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Protective role of misoprostol in prevention of gentamicin ototoxicity

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ABSTRACT

Objectives: To demonstrate potential protective effect of misoprostol on cochlear toxicity caused by gentamicin with electrophysiological tests and histopathological studies.
Materials and methods: The study included 80 ears of 40 rats with normal hearing threshold and DPOAE value in both ears. Animals were assigned into 4 groups. The rats were randomized into 4 groups. Group I (n = 10): Gentamicin, Group II (n = 10): Gentamicin plus misoprostol, Group III (n = 10): Saline; Group IV (n = 10): Misoprostol. All drugs used in the study were given once daily for 15 days. DPOAE and ABR measurements were repeated after drug administration. Subsequently, the rats' cochleae were examined histopathologically. Baseline DPOAE and ABR values were compared to those obtained after drug exposure and cochlear toxicity was evaluated in electrophysiological manner.
Results: When At baseline, there were no significant differences in DPOAE responses at frequencies of 1001, 1501, 2002, 3003, 4004, 6006 and 7996 Hz among groups. However In DPOAE test, statistically significant difference was observed between the pre-study basal values and post-study results in groups other than gentamicin + misoprostol group. Additionally, It was found that there was a significant difference in DPOAE response at frequency of 4004 Hz obtained at baseline and after drug exposure according to measurements of epithelial vacuolization in stria vascularis. While ABR threshold values were compared at baseline, there were no significant difference in ABR threshold values of left and right ear between groups. Histopathologically it was also found that there were significant differences measurements of epithelial vacuolization in stria vascularis and inflammation among groups ($p < 0.05$).
Conclusion: By these results, misoprostol, a potent antioxidant, has protective effect against cochlear damage, and that may be a safe alternative.

1. Introduction

Ototoxicity is a condition with onset of symptoms such as hearing loss, impaired balance and tinnitus due to damage of internal ear structures resulting from external stimuli including drugs, chemicals, noise or infection [1]. Aminoglycoside antibiotics were developed to use in the treatment tuberculosis and advanced bacterial infections. However, ototoxicity and nephrotoxicity are most important adverse effects limiting their use [2]. Ototoxicity caused by gentamicin is often bilateral, symmetrical and irreversible. Loss generally starts at higher frequencies; however, remaining frequencies are also affected by ongoing exposure. Incidence of gentamicin-induced ototoxicity varies from 2% to 25% [3,4].

Aminoglycoside agents enter into hair cells via mechanoelectrical transducer canals. Then, aminoglycoside-iron complex formed reacts with electron donor such as free oxygen radicals produced during arachidonic acid metabolism. c-Jun N-Terminal kinase (JNK) activated by free radicals released contributes to cellular apoptosis. Active protein-1 is downstream transcription factor of JNK. Gene translocations occur in nuclear factor kappa B (NFkB) in nucleus and sitochrome-c is released from mitochondria. Finally, apoptosis develops by cell membrane damage through caspases (caspase 8, 9 and 3) [5]. Primary target of aminoglycoside

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agents is hair cells which are sensorial epithelium at basal round of cochlea [6].

Several experimental and clinical studies showed protective effects against ototoxicity of many agents such as iron chelators, glutathione, alpha-tocopherol, alpha lipoic acid, D-methionine, dexamethasone, trimetazidine, geranylgeranylacetone or n-acetylcysteine.

Misoprostol (MP) has gained considerable interest as a scavenger of reactive oxygen species [7]. It also has other properties in addition to antioxidant such as anti-apoptotic or cytoprotective effects [8,9]. To best of our knowledge, there is no study investigating protective effect of MP, a prostaglandin E1 (PGE1) analogue, on gentamicin-induced ototoxicity. Therefore, this experimental study was aimed to investigate the effects of MP on gentamicin-induced histopathological changes and auditory parameters including auditory brainstem responses (ABR) and distortion product otoacoustic emission.

The aim of this study was to demonstrate potential protective effect of misoprostol on cochlear toxicity caused by gentamicin with electrophysiological tests and histopathological studies.

2. Material and method

This experimental study was approved by Ethics Committee on Animal Studies of Erciyes University, Medicine School (date: 12.03.2014; #14/039). It is conducted at Hakan Çetinsaya Experimental and Clinical Research Center of Erciyes University, Medicine School.

2.1. Animal preparation and experimental procedure

All rats underwent otoscopic examination (Zeiss S1, Germany). Emission and normal hearing were assessed by DPOAE and ABR measurements. The study included 80 ears of 40 rats with normal hearing threshold and DPOAE value. The rats (n = 40) were randomized into 4 groups as follows:

- **Group I (n = 10):** Gentamicin (80 mg/kg); (Genta ampul, İ.E. Uluguay) was given via intraperitoneal route,
- **Group II (n = 10):** Gentamicin (80 mg/kg) plus misoprostol (100 mcg/kg; Sigma Aldrich Chemical Co, St. Lousi, Mo, USA) was given via intraperitoneal route,
- **Group III (n = 10):** Saline (1 cc) was given via intraperitoneal route,
- **Group IV (n = 10):** Misoprostol (100 mcg/kg; Sigma Aldrich Chemical Co, St. Lousi, Mo, USA) was given via intraperitoneal route,

All drugs used in the study were given once daily for 15 days.

In all animals, sedation was achieved by using combination of ketamine hydrochloride (80 mg/kg, i.p., Ketalar, Eczacibaşı, Turkey) and xylazine (10 mg/kg, i.p.; Rompun, Bayer, Germany). After anesthesia, external auditory canal and tympanic membrane was evaluated in 40 rats by using an otoscope (Riester 2101, Germany) with an appropriate speculum in place. Cerumen was removed from external auditory canal. No rat with abnormal auditory canal or middle ear was detected. In all rats, DPOAE and ABR measurements were performed in both ears. Then, animals were assigned into 4 groups. After 7-days drug exposure, otoscopic examination was repeated under general anesthesia and 3 rats with pathology were excluded (1 rat in control group, 1 in gentamicin group and 1 in misoprostol group had otitis). Again, 3 rats died during study period (1 rat from control group, 1 from gentamicin plus misoprostol group, and 1 in gentamicin group died). Thus, DPOAE and ABR measurements were repeated in 34 rats (68 ears). Baseline DPOAE and ABR values were compared to those obtained after drug exposure and cochlear toxicity was evaluated in electrophysiological manner.

2.2. DPOAE test procedure

DPOAE measurements were performed by using Otodynamic ILO-288 Echoport device (Otodynamics Ltd., London, UK). Measurements were performed in a silent room. An appropriate plastic tube adaptor (1 cm in size) attached with plastic tympanometer probe was inserted to external auditory canal. Primary stimulus levels were equalized at 80 dB (L1/L2) were chosen for DP-gram measurements. Two distinct frequencies (f1 and f2) were set as f1/f 2 ratio of 1.22 to obtain maximum responses. DP gram measurements were performed at frequencies of 1001, 1501, 2002, 3003, 4004, 6006, 7996 Hz. DPOAE measurements were repeated 14 days after drug initiation and results were compared with baseline DPOAE measurements.

2.3. ABR test procedure

ABR test was performed in both ears under anesthesia. Measurements were performed by using Interacoustics (Interacoustics, Denmark). ABR responses were recorded by using silver subdermal needle electrodes (Technomed Europe, Netherlands). In the study, ipsilateral recording was performed via 3 electrodes by using one channel. Electrode positioning was as follows: active electrode (+) at vertex, grounding electrode over contralateral mastoid bone and reference electrode (−) over ipsilateral mastoid bone. Click stimulus was used as auditory stimuli. The band-pass filter 100–3000 Hz for click stimuli, and repetition rate of 21 s were set as filtering. The threshold was determined beginning from 70 dB by 20 dB decrements. Normal hearing is defined as detection of normal ABR configuration at 10 dBHL. Behavioral reproducibility was tested by two repetition at threshold level and proof is demonstrated. ABR threshold is defined as lowest level where wave V of ABR was observed. ABR measurements were repeated 14 days after drug initiation and results were compared with baseline ABR measurements.

2.4. Histomorphological examination

The cochleas were fixed in 10% formalin and embedded in paraffin in order to avoid cell destruction by autolysis or bacteria and to preserve tissue morphology and composition. To enable histopathological examination, the specimens were decalcified in a solution of formic acid and sodium citrate and the cochleas were bisected, creating a transverse section of the cochlea one paraffin-embedded block tissue was selected from each case and cut into 5 μm sections, stained with haematoxylin and eosin and examined under light microscopy (Nikon Eclipse Ni) and digital images were obtained by digital camera (Nikon DS2-Fi2). Tissue sections were deparaffinized with xylene and washed with ethanol.

Histologically, samples obtained from the mouse revealed normal microarchitecture of the organ of Corti. Histopathologically, the presence of stria vascularis edema, infiltration of leucoytes, neovascularization and fibroblast proliferation was scored subjectively as -, +, ++ or +++.

3. Results

3.1. ABR tests

When ABR threshold values were compared at baseline, there were no significant difference in ABR threshold values of left and