Electrophysiological and Histopathological Evaluation of Effects of Sodium-2 Mercaptoethanesulfonate Used for Middle Ear Surgery on Facial Nerve Functions

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OBJECTIVE: Sodium-2-mercaptopethanesulfonate (MESNA) is widely used in medicine because of its antioxidant and mucolytic effects. In recent years, it has been used in otologic surgery. Because it cleaves disulfide bonds, it is used to easily dissect the epithelial matrix in cholesteatoma and atelectasis. In this study, we hypothesized that MESNA does not have any toxic effect on the facial nerve, and the effects of MESNA on the facial nerve were examined histologically and electrophysiologically.

MATERIALS and METHODS: Twenty Wistar albino rats were used. Groups A and B were designated as the control and sham groups, respectively. The animals in groups C and D were administered 20% and 50% of MESNA solution, respectively, after the facial nerve was exposed in the parotid region. Electromyography (EMG) measurements were performed preoperatively and postoperatively at 4 weeks. The animals were subsequently euthanized; facial nerve samples were taken for histopathological examination.

RESULTS: When EMG parameters were compared within and between each group, preoperative and postoperative results were not statistically significantly different. Histopathological examination showed that MESNA did not cause any inflammation, granulation tissue, or foreign body reaction.

CONCLUSION: To the best of our knowledge, the effects of MESNA on facial nerve functions have not been investigated. In this study, the effects of MESNA after direct application to the facial nerve were examined electrophysiologically and histologically, and it was determined that MESNA did not cause any toxic effects. It was concluded that MESNA can, therefore, be safely used during middle ear surgery.

KEYWORDS: MESNA, rat, electromyography, histopathology

INTRODUCTION
Cholesteatoma is a common pathology in chronic otitis media (COM). Residual cholesteatoma and late-term disease recurrence are major problems related to COM surgery. Different applications may be used to reduce the incidence of residual cholesteatoma. Oto-endoscopy systems have been introduced for providing better visibility and eradicating “blind spots”. The use of a potassium titanyl phosphate laser provides more effective matrix dissection. In addition, the application of galectin-7 using an immunofluorescence technique enabled visualization of the microscopic epidermal remains.

Although new techniques and surgical procedures have been introduced, the incidence of residual cholesteatoma was reported to vary from 5% to 50%. The cholesteatoma matrix is mainly covered with keratin, and it contains numerous disulfide bonds. Chemical separation of these bonds allows dissection to be performed more easily and effectively during surgery. Sodium-2-mercaptopethanesulfonate (MESNA) is widely used in medicine because of its antioxidant and mucolytic effects. In recent years, it has been used in otologic surgery. Because it cleaves disulfide bonds, it is used to easily dissect the epithelial matrix in cholesteatoma and atelectasis. In this study, we hypothesized that MESNA does not have any toxic effect on the facial nerve, and the effects of MESNA on the facial nerve were examined histologically and electrophysiologically.

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Cholesteatoma is a common pathology in chronic otitis media (COM). Residual cholesteatoma and late-term disease recurrence are major problems related to COM surgery [1-3]. Different applications may be used to reduce the incidence of residual cholesteatoma. Oto-endoscopy systems have been introduced for providing better visibility and eradicating “blind spots” [4]. The use of a potassium titanyl phosphate laser provides more effective matrix dissection [5]. In addition, the application of galectin-7 using an immunofluorescence technique enabled visualization of the microscopic epidermal remains [6].

Although new techniques and surgical procedures have been introduced, the incidence of residual cholesteatoma was reported to vary from 5% to 50% [7]. The cholesteatoma matrix is mainly covered with keratin, and it contains numerous disulfide bonds. Chemical separation of these bonds allows dissection to be performed more easily and effectively during surgery [8]. Sodium-2-mercaptopetanethane-
toethanesulphonate (MESNA), a sodium salt of 2-thiosulphonate anion, is now used for this purpose during otologic surgery; it provides more effective dissection because it cleaves the disulfide bonds. In addition, because MESNA is a synthetic sulfur compound, it enables separation of mucus polypeptide chains by mucolysis of the disulfate bonds. Moreover, owing to its antioxidative, protective, and mucolytic features, it is commonly used in medicine. As a thiol-based cytoprotective agent, it is used for prophylactic treatment in cyclophosphamide-induced hemorrhagic cystitis. Because of its mucolytic effects, it is also used in respiratory medicine (in the treatment of asthma, chronic bronchitis, pharyngolaryngitis, etc.) as well as in some surgical treatments, for example, ear, nose, and throat, orthopedic, and gynecological fields.

Previous studies have reported that the use of MESNA in atelectatic and adhesive middle ear and cholesteatoma surgery is not associated with ototoxic effects and had neuroprotective effects; however, to the best of our knowledge, its direct effect on the facial nerve has not been demonstrated. The possibility of deafness development in the fallopian canal of the facial nerve is very high in the presence of pathologies progressing with dissolving bone structures, such as cholesteatoma; the use of MESNA for separating the cholesteatoma matrix in such surgeries inevitably results in contact with the facial nerve, directly or by passing through bony deafness. In this study, we investigated the effects of MESNA, which is commonly used during middle ear surgery, on the facial nerve using electrophysiological and histopathological methods.

MATERIALS and METHODS

This study was approved by the local ethics committee on animal experiments (No.01.11.2016-504). Twenty male Wistar rats raised in the animal experiments laboratory, aged 12-14 weeks and weighing 250-300 g were used in this study. Animals were placed in cages with free access to food and water. The cages had 50%-10% relative humidity at 23°C±3°C and artificial lighting was made available from 8:00 a.m. to 8:00 p.m. The rats were equally divided into four groups. Group A was the control group and group B constituted the sham group. After identifying the facial nerve, the animals in group C were given 20% MESNA solution after the facial nerve was located, and the wound was closed after waiting for approximately 5 min. Five animals in group D were administered 50% MESNA solution onto the facial nerve for 5 min, and the operation was subsequently terminated, closing the wound.

After EMG measurements were performed under anesthesia postoperatively at 4 weeks, all rats included in the study were euthanized by guillotine decapitation, and sufficient amounts of facial nerve and surrounding tissue samples were obtained from the region of intervention. Each tissue sample was fixed in formaldehyde solution for 24 h (10%) and embedded in paraffin blocks for histopathological examination. Sections of 4-6 micron thickness were obtained in pathology laboratories using a microtome. The sections were stained using hematoxylin and eosin and S-100 immunohistochemistry stains (S-100, BiocareMedical, USA) and evaluated by a single pathologist under a light microscope (Olympus BX53) to be photographed at 40×, 100×, and 200× magnification fields. All histopathological micromeasurements were quantitatively made using an Olympus DP73 camera and digital microscopy software; each parameter was individually classified as follows:

- Foreign body reaction (Yes/No)
- Inflammation (None: 0, Mild: +, Moderate: ++, Extensive: +++, and Widespread inflammation and necrosis: ++++)
- Granulation tissue (Yes/No)
- Status of the perineural sheath and myelin structure (myelin structure preserved/irregular myelin structure)
- Axonal degeneration (Yes/No)
All samples were assessed by a single pathologist using a protocol blinded to the type of treatment provided to each group.

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences version 22 software (IBM Corp.; Armonk, NY, USA). For the evaluation of test results, preoperative and postoperative mean and standard deviations were calculated for latencies, amplitude, wave duration, and supramaximal stimulus thresholds in the study and control groups. Histopathological data were evaluated together with the statistical results. For comparison of quantitative data between the groups, the Kruskal-Wallis test was used for data not normally distributed, and the Wilcoxon sign test was used for in-group comparisons. A p value of <0.05 was considered to be statistically significant.

**RESULTS**

One animal in group C (MESNA 20%) was excluded from the study because it died because of anesthesia provided before preoperative EMG measurements.

When the wave amplitude, as measured by EMG, was evaluated between the groups, there was no statistically significant difference between preoperative and postoperative amplitude levels of any of the groups (p>0.05). Compared with the preoperative amplitude level, there was no statistically significant change in the postoperative amplitude level of groups B, C, or D (p=0.893, p=0.068, or p=0.500, respectively, Table 1).

In terms of wave latency levels, as measured by EMG, no statistically significant difference was noted in preoperative and postoperative levels.
tive measurements between the groups (p>0.05). Compared with the preoperative latency level, there was no statistically significant change in the postoperative latency level of groups B, C, or D (p=0.068, p=0.713, or p=0.496, respectively, Table 2).

Table 3 denotes the evaluation of wavelength within each group and between all groups. Accordingly, there was no statistically significant difference in the preoperative and postoperative wavelengths between the groups (p>0.05). The increase noted in the wavelength in the postoperative period compared with that in the preoperative period did not show statistically significant difference in groups B, C, or D (p=0.051, p=0.068, or p=0.345, respectively).

When the preoperative and postoperative supramaximal stimulus threshold levels, as measured by EMG, were evaluated between study groups, no statistically significant difference was detected (p>0.05). Compared with the preoperative stimulus threshold level, there was no statistically significant change in the postoperative level in groups B, C, or D (p=0.068, p=0.157, p=1.000, respectively, Table 4).

Table 5 shows the results of histopathological examination. The degree of inflammation in all groups was 0. Foreign body reaction and granulation tissue were not detected in any of the groups. Perineural sheath and myelin structure were normal in all groups, and axonal degeneration was not observed (Figure 2-4).

**DISCUSSION**

MESNA is the sodium salt of 2-thiosulphonate anion. It is widely used in medicine because of its preservative, antioxidant, and mucolytic properties [12]. It is used as a cytoprotective agent to prevent hemorrhagic cystitis and as a mucolytic agent to improve pulmonary functions. In addition, the sulfhydryl group has the potential to clean the reactive oxygen metabolites and is used as a systemic protective agent against chemotherapy toxicity [9, 11].

In the field of otology, MESNA is used to easily dissect the tissue layers during ear surgeries, such as atelectatic ear and cholesteatoma, and its use has increased in recent years [7]. Some animal studies have shown that MESNA has no harmful effects on the cochlea and does not cause ototoxicity [8, 12]. However, the possibility of dehiscence development...
directly or by passing through bony dehiscence. In a study performed such surgeries inevitably comes in contact with the facial nerve, either lesteatoma, and MESNA used to separate the cholesteatoma matrix in pathologies progressing with dissolving bone structures, such as cho-

in the fallopian canal of the facial nerve is very high in the presence of (b) S-100. Nerve samples are indicated using arrows

At 200× magnification, examples of a nerve stained with (a) H&E, (b) S-100 staining; (a) group A, (b) group B, (c) group C (MESNA 20%), and (d) group D (MESNA 50%). There is no inflammation, foreign body reaction, or formation of granulation tissue in any sections. Myelin sheaths are found to be intact without axonal degeneration. Nerve samples are indicated using arrows

CONCLUSION
To the best of our knowledge, this is the first study to investigate the effects of MESNA during otologic surgery, which enables cleavage of disulfate bonds and therefore, allows easy and safe dissection, particularly in cholesteatoma and adhesive otitis media, on the facial nerve in the parotid region by physiological and histopathological methods. The findings suggest that a single application of MESNA does not cause any histopathological and electrophysiological changes that indicate toxicity on the facial nerve.

Ethics Committee Approval: Ethics committee approval was received for this study from Yeditepe University Local Ethics Committee on Animal Experi-
ments (Approval No: 01.11.2016-504).

Informed Consent: N/A.

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REFERENCES