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Evaluation of ototoxicity of intratympanic administration of Methotrexate in rats

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ABSTRACT

Objective: Methotrexate is a dihydrofolate reductase enzyme inhibitor with very high selectivity, and it is an antiproliferative folic acid antagonist used for the treatment of autoimmune diseases. In this study, our objective was to evaluate the effect of intratympanic Methotrexate application in the inner ear.
Methods: This study was planned as an animal study. This study performed in a tertiary referral center. 24 healthy female rats were used in our study. They were separated into three groups. 0.2 cc intratympanic saline was applied to both ears of Group 1. Paracentesis was applied to the tympanic membrane in both ears of Group 2. 0.2 cc intratympanic Methotrexate was applied to both ears of Group 3. At the beginning of the study, Distortion-product otoacoustic emissions (DPOAE) and Auditory brainstem response (ABR) of all rats were measured and then again on the 5th, 10th and 15th day. Histologic examinations of all groups were compared.
Results: There was not any significant difference between basal DPOAE and ABR measurement values of the groups and the results were measured again on the 5th, 10th and 15th day ($p > 0.05$). There was no difference between the groups in terms of histology.
Conclusion: The intratympanic Methotrexate injection does not have any ototoxic effect on inner ear. We assume that intratympanic Methotrexate could be used safely on inner ear diseases in which steroid treatment is contraindicated or not effective.

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1. Introduction

Immune-mediated cochleovestibular diseases [1], autoimmune sensorineural hearing loss [2], immune-mediated inner ear disease [3] [4], idiopathic rapidly progressing bilateral sensorineural hearing loss [5], sudden sensorineural hearing loss [5] and bilateral immune-mediated Meniere's Disease [6] are known as autoimmune vestibulocochlear diseases with the suspicion of having autoimmune pathology. Methotrexate (MTX) is an alternative treatment for those which steroid treatment is ineffective or patients for who can not receive steroid treatment due to their systemic diseases [1].

MTX is a folic acid (folate) analogue used for the treatment of many malignancies, rheumatoid arthritis, severe psoriasis and psoriatic arthritis. It prevents folinic acid development by inhibiting the intracellular dihydrofolate reductase enzyme that generates tetrahydrofolate from folate. As a result, DNA, RNA and protein synthesis are blocked [7], [8]. It also has an immunosuppressive effect by inhibiting the inflammatory response, as well as an antiproliferative effect [9].

Ototoxicity is a clinical status occurring as a result of cellular degeneration of the inner ear tissues and their functional lesions due to medications and therapeutic agents by means of cochlear and/or vestibular [10].

Systemic usage of MTX is an option, but it is not the first choice of clinicians and patients due to its toxic risks [1]. In this study, whether MTX has an ototoxic effect on the inner ear after intratympanic injection will be evaluated by means of Otoacoustic emission (OAE) and the auditory brain stem responses (ABR) measurement.

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2. Materials and methods

2.1. Animals

This is a prospective study, the approval dated 21.02.2012 and decision number 2012/35 was received from the Ethical Board for Animal Research at the local Clinical Research Ethics Committee. The study was performed using 24 adult male Wistar Albino rats of healthy weight 200–240 g. Rats having a pathology (cerumen, serous otitis, acute otitis, adhesive otitis etc.) discovered during endoscopic ear examination on rats with positive Preyer's reflex were excluded from the study. Rats were held in an environment light for 12 h and dark for 12 h, with 21C° ±1 temperature and a background noise level below 50 dB where they could eat and drink water freely. Animals were treated in accordance with the regulation for Care and Use of National Laboratory Animals.

2.2. Anesthesia

Before DPOAE and ABR records, anesthesia was administered with Ketamine hydrochloride (45 mg/kg) and Xylazine (5 mg/kg) intraperitoneally.

2.3. Experimental design

Twenty-four healthy female rats were used in our study. Rats were separated into three groups of eight rats each. Group 1 (Control), Group 2 (Sham) and Group 3 (MTX). 0.2 cc intratympanic saline was applied to both ears of Group 1 after basal measurements were taken at the beginning of study. Paracentesis was applied to tympanic membrane in both ears of Group 2. No other practice was performed. 0.2 cc MTX was applied to both ears of Group 3. DPOAE and ABR of all the rats were measured at the beginning of the study and on the 5th, 10th and 15th day.

2.4. The DPOAE measurements

GSI Audera device was used for DPOAE measurement on assessing rats' peripheral auditory system. Minimum size tympanometry rubber probes were used for rats. OAE's were measured in General Diagnostic mode both as Dpgram and Input-output (I/O). OAE's were measured using stimulations with different frequencies and intensities. Primary stimulation intensities were equalized at 65-55 dB (L1 = L2). They were regulated as two separate frequencies (f1 and f2) with f2/f1 = 1.2. DPgram measurement was taken in 3000, 4008, 5004, 6000, 6996, 8004, 9012, 10008, 11004 and 12000 Hz frequencies. DPOAEs with 3 dB or above noise level in the 2f1-f2 frequency were considered as positive. The person doing the hearing testing was blinded to these groups.

2.5. The ABR measurements

Viasys Medelec Synergy with subcutaneous needle electrodes (Technomed Europe) was used for ABR measurements in a quiet room. Sounds were given using as toneburst stimuli of 8 kHZ via ER 3A insert earphones on alternating polarity. Filter was calibrated as 30–1500 Hz; incidence as 21/sn; time window as 25 msn. 1024 samples were taken for signal averaging. Sound stimuli were initially given at the 80 dB nHL level, and then reduced by 20 dB until the intensity level approached the threshold. The threshold was located by choosing intensity steps of 10 dB when it came closer to the threshold. Behavior reproducibility was tested by generating at least two traces for each measurement, and threshold determination was cross-checked. ABR threshold was determined as the lowest intensity level in which ABR's third wave could be observed. The person doing the hearing testing was blinded to these groups.

2.6. Histologic examination

The rats were then injected with 3% glutaraldehyde via the intracardiac route and decapitated. A retroauricular incision was made in the posteroinferior region. After blunt dissection of the muscles, the tympanic bulla was exposed and opened. Multiple biopsies were taken from the cochlea under microscopic examination; these were fixed in 10% buffered formaldehyde and kept in this solution at 4 °C for 24 h. All specimens were then dehydrated, embedded in paraffin and serially sectioned into 4-μm slices. Sections were stained with hematoxylin and eosin, and were examined under light microscopy by the same pathologist.

3. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 13.0 software for Windows (SPSS Inc, Chicago, Illinois, USA). All quantitative variables were estimated using measures of central location (i.e. mean and median) and measures of dispersion (i.e. standard deviation (SD)). Data normality was checked using the Kolmogorov-Smirnov tests of normality.

Student *t*-test was used for between-groups comparison of DPOAE and ABR values. Repeated ANOVA test was used to evaluate groups within themselves based on DPOAE and ABR values. $p < 0.05$ was taken as significant.

4. Results

4.1. DPOAE

There was not any significant difference statistically between Group 1's initial measurement values and DPOAE values taken on the 5th, 10th and 15th day ($p > 0.05$) (Fig. 1). There was not any significant difference statistically between Group 2's initial measurement values and DPOAE values taken on the 5th, 10th and 15th day ($p > 0.05$) (Fig. 2). There was not any significant difference statistically between Group 3's initial measurement values and DPOAE values taken on the 5th, 10th and 15th day ($p > 0.05$) (Fig. 3). According to between-groups evaluations, there were not any significant difference statistically between both groups' initial measurement values and DPOAE values taken on the 5th, 10th and 15th day ($p > 0.05$) (Table 1).

4.2. ABR

ABR threshold values of all groups were compared in Table 1. There was not any significant difference statistically between Group 1's initial threshold values and values taken on the 5th, 10th and 15th day ($p = 0.263$) (Table 1) (Fig. 4). There is not any significant difference statistically between Group 2's initial threshold values and values taken on the 5th, 10th and 15th day ($p = 0.415$) (Table 1) (Fig. 4). There is not any significant difference statistically between Group 3's initial threshold values and values taken on the 5th, 10th and 15th day ($p = 0.329$) (Table 1) (Fig. 4). According to between-groups evaluations, there were not any significant difference between groups' initial ABR threshold values ($p = 0.426$) and values taken on the 5th ($p = 0.368$), 10th ($p = 0.659$) and 15th day ($p = 0.524$) (Table 1) (Fig. 4).

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