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Curcumin protects against acoustic trauma in the rat cochlea☆

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ABSTRACT

Objectives: In this study we evaluated the therapeutic utility of curcumin in a rodent model of acoustic trauma using histopathology, immunohistochemical, and distortion product otoacoustic emission (DPOAEs) measurements.
Methods: 28 Wistar albino rats were included in the study and randomly assigned to 4 treatment groups. The first group (group 1) served as the control and was exposed to acoustic trauma alone. Group 2 was the curcumin group. Group 3 was the curcumin plus acoustic trauma group. Group 4 was the saline plus acoustic trauma group. Otoacoustic emission measurements were collected at the end of the experiment and all animals were sacrificed. Cochlea were collected and prepared for TUNEL (TdT-mediated deoxyuridinetriphosphate nick end-labelling) staining assay.
Results: Group 3 maintained baseline DPOAEs values at 3000 Hz, 4000 Hz and 8000 Hz on the 3rd and 5th day of the experiment. DPOAEs results were correlated with the immunohistochemical and histopathological findings in all groups. In comparison to the histopathologic control group, Group 1 exhibited a statistically significant increase in apoptotic indices in the organ of Corti, inner hair cell, and outer hair cell areas ($p < 0.05$). Relative to the control group, rats in Group 3 showed little increase in inner hair cell and outer hair cell apoptotic indices.
Conclusions: Our results support the conclusion that curcumin may protect the cochlear tissues from acoustic trauma in rats. Curcumin injection prior to or after an acoustic trauma reduces cochlear hair cell damage and may protect against hearing loss.

1. Introduction

Presbycusis and excessive noise exposure are the most prevalent forms of adult hearing loss [1]. Noise pollution is pervasive in modern life and is a particular concern for members of the military and industrial workers. In some cases, individuals may be exposed to hazardous levels of noise in conjunction with ototoxic substances. Noise-induced hearing loss (NIHL) is among the most prevalent forms of workplace injury [2]. In recent years, civilians and soldiers are exposed to acoustic trauma resulting from exposure to terrorist explosions.

Several mechanisms have been proposed to explain NIHL, however the pathogenesis of this conditions remains poorly understood. Apoptosis in the Organ of Corti is among the key pathological findings in individuals with hearing loss [3].

Oxidative stress has been associated with acoustic trauma and may contribute to cochlear damage. Oxidative stress is generally characterized by the presence of lipid peroxidation, a process by which free radicals and reactive oxygen species (ROS) break down lipid molecules leading to cell death [4,5]. Various neurodegenerative syndromes are characterized by free oxygen radical damage. Inflammation of the cochlear may also contribute to noise-induced hearing loss. Previous studies have demonstrated up-regulation of proinflammatory cytokines, chemokines and cell adhesion molecules, and proliferation of inflammatory cells in the ear following excessive noise exposure [6].

Antioxidants may be useful in limiting or reversing the oxidative damage caused by noise-induced hearing loss. Several antioxidants and other agents have been evaluated in the treatment of noise-induced hearing loss, including: ascorbic acid [7], N-acetyl cysteine, salicylate [8], melatonin, caroverine, tacrolimus [9],

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corticosteroids [1,10], rosmarinic acid [4], low-level laser therapy [11], atorvastatin [12], adenosine amine congener (ADAC) [13], vitamin E (a-tocopherol) and vitamin A (retinol), and diverse polyphenols, including flavonoids and organosulfur compounds, a combination of vitamins A,C, E and magnesium, Coenzyme Q [5], thymoquinone [3], leupeptin [14], 2-aminoethyl diphenylborinate (2-APB) [15], activated protein C [16].

Curcumin is derived from golden spice turmeric (*Curcuma longa*) a perennial plant from the Zingiberaceae family that is cultivated in Southeast Asia and India. Tumeric is best known as the spice that lends curry its characteristic yellow color. Antimicrobial, antioxidant, anti-carcinogenic, and anti-inflammatory activities have been attributed to curcumin over the past decade. Curcumin has an established record of safety in humans, even when delivered at very high doses [17–19].

No previous study has shown the protective effect of curcumin against acoustic trauma by using both audiological and immunohistochemical means.

We hypothesized that curcumin, an antioxidant and anti-inflammatory agent, may be useful for the treatment of NIHL.

In the present study, we evaluated the effects of curcumin in a rodent model of NIHL using histopathological and immunohistochemical analysis and distortion product otoacoustic emissions (DPOAEs).

2. Methods

The Ethical Committee on Animal Research of Tokat Gaziosmanpasa University reviewed and approved all study procedures. The research was conducted at the experimental research laboratory of Gaziosmanpasa University.

2.1. Animals

The study consisted of 28 male Wistar albino rats weighing 240–320 g each. Animals were maintained at 21 ± 1°Cin the separate cages under a 12 h light/dark cycle. Food and water were provided *ad libitum*. The ambient noise level was 50 dB. An ear microscope was used to examine the tympanic membranes and external ear canals of all rats prior to the start of the study. Cerumen was removed from the ear canal. Exclusion criteria were as follows: signs of otitis media, tympanic membrane perforation, and opacification.

2.1.1. Experimental groups

The 28 rats were randomly assigned to 1 of 4 treatment groups as follows: Group 1 (n = 7) was exposed to acoustic trauma only. Group 2 (n = 7) underwent intra-peritoneal (i.p.) injection of curcumin solution at 200 mg/kg (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) once daily for 4 days. Group 3 (n = 7) underwent i. p. injection of at curcumin solution 200 mg/kg (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), 1 h prior to acoustic trauma and once per pay for each of the subsequent 3 days. Group 4 (n = 7) underwent i. p. injection of saline solution 1 h prior to noise exposure and once daily for each of the subsequent 3 days.

2.2. Preparation of curcumin solution

2.5 g of curcumin powder was dissolved in dimethyl sulfoxide (DMSO) and ethanol, incubated on the shaker at room temperature for 10 min and centrifuged at 5000 rpm for 10 min before removal of the DMSO fraction. The remaining pellet was then dispersed in 96% ethanol and the procedure was repeated. The pellet was then diluted in 25 ml sterile saline solution after removal of the ethanol fraction. The curcumin stock solution was prepared at a 2.5 g/25 ml. Fractions were prepared fresh for each experimental treatment to minimize the damage caused by extended storage.

2.3. Study design

An intraperitoneal anesthetic cocktail consisting of 10 mg/kg xylazine and 60 mg/kg ketamine was given to all experimental animals prior to ear examinations. Ear microscopic examination, pre-treatment and post-treatment distortion product otoacoustic emission (DPOAEs) measurements were collected after the animals were anesthetized. Curcumin and saline were injected intraperitoneally. DPOAEs measurements were obtained prior to the study and at 5 days after noise exposure. Euthanasia was performed under deep anesthesia. The temporal bones and cochleas were excised from all experimental animals. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed on the cochlear tissue to identify apoptotic cells.

2.4. DPOAEs measurements

DPOAEs was conducted in a noise-controlled environment under anesthesia to assess hearing in all animals. A Madsen Capella device (Otometrics, Denmark) with an infant-sized probe was used to perform the distortion otoacoustic emissions testing. Distortion product otoacoustic emission (DPOAEs) levels in response to 2 primary tones (L1, L2 = 65, 55 dB SPL and f2/f1 = 1.22). DPOAEs was measured at frequencies from 2 kHz to 8 kHz (2002, 3003, 4004, 6006, 8003 Hz) in all groups.

2.5. Noise exposure

Noise-induced trauma was generated by exposing group 1, 3, and 4 animals to white noise at a frequency of 110 dB for 8 h. Otoacoustic analyses were carried out prior to noise exposure, and on the 3rd, 5th days following acoustic trauma.

2.6. TUNEL assay

TUNEL (TdT-mediated deoxyuridinetriphosphate nick end-labelling) staining was used to evaluate the extent of apoptosis in the organ or Corti in all experimental animals. Staining was completed using a TUNEL staining assay kit (In Situ Cell Death Detection Kit, AP; Roche) according to the manufacuter's protocol. 5 μm thick sections were cut from 4% neutral buffered formalin fixed and paraffin embedded cochlear tissues using a microtome and were deparaffinized in xylenes and rehydrated through a graded ethanol series and double distilled water. The investigators were blinded to the animal grouping during analysis of the TUNEL-stained tissue sections. The apoptotic index of the organ of Corti was calculated by quantifying the number of TUNEL positive cells over the total cell number in each section using light microscopy and a 40× objective lens. 5 slides were analyzed in each rat and there were 3 sections on each slide and an average of 4 organs of Corti in each section.

2.7. Statistical analysis

Statistical Package for Social Sciences (SPSS) v 22 (IBM Corporation, Armonk, NY, United States) Software for Windows was used for all statistics analysis. The Mardie (Dornieden and Hansen Omnibus) test was used to fit data to the multivariate normal distribution. Levene's test was used to evaluate the homogeneity of variance. General Linear Model Repeated-ANOVA test (Wilk's lambda & Huynh-Feldt) was used to examine repeated quantitative