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Quercetine attenuates the gentamicin-induced ototoxicity in a rat model☆

Mustafa Sagit [a,*], Ferhat Korkmaz [b], Seren Gulsen Gürgen [c], Ramazan Gundogdu [a], Alper Akcadag [d], Ibrahim Ozcan [a]

[a] Kayseri Training and Research Hospital, Department of ENT, Kayseri, Turkey
[b] Sanliurfa Training and Research Hospital, Department of ENT, Şanlıurfa, Turkey
[c] Celal Bayar University, School of Vocational Health Service, Department of Histology and Embryology, Manisa, Turkey
[d] Kayseri Training and Research Hospital, Subdepartment of Audiology, Kayseri, Turkey

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ABSTRACT

Objectives: The aim of this study is to evaluate the protective role of quercetin in gentamicin-induced ototoxicity through an auditory brainstem response (ABR) test and a histopathological evaluation of the cochlea.

Methods: In this study, 48 female adult Sprague–Dawley rats aged 20–22 weeks and weighing 200–250 g were used. An ABR test was carried out on all rats prior to drug administration, after which, the rats were divided into four groups of 12 animals each. Drug administration was gentamicin 120 mg/kg plus ethanol in group one; gentamicin 120 mg/kg plus quercetin 15 mg/kg in group two; quercetin 15 mg/kg in group three; and ethanol in group four. The drugs were administered intraperitoneally once a day for two weeks, and the ABR test was repeated after drug administration. Subsequently, the rats were sacrificed and their cochleae were dissected and examined histopathologically.

Results: There was no significant difference between the pre-treatment ABR measurement values of the groups. However, a significant increase was detected in the ABR values in the group of rats that were administered gentamicin plus ethanol, while no statistically significant increase was found in the ABR values in the groups administered with gentamicin plus quercetin; quercetin alone; and ethanol alone. The number of TUNEL positive cells in the inner and outer hair cells in the Corti organ was found to be fewer, and Caspase 3 and 9 expressions were found to be weaker in the group receiving gentamicin plus quercetin than in the group receiving gentamicin plus ethanol.

Conclusions: Auditory function was detected to be significantly protected and apoptotic cells were found to be decreased when quercetin was administered together with gentamicin. From these results it was concluded that quercetin, a powerful antioxidant, attenuates ABR thresholds and histopathological lesions in the cochlea in gentamicin-induced ototoxicity in rats.

1. Introduction

Ototoxicity is a clinical condition related to hearing loss due to damaged inner ear structures secondary to drugs, chemical material and external stimuli such as noise and infection, and leads to such symptoms as balance disorder and tinnitus [1]. The aminoglycoside group of antibiotics is in wide use around the world, having been developed to tackle tuberculosis and advanced bacterial infections, intratympanic gentamicin therapy is also used for Meniere's disease, although their use is limited due to major side effects of ototoxicity and nephrotoxicity [2,3]. Ototoxicity secondary to gentamicin use is frequently bilateral, symmetrical and irreversible. Hearing loss starts initially in the high frequencies; however other frequencies are also affected with continued exposure. The frequency of ototoxicity due to gentamicin varies between 2% and 25% [4,5].

Many clinical and experimental studies have been made with the aim of protecting the inner ear from the potential toxic effects of gentamicin, in which agents such as iron chelators (deferoxamine and dihydroxybenzoate), glutathione, alpha-tocopherol,

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* Corresponding author. Tel.: +90 352 336 88 84; fax: +90 352 320 73 13.
E-mail address: musagit@yahoo.com (M. Sagit).

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alpha lipoic acid, D-methionine, dexamethasone, trimetazidine, geranylgeranyl acetone, *N*-acetylcysteine, estradiol (E2) and thymoquinone have demonstrated a protective role against the ototoxicity of gentamicin [6–17].

Flavonoids are compounds with useful biochemical and antioxidant activity that are widely and abundantly present in vegetarian food, being found mainly in vegetables, fruits, tea, onions and legumes, and give color to most flowers and fruits. One of the most important effects of flavonoids is the function of removing free radicals, while the best-defined characteristic of almost all flavonoid groups is their anti-oxidant capacity [18]. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is an main member of flavonoids, having a powerful antioxidant efficacy compared to others. Quercetin has various biological effects, being anti-inflammatory, anti-proliferative, anti-viral, anti-allergic, anti-thrombotic, anti-atherosclerotic and anti-tumoral, in addition to its anti-oxidant features [19–23]. Quercetin has been demonstrated to have hepatoprotective, neuroprotective and nephroprotective functions in many studies, although it is unknown whether quercetin has an otoprotective effect when used alone [24–26].

In the present study, it was aimed to evaluate the possible protective role of quercetin in gentamicin-induced ototoxicity using an auditory brainstem response (ABR) test and a histopathological evaluation of the cochlea.

2. Material and methods

This study was approved by the Ethics Committee on Animal Experiment and Research of Erciyes University (13/59). The study was carried out at the Laboratory for Experimental Animals of Erciyes University.

2.1. Animals

The study was performed on 48 (96 ears) female adult Sprague–Dawley rats, produced in the Experimental Clinical Research Center of Erciyes University, aged 20–22 weeks and weighing 200–250 g. The study animals were accommodated in safe cages with no limitations in food (pellet and water) at a constant temperature of 21 °C on 12-h day/12-h night cycle.

2.2. Study design and experiment groups

Animals were sedated using an intraperitoneal (i.p.) combination of ketamine hydrochloride 40 mg/kg (Ketalar, Eczacıbasi, Turkey) and xylazine 10 mg/kg (Rompun, Bayer, Germany). The external ear canals and tympanic membranes of all rats were examined microscopically at the initiation of the study, and any debris and/or earwax found present in the external ear canal were removed. At the beginning of the study totally three rats that were found to have serous otitis media (2 rats) and tympanic membrane perforation (1 rat) were excluded from the study. Following an otomicroscopic examination of all rats, the presence of normal hearing was analyzed by measuring ABR thresholds in bilateral ears. Included in the study were 96 ears of 48 rats that were found to have a normal hearing threshold in ABR measurements, and these 48 rats were subsequently divided randomly into four groups.

Group 1 ($n = 12$) received i.p. gentamicin 120 mg/kg (Genta 40 mg ampule, I.E Ulagay, Turkey) plus i.p. 1 ml 20% ethanol solution; group 2 ($n = 12$) received i.p. gentamicin 120 mg/kg plus quercetin 15 mg/kg (Sigma-Aldrich Chemical Co., St. Louis, MO, USA; dissolved in 1 ml 20% ethanol solution); group 3 ($n = 12$) received i.p. quercetin 15 mg/kg and group 4 ($n = 12$) received i.p. 1 ml 20% ethanol solution.

Drugs were administered once daily to all groups according to the above mentioned protocol for two weeks. At the 14th day, ABR measurements were repeated after drug administration under general anesthesia, and the rats were subsequently sacrificed. The temporal bullae of the rats were bilaterally dissected and stored in 10% formaldehyde for histopathological examination. The ABR values before and after drug administration were compared; thus cochlear toxicity was examined both electrophysiologically and histomorphologically. The individuals carrying out the ABR measurements and histopathological examinations were blind to the experiment groups.

2.3. ABR test

ABR measurement was carried out in a quiet room in both ears of the anesthetized rats using an interacoustics EP-25 equipment (Interacoustics, Denmark, 2001) and ABR 3A ear phones. ABR responses were recorded using subdermal needle electrodes (Technomed Europe, Holland). Active electrodes were placed on the vertex, the ground electrode on the contralateral mastoid and the reference electrode on the ipsilateral mastoid. Click stimulus was used as the auditory stimulant. For the click stimulus, a 100–3000 Hz band-pass filter at a repeat rate of 21 s^{-1} was set. Normal hearing was recorded when a normal ABR configuration was detected at 10 dBnHL. Hearing thresholds were defined starting from 70 dBnHL, decreasing in 20 dB increments each time. When a behavior was not achieved at the level of 70 dBnHL, the level of stimulus was set to 90 dBnHL. Behavior repeatability was tested by repeating the measurement at least twice and the threshold was elicited. The ABR threshold was defined as the lowest level of intensity at which a V wave was observed.

2.4. Histomorphological examination

Tissues extracted were stored in neutral formalin for 24 h and fixed, after which they were stored in an EDTA solution of 0.1 mol/l for three weeks for the decalcification of bone tissues. Tissues were washed under running water for 24 h following this procedure, and then dehydrated using an ethanol series classified according to the routine protocol. The tissues were then cleared in xylene and dried for placement in paraffin wax.

2.4.1. TUNEL method

Terminal deoxynucleotidyl nick-end labeling (TUNEL) stain is the method that shows the last stage of the nuclear fragmentation of apoptosis. The cytoplasm of cells underwent apoptosis are observed reduced, membrane vesicles and brown like as apoptotic bodies with TUNEL method.

Sections that were deparafinized and rehydrated as stated above were stained using a commercial kit (Apoptag, S7101, Chemicon, CA, USA) according to the manufacturer's instructions. The sections that were stained using the TUNEL technique were evaluated using a CX41 radiant field microscope (Olympus, Tokyo, Japan). TUNEL scoring was made by two independent investigators who were blind to the experiment information. The number of positive immune reactive cells was analyzed, starting from the apical region and ending at the basal region. The mean number of apoptotic cells was defined by counting the TUNEL positive cells in randomly selected areas for each case. In each case, the number of TUNEL positive or negative cells was calculated so that the total number would be 100 cells, and TUNEL positive cells were expressed in percentages. Cells in necrotic areas, in areas with weak morphology or at the borders of the sections were excluded. Figures were obtained from the basal turn of the cochlea.