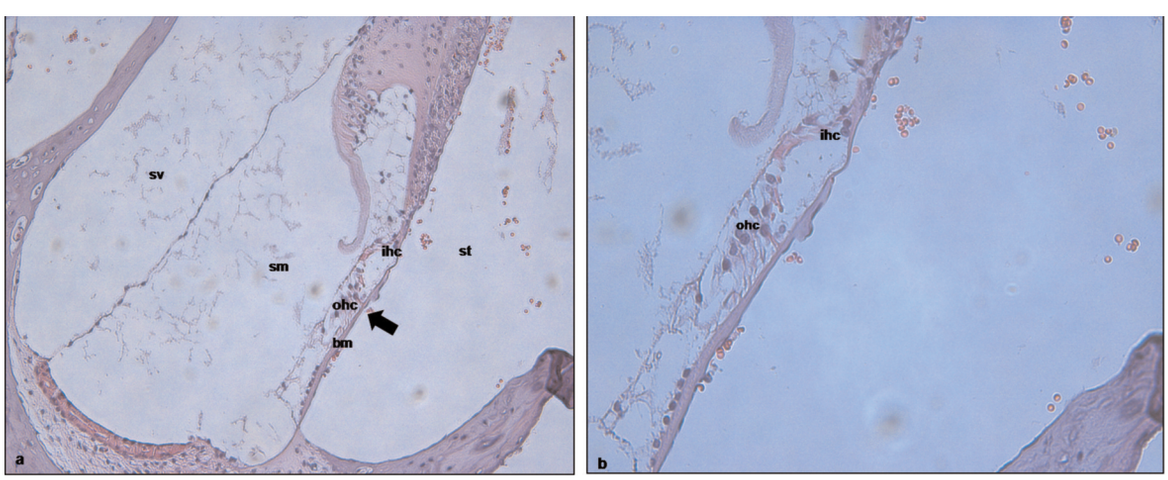

Figure 2. Acoustic trauma group. Light microscopic examination of the cochlea revealed cochlear damage. Hair cell loss was observed primarily in the inner hair cells, although the outer hair cells were affected. sv, scala vestibuli; sm, scala media; st, scala tympani; arrow head, organ of Corti; ohc, outer hair cell; ihc, inner hair cell; HE, hematoxylin-eosin; scale bar, 100 μm (a), 50.0 μm (b).

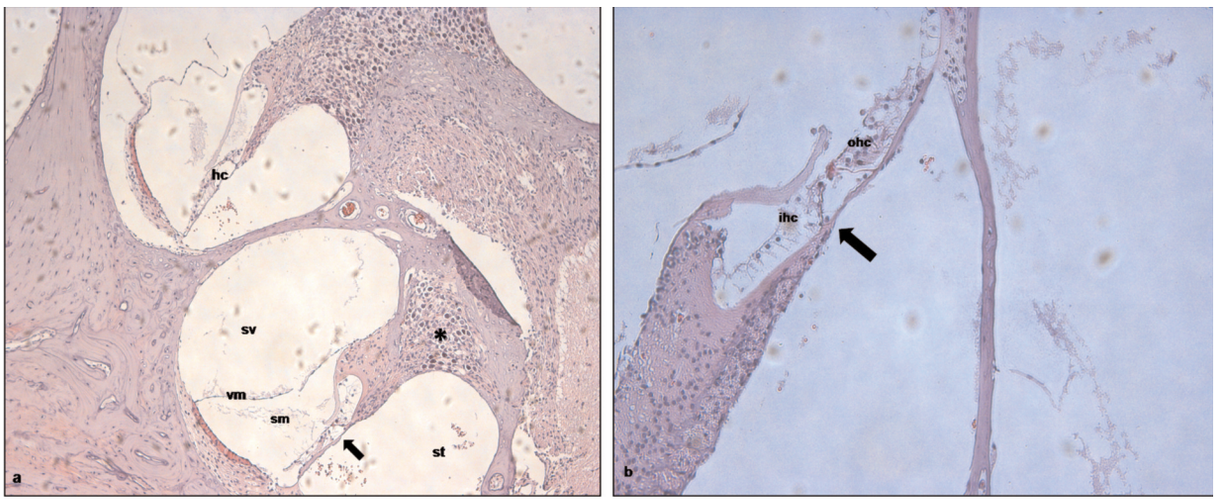

Figure 3. Resveratrol + acoustic trauma group, Light microscopic view of the cochlea. The cochlea structure appeared preserved and near-normal compared with the acoustic trauma group. sv, scala vestibuli; sm, scala media; st, scala tympani; arrow head, organ of Corti; ohc, outer hair cell; ihc, inner hair cell; *, spiral ganglion; HE, hematoxylin-eosin; scale bar, 200 μm (a), scale bar: 50.0 μm (b).

one-time exposure to excessive sound pressure or high-intensity sounds such as explosions, gunfire, a large drum hit loudly, and firecrackers. Gradually developing NIHL refers to permanent cochlear damage resulting from repeated exposure to loud sounds over a period of time rather than a single exposure. Noise-induced impairments are generally associated with a notch-shaped high-frequency sensorineural loss that is worst at 4000 Hz, although the loss is always greater at the frequencies 3000 or 6000 Hz[14,15].

Oxidative stress is mediated by reactive oxygen species (ROS), which cause cellular and molecular damage. ROS inhibitors/scavengers, such as antioxidants, interfere with the oxidative process and have shown promising protective effects in specific systems. Resveratrol, an active component in red wine, is among the antioxidants that

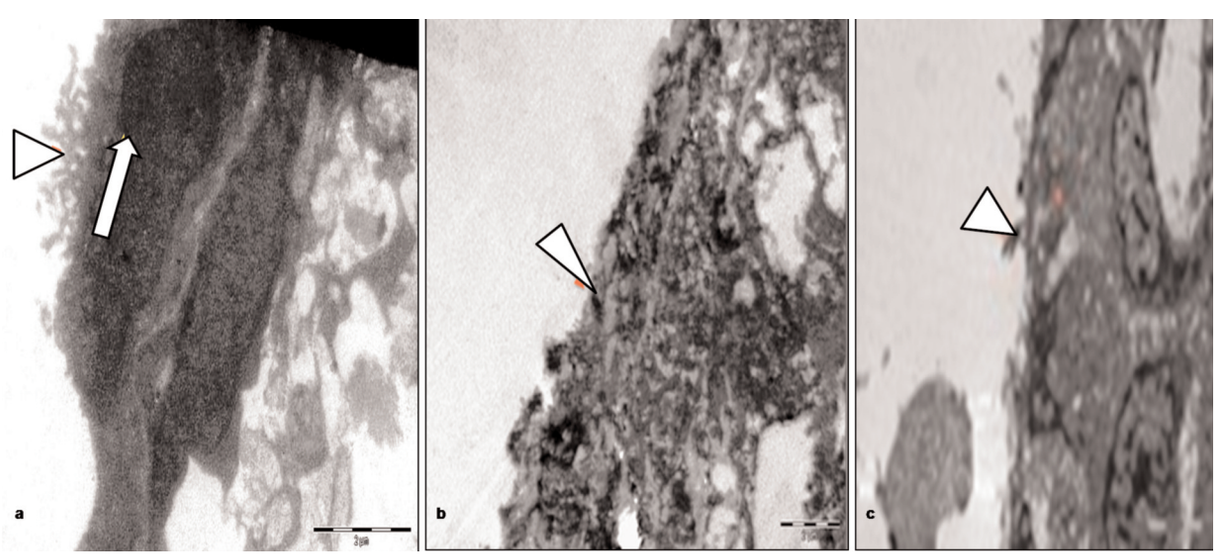

Figure 4. Transmission electron microscopy (TEM) image of the cochlea. ***A***, Control group: the outer hair cells (→) and stereocilia structure (▶) appeared normal. ***B***, Acoustic trauma group: significant stereocilia loss (▶) was detected. ***C,*** Resveratrol + acoustic trauma group: Although stereocilia loss was detected, the hair cells and stereocilia structure (▶) appeared near-normal compared with the acoustic trauma group (TEM-uranyl acetate + lead citrate).

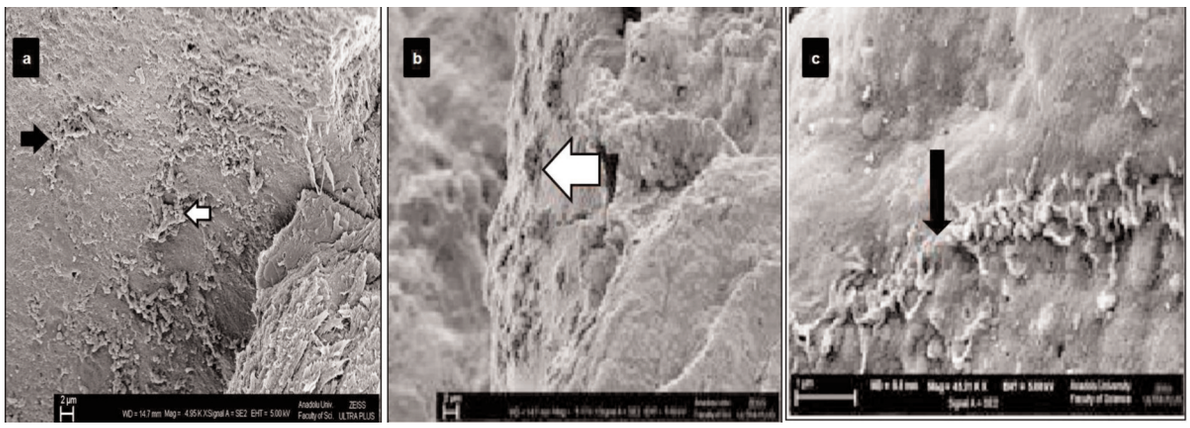

Figure 5. Scanning electron microscopy (SEM) micrograph of a surface view of the organ of Corti. ***A***, Control group: the outer (white arrow) and inner (black arrow) hair cells and stereocilia structure appeared normal. ***B,*** Acoustic trauma group: significant stereocilia loss (→) was detected. ***C,***) Resveratrol + acoustic trauma group: Although stereocilia loss (→) was observed, the hair cell stereocilia structures were near-normal in appearance compared with the acoustic trauma group.

have received recent attention[16]. It was reported that resveratrol protected human umbilical vein endothelial cells against apoptosis induced by hydrogen peroxide through enhancing the antioxidant defenses, and inhibiting the degression of the mitochondrial membrane potential[17].

An *in vitro* study of the antioxidant activity of resveratrol in human erythrocytes found that the antioxidant protected erythrocytes from hydrogen peroxide-induced lipid peroxidation, although to a lesser degree than that of the polyphenols quercetin and pterostilbene (a dimethoxylated resveratrol analog). However, a synergistic antioxidant effect was observed for resveratrol in combination either quercetin or pterostilbene[18,19].

We investigated the preventive effect of resveratrol in rats exposed to AT. To our knowledge, ours is the first study to use TEM and SEM to compare structural damage to the cochlea of rats exposed to AT and those pretreated with resveratrol prior to AT. TEM and SEM revealed no cochlear damage in the control group. In the AT group, we observed significant loss of the inner hair cell stereocilia in some regions and the outer hair cell stereocilia were severely damaged and

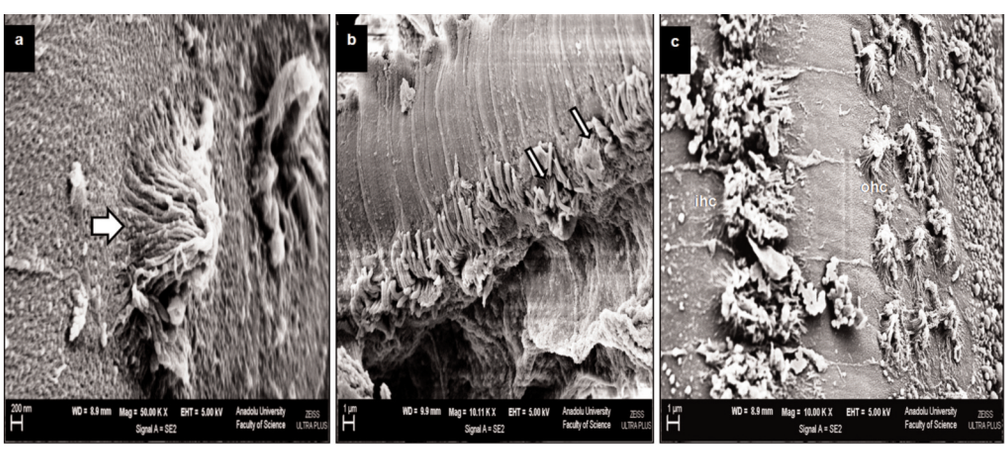

Figure 6. Scanning electron microscopy (SEM) view of the cochlea. ***A,*** Control group: the inner and outer hair cells were normal and the stereocilia were vertically arranged. ***B,*** Acoustic trauma group: significant loss of stereocilia was detected, and the remaining stereocilia (→) were arranged in an asymmetrical array. ***C,*** Resveratrol + acoustic trauma group: Although stereocilia loss was present, the hair cells and stereocilia structures were near-normal compared with the acoustic trauma group. Ohc, outer hair cells; ihc, inner hair cells.

lying flat or missing. The damage was most evident in the outer hair cells in the basal turns of the cochlea. Although a slight loss of inner and outer hair cells and damage to the outer hair cell stereocilia was detected in the RS+AT group, the cochlear damage was less than that observed in Group 2 (AT).

Examination of the cochlear structure using light microscopy, TEM, and SEM revealed that the hair cells, stereocilia, and Deiters' cells were normal in the control group. Examination of the AT group cochleae under the light microscope revealed loss of inner hair cells in particular, although outer hair cells were affected. TEM and SEM revealed severe stereocilia loss in the AT group cochleae, and SEM further revealed that the stereocilia were arranged in an asymmetric array. Examination of the RS+AT group tissue under the light microscope revealed that the cochlear structure was well preserved and appeared to be near normal compared with that of the AT group. TEM and SEM revealed stereocilia loss; however, the hair cells and stereocilia structure were well preserved compared with those of the AT group.

Bonabi et al[20] reported that the antioxidant efficacy of resveratrol was associated with nuclear factor-kappa B (NF-κB) activity. The authors showed that NF-κB is critical for the survival of immature mammalian hair cells and, thus, conducted a study to determine whether resveratrol exerted a protective effect against gentamicin-induced auditory hair cell damage and death. They cultured organ of Corti explants from newborn Sprague-Dawley rats in medium and allowed them to recover overnight. Then, two groups of explants were treated with different concentrations of resveratrol plus gentamicin for 24 h. The researchers included gentamicin-only and untreated explant groups for comparison and control purposes. They found that both concentrations of resveratrol had a moderate but statistically significant protective effect against gentamicin-induced toxicity *in vitro*.

Resveratrol has been used to treat animal models of AT. Seidman et al[16] investigated the ability of resveratrol to protect the auditory system from ROS-mediated noise damage. The experimental group (n = 5) underwent 7 weeks of resveratrol treatment (430/μg/kg/day), by gavage, and the control group (n = 5) was administered normal saline solution by gavage. Baseline auditory brainstem responses (3, 6, 9, 12, and 18 kHz) were determined for both groups. After 21 days, animals were exposed to noise (105 dB, 4500-9000 Hz for 24 h). Post-noise auditory brainstem responses were assessed at four recovery time points: immediate and 3 days, 7 days, and 4 weeks after noise exposure. Rats treated with resveratrol for 7 weeks were more resistant to AT, and the threshold shift was significantly lower at 6- and 9-kHz frequencies. The protective effect of resveratrol may be related to a decrease in cochlear hair cell damage following noise exposure. Seidman et al suggested that specific antioxidant therapy might play a significant role in the prevention of ischemic-, noise-, and age-related hearing loss. Their study demonstrated a protective effect of resveratrol on NIHL.

In a different study, Seidman et al[21] investigated the mechanisms underlying the potential protective effect of resveratrol by assessing its effect on cyclooxygenase-2 (COX-2) protein expression and ROS formation following noise exposure. AT exposure resulted in a progressive upregulation of COX-2 protein expression commencing at 8 h and peaking at 32 h. Similarly, ROS production increased after noise exposure. However, treatment with resveratrol reduced noise-induced COX-2 expression and ROS formation in the blood compared with the controls. The authors concluded that noise exposure induced a marked increase in COX-2 levels, and that resveratrol may mitigate the effect of NIHL via its ability to reduce COX-2 expression.

Conclusions

Our findings suggest that resveratrol may have a protective effect against AT damage in the cochlea, which is most likely mediated by its antioxidant activity. We administered resveratrol for 7 days previously to the AT. Our results may be useful for studies in humans exposed AT and NIHL related to chronic exposure to occupational noise. As resveratrol has no known side effects, we recommend investigating use of the antioxidant in individuals exposed to chronic workplace noise and soldiers exposed to potential AT from firearms. Detailed studies are needed to determine the optimum doses.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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