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Different concentrations of mesna application have an effect on the internal ear?

Remzi Dogan[a,*], Sabri Baki Eren[a], Ozge Gedik[b], Alper Yenıgun[a], Mehmet Serif Aydın[c], Busranur Eser[d], Orhan Ozturan[a]

[a] Bezmialem Vakif University, Department of Otorhinolaryngology, Fatih, Istanbul, Turkey
[b] Bezmialem Vakif University, Health Sciences Faculty, Audiology Department, Fatih, Istanbul, Turkey
[c] Medipol University, Regenerative and Restorative Medicine Research Center, Beykoz, Istanbul, Turkey
[d] Medipol University, Health Sciences Faculty, Audiology Department, Beykoz, Istanbul, Turkey

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ABSTRACT

Objective: The use of Mesna as a chemical dissector in higher concentrations may reduce the length of time of operation by providing more effective dissection as well as being used in otologic pathologies such as tympanosclerosis. In this study, it was aimed to assess the effect of Mesna on the internal ear, which was applied intratympanically in higher concentrations than the conventional use.

Methods: Twenty-four female rats were included in our study. The rats were randomly divided into three groups (Group 1: Mesna 50%, Group 2: Mesna 100%, Group 3: Saline). At the beginning of the study, DPOAE and ABR measurements were carried out on every rat on days 7 and 14. At the end of the study, cochleas of the rats were excised and histopathological assessments were carried out.

Results: Basal values and DPOAE and ABR values on day 7 and 14 of Group 1, Group 2, and Group 3 were similar to each other. No significant difference was detected among the three groups in the histopathological assessment carried out at the end of the study.

Conclusion: It was revealed by audiological and histopathological parameters that the use of Mesna at 50% and 100% concentrations did not create toxicity effects on the internal ear. Mesna would be more effective by being used in higher concentrations in audiological surgeries, that its duration of operation world reduce and could being used in different indications including tympanosclerosis.

1. Introduction

Sodium 2-mercaptoethanesulfonate (C2H5NaO3S2, Mesna) is a synthetic sulphur compound. Mesna is used in medicine due to its three effects (mucolytic, protective from mucosal damage and an antioxidant) [1]. The mucolytic effect of Mesna arises from being a thiol compound that can decompose disulfide bonds of mucosal polypeptide chains [2]. Mesna's protective effect from mucosal damage is used to prevent toxic effects formed by agents which are used in the treatment of a disease or disorder [1]. In particular, it is practically used in order to prevent toxic effects which the antimetabolites toxic to mucosal membranes such as ifosfamide or cyclophosphamide might cause in the bladder [1]. Thanks to having sulfhydryl groups, Mesna has been demonstrated in conducted studies that it reduces oxidative stress, prevents reactive oxygen radicals and ischemia-reperfusion damage [3,4].

Mesna has been used in otorhinolaryngology practice for the last 20 years and is preferred particularly in otologic surgery [2,5–10]. In ear surgeries, complete removal of chemical-dissector-purpose cholesteatoma tissue by decomposing sulfhydryl bonds is used to elevate retracted membrane in adhesive otitides and prevent cholesteatoma recurrences [2,7–9]. Moreover, it was empirically shown to prevent the formation of cholesteatoma tissue [10]. Mesna use in otologic surgery is prepared with 10% or 20% concentrations [2,5–10]. In order to demonstrate whether there is any toxic effect of Mesna application on the internal ear in 10% and 20% concentrations, experimental studies were performed [5,6]. In those studies, it was demonstrated that Mesna had no ototoxic effects [5,6]. However, we do not have any data regarding a Mesna application performed in higher concentrations. Use of Mesna in higher concentrations for the purpose of a chemical dissector may both reduce the duration of the operation and be used as a new method in the treatment of a problematic middle ear pathology i.e. tympanosclerosis.

For this purpose, in our study we aimed to assess the effect of Mesna applied intra-tympanically in higher concentrations (50% and 100%)

* Corresponding author.
E-mail address: dr.remzidogan@gmail.com (R. Dogan).

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on the internal ear with audiological (DPOAE, and ABR) and histopathological parameters.

2. Material and method

2.1. Study design

The study was initiated following the consent from the local ethics committee for testing animals. Twenty-four healthy female Sprague Dawley rats (200–240 gr) were included in the study. Endoscopic ear examination was applied to rats with positive Preyer reflex, the ones having middle and outer ear pathology in their examination were left out of the study. Rats were kept in an environment of 12 h under light and 12 h in dark, at 21C° ± 1 temperature, where they could reach free food and water and background noise level was below 50 dB. The animals were used in accordance with the National Care and Use of Laboratory Animals Regulation. Rats were divided into three groups with eight in each group.

Group 1 (50% Mesna Concentration): After basal measurements were carried out, 0.2 cc Mesna was intra-tympanically applied to the ears of rats in 50% concentration under microscope, no other additional operation was made, audiological tests were repeated on days 7 and 14 of the study.

Group 2 (100% Mesna Concentration): After basal measurements were carried out, 0.2 cc Mesna was intra-tympanically applied to the ears of the rats in 100% concentration under microscope, no other additional operation was made, audiological tests were repeated on days 7 and 14 of the study.

Group 3 (Control-Saline): After basal measurements were carried out, 0.2 cc Saline (0.09%) was intra-tympanically applied to the ears of rats under microscope, no other additional operation was made, audiological tests were repeated on days 7 and 14 of the study.

2.2. Assessment parameters

2.2.1. Audiological assessment

At the beginning of the study, before audiological assessment was completed for each rat on days 7 and 14, intraperitoneal anesthesia was provided with Ketamine hydrochloride (40 mg/kg) and Xylazine (5 mg/kg). DPOAE and ABR measurements were performed.

2.2.1.1. DPOAE. The smallest tympanometry probe was attached to the tip of the device, and measurements were carried out in a noise-insulated cabin. The measurement process was initiated after confirming that the ear probe was in a position appropriate for measurement and that the probe indicator and stimulation waveform were in the correct configuration. DPOAEs were measured using stimulations with different frequencies and intensities. Primary signal levels were adjusted to L1 = 65 dB and L2 = 55 dB for DPgram measurements. A f2/f1 ratio of 1:20 was used. DPgram measurements were carried out at frequencies of 358, 498, 701, 997, 1401, 1977, 2834, 4002, 5636, 7988,11288, 15991 and 22608 Hz. The detection threshold was defined as the primary signal level at which the DPOAE was distinguishable, at 3 dB above the noise floor. For all measurements, the responses up to the highest level of stimulation were recorded, and the test was concluded.

2.2.1.2. ABR. An Intelligent Hearing System (IHS) instrument was used for ABR measurements. Measurements were performed on both ears of the anaesthetized rats in a noise-insulated cabin. ABR responses were recorded using needle electrodes placed under the skin. The stimuli were delivered through insert earphones. ABR click, 8 Hz, 16 Hz, 20 Hz, 32 Hz stimuli with alternating polarities were used. The filter was adjusted to 30–1500 Hz, the repetition rate was 21.1/s and the time window was 25 ms. A total of 1024 stimuli were administered, and the signal was averaged. The threshold was defined as the lowest intensity level that could be observed and repeated. An 80 dB nHL stimulus was applied initially; the intensity was then reduced in 20 dB steps until the threshold value was reached. The magnitude of the intensity steps was reduced to 10 dB as the threshold was approached, and the threshold value was determined. At least two traces were created for each measurement, and crosschecking was performed by attempting to reproduce the threshold. The ABR threshold was defined as the lowest intensity at which wave II of ABR was observed. Baseline ABR measurements carried out prior to noise exposure were compared with those on days basal, 7, and 14. A single individual performed all of the audiological measurements.

2.3. Histopathological assessment

After the audiological stage of the study was finished, the cochleas of the rats were excised and histopathological assessments were made. Cochlea tissues received from test groups were fixed in 10% neutral buffered formalin (NBF) for 72 h. Tissues were decalcified in EDTA (Ethylene diamine tetra acidic acid) solution (in 100 ml 0.1 M phosphate buffer) under control by being replaced every 2 days at +4 °C for a 1 month period. After that, they were dehydrated by being exposed to an increasing alcohol series (70%, 90%, 96%, and 100%) and made pellucid by xylene. Following this procedure, they were kept in 60 °C paraffin for 1 night, embedded inside the paraffin and blocked. Sections with 5 μm thickness were taken from paraffin blocks onto the slide. For microscopic examination, they were painted by Hematoxylin & Eosin combined painting method. Painted sections were examined by light microscope (Axio Zoom V16, Zeiss). Hairy cells, spiral ganglion cells and nerve fibers of all cochleas were examined in terms of hydropic degeneration and nuclear loss. Degree of the changes were separated as no change (−), mild (+), mid-level (++) and severe (+++). Changes in the cochleas were compared to the control group and assessed statistically.

3. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 16.0 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.

Kruskal Wallis Variance analysis was used in comparison of histopathological parameters and DPOAE and ABR values between the groups. Between groups, difference was determined by the Mann Whitney *U* test, $p < 0.05$ was considered significant.

The Friedman test was used in the internal assessment of the groups for DPOAE and ABR values. For the assessment of internal significance of the groups, the Wilcoxon test was used and $p < 0.05$ was considered significant.

4. Results

4.1. Audiological assessment

Audiological assessment: There was no significant difference between basal, day 7 and day 14 DPOAE amplitudes and ABR thresholds of Group 1 (Mesna-50%) ($p > 0.05$) (Figs. 1 and 2). There was no significant difference between basal, day 7 and day 14 DPOAE amplitudes and ABR thresholds of Group 2 (Mesna-100%) ($p > 0.05$) (Figs. 1 and 2). There was no significant difference between basal, day 7 and day 14 DPOAE amplitudes and ABR thresholds of Group 3 (Control-Saline) ($p > 0.05$) (Figs. 1 and 2). There was no significant difference between basal DPOAE amplitudes and ABR thresholds of each group ($p > 0.05$). There was no significant difference between day 7 DPOAE amplitudes and ABR thresholds of each group ($p > 0.05$). There was