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Protective role of intratympanic nigella sativa oil against gentamicin induced hearing loss

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ABSTRACT

Objective: Aminoglycosides, used to combat with life-threatening infections, have a substantial risk of hearing loss. Nigella sativa is an annual herbaceous plant and used for treatment of many diseases for ages. We aimed to investigate the protective role of intratympanic nigella sativa oil against gentamicin induced hearing loss in an animal model.

Methods and materials: Twenty eight guinea pigs were randomly divided into four groups: i-control, ii-Intratympanic nigella sativa oil (IT-NSO), iii- Intraperitoneal gentamicin (IP-G) and iv- Intraperitoneal gentamicin and intratympanic nigella sativa oil (IP-G + IT-NSO). Preoperative and postoperative hearing thresholds were determined with auditory brainstem response with click and 8 kHz tone-burst stimuli. Histological analysis of the cochlea specimens were performed under light microscope. Semiquantitative grading of the histological findings was carried out and compared between the groups.

Results: Highest posttreatment hearing thresholds were detected in IP-G group. Posttreatment mean hearing threshold of the IP-G group with click stimulus was significantly higher than the IP-G + IT-NSO group ($p = 0.004$). whereas the difference was not significant with 8 kHz tone-burst stimulus ($p = 0.137$). Both IP-G and IP-G + IT-NSO groups had significantly higher hearing thresholds compared to control and IT-NSO groups ($p > 0.05$). Histological examination of the control and IT-NSO groups demonstrated normal appearance of cochlear nerve, stria vascularis and organ of Corti. IP-G group showed the most severe histological alterations including hydropic and vacuolar degenerations, hair cell damage and deformation of the basilar mambrane. Histological evidence of damage was significantly reduced in IP-G + IT-NSO group compared to IP-G group.

Conclusion: Addition of intratympanic NSO to systemic gentamicin was demonstrated to have beneficial effects in hearing thresholds which was supported by histological findings.

1. Introduction

Aminoglycosides are relatively potent antibiotics used against aerobic Gram-negative bacteria including *Enterobacteriaceae* and *Pseudomonas* spp., tuberculosis, neonatal sepsis and some other life threatening infections [1–4]. Aminoglycosides display concentration dependent bactericidal activity rather than bacteriostatic potential by inhibiting protein synthesis at the ribosomes as well as by pore-forming effect on cell membranes [2,4–6]. Relative selectivity for bacterial ribosomes lowers the affinity of aminoglycosides for mammalian ribosomes [4]. Ototoxicity and nephrotoxicity are the main adverse effects that should be managed carefully, however neuromuscular block may also be prominent with aminoglycosides [2,6]. Ototoxicity appears only after days or weeks of beginning of treatment [6]. Risk for ototoxicity becomes more prominent with increasing dose, frequency of administration and duration of treatment [7]. Genetic predisposition, which was described as a mitochondrial DNA A1555G point mutation, is also a well documented risk factor for aminoglycoside ototoxicity [4].

The severity of hearing impairment may even increase following

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cessation of aminoglycoside administration [6]. However, broad spectrum of activity and low cost still make this class of antibiotics one of the most commonly used agents especially in developing countries [8–10].

The incidence of aminoglycoside induced hearing loss may be seen in up to 25% of patients [2,9]. Hair cells are the main targets of aminoglycoside ototoxicity, however spiral ganglion and stria vascularis may also be affected [1,4].

Gentamicin induced hearing loss appears secondary to apopotic mechanisms triggered by reactive oxygen species which lead to hair cell death [2,11,12]. Mitochondrial damage is also an important feature of hair cell death [13,14]. Oxygen free radicals are generated by iron-gentamicin complexes [8,10]. Gentamicin-induced hearing loss is generally symmetrical, bilateral and irreversible. Outer hair cells especially at the basal turn are more vulnerable to gentamicin toxicity [15,16]. Hence, higher frequencies are affected initially, however, hearing thresholds at lower frequencies also worsen at later stages [1,17].

Nigella sativa is an annual herbaceous plant and used for treatment of many diseases for ages [18]. It is also known as black seed or black cumin and has a bitter taste [19]. Thymoquinone is the predominant active compound of nigella and has considerable antioxidant effects. The effectiveness of nigella sativa against hypertension, hyperlipidemia, diabetes mellitus, asthma and infectious, neoplastic and immune-mediated diseases was previously demonstrated in many clinical and experimental studies [19–22].

This study was designed to evaluate the effectiveness of intratympanic nigella sativa against the ototoxicity of systemic gentamicin administration.

2. Methods

Twenty eight Dunkin-Hartley male adult albino guinea pigs (8 weeks, 600–800 g) were used in this study. Local Committee on Animal Research approved the surgical interventions (2013/94). The study was conducted in accordance with the guidelines of animal care and use for experimental procedures. Animals had free access to food and water and kept under standard laboratory conditions. Guinea pigs were randomly divided into 4 groups, with seven animals in each group:

i- Intratympanic (IT) saline (control),
ii- IT nigella sativa oil (IT-NSO) once a week for three weeks,
iii- IP gentamicin (IP-G) (100 mg/kg) daily for three weeks,
iv- IP gentamicin (100 mg/kg) daily for three week + IT NSO once a week for three weeks (IP-G + IT-NSO).

Animals were anesthesized by ketamine 40 mg/kg (Ketalar, Eczacibasi, Turkey) and xylazine 10 mg/kg (Rompun, Bayer, Germany), and all of the procedures, except for intraperitoneal injections, were performed under general anesthesia. All intratympanic injections were performed to the right ear.

Auditory brainstem evoked potentials were measured (GSI Audera ABR system, Grason-Stadler Inc., USA), in a sound-proof room. Auditory brainstem response (ABR) was recorded with sterile needle electrodes. The active electrode was placed at the vertex, the reference electrode at the contralateral mastoid and the ground electrode at the ipsilateral mastoid. Following cleansing of any debris from the external ear canal and inspecting the tympanic membrane, insert earphones were used for transmission of acoustic signals. Click and tone-burst at 8 kHz stimuli were used with 1024 sweeps, at a rate of 19.6/sn. The hearing thresholds were determined by the lowest threshold (expressed in dB SPL) exploiting a wave V. ABR measurements were performed both before and 1 week after the end of drug administrations. Pretreatment and posttreatment hearing thresholds were recorded.

Intraperitoneal gentamicin dosage was 100 mg/kg per day, for a total of three weeks. Nigella sativa oil (Origo, Gaziantep, Turkey) (4 ml/kg) was injected directly to the tympanic bulla, once a week for a total of three weeks, through the tympanic membrane following sterilization via bacterial filter passage. The volatile component of NSO contains 18.4–24% of thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) and 46% of monoterpenes [23,24]. The dose and frequency of NSO and gentamicin administrations were adjusted in accordance with previous studies on humans and animals [8,18,25,26].

At the end of the procedures, all animals were decapitated following intraperitoneal sodium thiopental (120 mg/kg). Tympanic bullae were quickly removed and fixed in 2.5% glutaraldehyde solution, buffered with 0.2 M NaH_2PO_4 + $NaHPO_4$ (pH = 7.2) at 4 °C for five hours. For decalcification, specimens were stored in 10% formic acid solution (renewed every other day) at 4 °C for twelve days and then the cochleae were dissected from the tympanic bullae and divided into two halves at the level of the modiolus. Specimens were fixed in 1% osmium tetraxoide (OsO4) at 4 °C for three hours, dehydrated in acetone series and embedded in araldite CY 212. Araldite-embedded cochlea specimens were cut into 1 μm thick sections, mounted on slides and stained with Toluidine blue. The sections were examined with a Nikon Optiphot-2 light microscope and analysed in Nikon DS-L3 Image Analysis System (Nikon Corporation, Tokyo, Japan).

Assessment of tissue alterations in Corti organ, stria vascularis and cochlear nerve fibers for each specimen was conducted by an experienced histologist who was blinded to the treatment groups. The average number (100 μm^2 at random in five different areas) and the diameter of myelinated axons (randomly, in 100 axons) were calculated along with investigation of the edema of the cochlear nerve. Epithelial damage and edema of the stria vascularis and degeneration of the organ of Corti were also assessed. Changes in organ of Corti (hydropic and vacuolar degeneration and loss of hair cells), stria vascularis (edema, vacuolization and loss of cells) and cochlear nerve were scored in a semiquantitative way as no change (0), mild (1), moderate (2) or severe (3). Hence, six histological variables were investigated: i-axon number, ii-axon diameter, iii-edema of the cochlear nerve, iv-epithelial damage in the stria vascularis, v-edema in the stria vascularis and vi-degeneration of the organ of Corti.

SPSS version 15.0 (IBM Corporation, USA) was used for statistical analysis. One Way Anova and Kruskal Wallis tests were used for comparison of numerical variables. For subgroup analyses Tukey and Mann Whitney U tests were chosen for parametric and nonparametric evaluations, respectively. Chi-square test was used for categorical variables. A p value less than 0.05 was considered statistically significant.

3. Results

All animals tolerated the interventions well and completed the study uneventfully. Recordings of hearing thresholds both before and after interventions and histologic evaluations were performed promptly without any technical problem. Tympanic bullae of the groups treated with intratympanic nigella sativa oil were noted to include some remnants of nigella sativa oil.

The mean hearing thresholds before and after drug administrations are given in Table 1 and Fig. 1. Pretreatment hearing thresholds were not significantly different between the groups ($p > 0.05$). Highest posttreatment hearing thresholds with both click and 8 kHz tone-burst stimuli were detected in IP-G group. Both IP-G and IP-G + IT-NSO groups had significantly higher hearing thresholds with click and 8 kHz tone-burst stimuli than the