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Utilizing prestin as a predictive marker for the early detection of outer hair cell damage

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ABSTRACT

Purpose: To evaluate prestin as a biomarker for the identification of early ototoxicity.

Materials and methods: Rats (n = 47) were randomly assigned to five groups: low-dose (LAG) or high-dose (HAG) amikacin (200 and 600 mg/kg/day, respectively, for 10 days), low-dose (LCIS)or high-dose (HCIS) cisplatin (single doses of 5 and 15 mg/kg, respectively, for 3 days), and control (n = 8). At the end of the experiment, measurement of distortion product-evoked otoacoustic emissions (DPOAE) were performed to evaluate hearing, then blood samples and both ear tissues were collected under anesthesia. Prestin levels were determined by ELISA. Cochlear damage was evaluated histologically using a 4-point scoring system.

Results: The mean serum prestin levels were 377.0 ± 135.3, 411.3 ± 73.1, 512.6 ± 106.0, 455.0 ± 74.2 and 555.3 ± 47.9 pg/ml for control, LCIS, HCIS, LAG and HAG groups, respectively. There was significant difference between prestin levels of Control–LCIS-HCIS groups ($p = 0.031$) and prestin levels of Control-LAG-HAG groups ($p = 0.003$). There were also significant differences in prestin levels between the low- and high-dose cisplatin and amikacin groups ($p = 0.028$ and $p = 0.011$, respectively). Each group had significantly lower DPOAE results at 4, 6 and 8 kHz than control groups ($p < 0.001$). The LAG, HAG, LCIS and HCIS groups had significantly higher cochlear damage scores than the control group ($p < 0.05$).

Conclusions: Higher doses of cisplatin and amikacin were associated with the greatest increases in serum prestin level and cochlear damage score. The results of this study suggest that prestin is a promising early indicator of cochlear damage.

1. Introduction

Ototoxicity is an adverse effect related to the inner ear resulting from exposure to different chemical substances, especially local or systemic drugs used for therapeutic purposes [1]. This can cause balance disorders, hearing loss and dizziness [2]. Ototoxicity-related hearing loss occurs as a result of damage advancing bilaterally from the basal end of the cochlea to its apex. Therefore hearing loss appears at high frequencies firstly then at low frequencies [3]. Aminoglycosides and cisplatin are among the most well-known ototoxic drugs. Aminoglycosides are broad-spectrum antibiotics that are used for the treatment of a great number of infections such as sepsis, tuberculosis and pulmonary diseases [2,3]. Cisplatin is a chemotherapeutic drug commonly used for the treatment of head/neck, ovarian, testicular, bladder and gastrointestinal system cancers, as well as cancer metastasis [4]. However, side-effects such as nephrotoxicity restrict the use of these drugs [3]. The most frequently reported ototoxic effect of aminoglycosides and cisplatin is cochlear outer hair cell (OHC) damage [5]. Thus, OHC damage can be a symptom of hearing loss [6].

Biomarkers are indicators of normal or pathological processes in the body, and can be used to monitor the effects of medical treatment [7]. Recently, there has been an increase in the search for specific biomarkers that can be used to assess diseases of the inner ear [8, 9]. Prestin is a protein specific to the inner ear. It can be found in the basolateral membrane of OHCs where it plays an important role in voltage-dependent electromotility and cochlear sensitivity [10,11]. Experimentally induced prestin damage has been demonstrated to lead to reduced electromotility of OHCs and approximately 40–60 dB decrease in cochlear sensitivity [12]. Following damage to the OHCs, structural proteins inside the cell such as prestin are transmitted into the systemic circulation [13,14].

As prestin is an inner ear-specific protein, it could represent a

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biomarker in the management of situations that can cause hearing loss. Thus, we hypothesized that following damage to the inner ear as a result of ototoxic drugs, the prestin level would be increased. Thus, the purpose of this study was to monitor early damage after ototoxic drug use through prestin, a potential biochemical marker specific to the inner ear.

2. Materials and methods

2.1. Chemicals and animals

Amikacin (AG; Amikozit®; Zentiva Health Products Trade Inc., Istanbul, Turkey), cisplatin (CIS; Cisplatin®; Kocak Farma, Istanbul, Turkey), ketamine (Ketalar®;Pfizer Drug Inc., Istanbul, Turkey) and xylazine (Rompun®; Bayer Drug Inc., Istanbul, Turkey) were used in this study. Male Wistar rats (16–20 weeks old) were obtained from the experimental animal center of our university, and all experiments were performed according to the principles and guidelines of the university's Animal Ethical Committee following approval (HADYEK 64583101/2017/089). The rats were weighed and their ears were physically examined, then they were randomly assigned to one of five groups on the first day of the study:

1. Control group (n = 8). The rats in this group were administered 0.5 ml serum physiologic intraperitoneally for 10 days, and served as the healthy animal group.
2. Low-dose aminoglycoside (LAG) group (n = 10). The rats in this group were administered a low dose (200 mg/kg/day) of amikacin intraperitoneally for 10 days, then sacrificed.
3. High-dose aminoglycoside (HAG) group (n = 6). The rats in this group were administered a highdose (600 mg/kg/day) of amikacin intraperitoneally for 10 days, then sacrificed [15].
4. Low-dose cisplatin (LCIS) group (n = 9). The rats in this group were administered a single low dose (5 mg/kg) of cisplatin intraperitoneally and sacrificed on day 4.
5. High-dose cisplatin (HCIS) group (n = 10). The rats in this group were administered a single high dose (15 mg/kg) of cisplatin intraperitoneally and sacrificed on day 4 [16].

In the HAG group, 4/10 of the rats died on days 8 and 9, therefore, this group was left with six animals. No animals were lost in the other groups. At the end of the experiment, under anesthesia with ketamine and xylazine (50 and 5 mg/kg, respectively), blood samples were obtained by cardiac puncture. Both ear tissues were harvested and kept in a 10% formalin solution.

2.2. Auditory assessment

2.2.1. Distortion product-evoked otoacoustic emission test protocol

Distortion product-evoked otoacoustic emissions (DPOAEs) were measured for both ears of rats in all groups with normal otoscopic findings. DPOAE tests were performed on 11thday in aminoglycoside groups and 4th day in cisplatine groups. All DPOAE tests were performed in a quiet room following ketamine/xylazine anesthesia. An Otodynamics Echoport USB cochlear emission analyzer was used to measure DPOAEs, and the data was processed using Otodynamics ILO software (MAICO MI 34, Berlin, Germany). The sound stimulus consisted of two different pure frequencies (F1 and F2; F1/F2 ratio = 1.22) at 70 dB sound pressure level (SPL). The DPOAE was considered positive for signal-noise ratios of 6 dB SPL, as specified by the company. DPOAEs were measured at five different frequencies ranging from 1 to 8 kHz (2001, 3154, 4003, 6298 and 7998 Hz).

2.3. Enzyme-linked immunosorbent assay (ELISA)

The blood sample collected by cardiac puncture was transferred to a tube without anticoagulant and centrifuged at 1000*g* for 10 min. The supernatant was collected and stored at −80 °C for ELISA analysis. Prestin levels were determined via a commercial rat ELISA kit (catalogue number E-EL-H16253; Elabscience Biotechnology, Houston, TX, USA). The absorbance was read using a spectrophotometer DAR 800; Diagnostic Automation, Calabasas, CA, USA). The limit of detection for prestin was given as 9.38 pg/ml. The calculated overall intra-assay coefficient of variation was 6.36%, and the inter-assay coefficient of variation was 6.09%. The detection range was 15.63–1000 pg/ml. The sensitivity of the prestin ELISA was 9.38 pg/ml.

2.4. Histological procedure

Histological assessment was done by an experienced pathologist who was blinded to the groups. After removal of the temporal bones, they were placed in a 10% formaldehyde solution. Following fixation, they were decalcified in 10% formic acid, replaced daily, at room temperature for 7 days. The samples taken from the materials were placed in tapes to undergo a routine tissue tracking process in an automatic tissue tracking device for 14–16 h. After routine tracking, 5-μm serial slices were taken from samples embedded in paraffin blocks using amicrotome (Shandon HM 430 Sliding Microtome; Thermo Fisher Scientific Inc., MA, USA). The slides were stained with hematoxylin and eosin. The sections were analyzed with a light microscope under 400× magnification (Olympus BX51; Olympus Co., Tokyo, Japan) and photomicrographs were taken with a high-resolution video camera (Olympus DP 25, Olympus Co.).

The stria vascularis (SV), organ of Corti (CO) and spiral ganglion (SG) were evaluated as the histopathological assessment criteria of ototoxicity. The 4-point scoring system for cisplatin-induced ototoxicity defined by Freitas et al. was used [17].

Intermediate cell contraction, cytoplasmic vacuolization and the degree of marginal cell loss were the criteria for SV. The SV histopathology scoring system was as follows: 0, no contraction; 1, mild contraction; 2, moderate contraction; 3, critical contraction.

For the CO, the scoring was based on the number of OHCs with an intact nucleus. The CO histopathology scoring system was as follows: 0, three intact OHCs; 1, two intact OHCs; 2, one intact OHC; 3, no intact OHCs.

For the SG, cytoplasmic vacuolization and the degree of nuclear degeneration were analyzed. The SG histopathology scoring system was as follows: 0, no change; 1, mild change; 2; moderate change; 3, severe change.

2.5. Data presentation and statistical analysis

SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. The normality of the continuous variables was evaluated with the Kolmogorov-Smirnov test. The descriptive statistics were expressed as the mean ± standard deviation (SD). One-way ANOVA test was used to compare the variables between groups. Independent samples *t*-test was used for comparison between the groups. Correlation analysis between prestin and DPOAE test results was performed by using Spearman tests. For all analyses, a two-tailed p-value of < 0.05 was considered statistically significant.

3. Results

The mean serum prestin levels were 377.0 ± 135.3, 411.3 ± 73.1, 512.6 ± 106.0, 455.0 ± 74.2 and 555.3 ± 47.9 pg/ml in the control, LCIS, HCIS, LAG and HAG groups, respectively. Fig. 1 shows the serum prestin levels of the groups and the statistical significance between Control–LCIS-HCIS and Control-LAG-HAG groups according to one-way ANOVA test. There was significant difference between prestin levels of Control–LCIS-HCIS groups (p = 0.031) and prestin levels of Control-LAG-HAG groups (p = 0.003). Statistically significant differences were