Original Article

Histopathological Evaluation of Mesna Application at Different Concentrations on Middle Ear Mucosa of Rats in Early and Late Stages

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Abstract

Background: The aim of this study was to determine the safe concentration of Mesna, which is widely used in otologic surgeries, by examining the early and late effects on middle ear mucosa, at different concentrations. **Methods:** Fifty-nine healthy ears of 32 rats were included in the study. The rats were divided into eight groups and Mesna at 25%, 50%, and 100% concentrations along with 100% saline were applied. On the third day of the experiment, animals in the first four groups were sacrificed to assess early effects, and on the twentieth day, animals in the last four groups were sacrificed to assess late effects. The middle ear mucosa samples were dissected and delivered blindly to the pathology department. **Results:** Thirty-one rats completed the study. The histopathological effects of Mesna when applied in 25% and 50% concentrations were similar to those of saline in the early period. However, the application of 100% Mesna caused severe inflammation and a statistically significant difference was observed (P = 0.004). Furthermore, vascular proliferation was significant in this group (P = 0.014). There was no significant difference between the groups in terms of late effects. **Conclusion:** In clinical practice, using up to 50% concentration of Mesna can be said to be reliable in obtaining a faster and more efficient chemical dissection. However, an inflammation of the middle ear mucosa was observed in the early period following the application of 100% concentration. Therefore, further studies are needed on its safe use in this concentration.

Keywords: *Ear, inflammation, histology, Mesna, middle mucosa, rats*

Introduction

Sodium 2-mercaptoethanesulfonate (Mesna) is a synthetic thiol compound. It was first used in lung diseases due to its mucolytic effects.[1] However, its most common use is to prevent hemorrhagic cystitis caused by some antineoplastic drugs.[2,3] In recent years, it has been used in various surgeries for chemical dissection due to its ability to break the disulfide bonds that are abundant in adhesions.[4-7]

Mesna is most frequently used in otological surgeries in otorhinolaryngology.[8-11] While it provides elevation of the tympanic membrane in adhesive otitis media, it is also used for chemical dissection in cholesteatoma cases.[8,9] It has been reported that Mesna at concentrations of 10–20% can be used safely by showing that it has no ototoxic effect on the inner ear.[12,13] For this reason, the preferred usage concentration in clinical practice is 10–20%. A recent study suggests that using higher concentrations may be beneficial in reducing the duration of surgery and in the treatment of pathologies such as tympanosclerosis.[14] In this study, Dogan *et al*.[14] evaluated the effect of Mesna on the inner ear and reported that even at high concentrations, no evidence of ototoxicity was observed. However, as far as we are aware, there has been no study showing the effects of Mesna applications on the middle ear mucosa.

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The purpose of this study was to investigate the histological reactions to Mesna given in three different concentrations in healthy middle ear mucosa of rats.

Methods

This experimental research was carried out with the approval of the Kocaeli University Animal Experiments Local Ethics Committee (KOU 1/1-2018) between April 15 and May 6, 2019.

Animals

Thirty-two 6-month-old male Wistar Albino rats were included in the study. The weight of the rats varied between 300 and 350 grams. The rats were housed in a 12-hour dark/12-hour light environment with an average temperature of 20–22°C, and they had free access to water and food.

Anesthesia

All rats were administered a general anesthetic with the use of Ketamine HCl (Alfamine Bulb, Atafen, Istanbul) at 60 mg/kg and Xylazine HCl (Rompun Bulb, Bayer, Istanbul) at 6 mg/kg intraperitoneally. No maintenance dose was required due to the short duration of the intratympanic (IT) drug administration.

Groups and drug administration

Otoscopic examinations of all experimental animals were performed under a microscope, and if there was a plug, it was cleaned. Ears with perforation, adhesion, and effusion (n = 5) were excluded. In order not to affect the distribution, rats with just one usable ear were placed in different groups (Groups 2, 3, 5, 6, and 7).

Thirty-two rats were divided into eight groups, with four experimental animals in each group:
Group 1: Control group early period (n: 8).
Group 2: 25% Mesna early period (n: 7).
Group 3: 50% Mesna early period (n: 7).
Group 4: 100% Mesna early period (n: 8).
Group 5: Control group late period (n: 7).
Group 6: 25% Mesna late period (n: 7).
Group 7: 50% Mesna late period (n: 7).
Group 8: 100% Mesna late period (n: 8).

In Group 1 and Group 5, 100% normal saline (isotonic saline 0.09% NaCl solution) was applied as IT with a 2.5 cc dental injector, approximately 0.5 cc, enough to fill the middle ear cavity.

In Group 2 and Group 6, 25% Mesna (Ureomitexan, MESNA, Baxter oncology, Germany) (25% MESNA and 75% normal saline) was given as IT to fill the middle ear cavity with a 2.5 cc dental injector. Approximately 0.5 cc was applied.

In Group 3 and Group 7, 50% Mesna (50% MESNA and 50% normal saline) was applied as IT with a 2.5 cc dental injector to fill the middle ear cavity, approximately 0.5 cc.

Subjects in Group 4 and Group 8 were administered 100% Mesna IT with a 2.5 cc dental injector, approximately 0.5 cc, enough to fill the middle ear cavity. One rat in Group 8 died on the 10th day after the procedure due to an unknown cause.

Surgery procedure

The surgical procedure was performed by the same surgeon. To examine the early histopathological effects, the rats in the first four groups were sacrificed with the use of a high dose of anesthetic on the third postoperative day. Under the microscope, after removing the cartilage and bone part of the external auditory canal, the tympanic membrane was observed in all its quadrants. Then, the membrane was removed and the mucosa over the bulla was dissected [Figure 1]. The samples taken were put into a 10% formaldehyde solution, and fixation was achieved. The prepared samples were delivered to the pathology department in a blind way.

The rats in the remaining four groups for late effects were sacrificed with the use of a high dose of anesthetic on the 20th day of drug administration. The middle ear mucosa samples were prepared by repeating the same procedures described in the early period.

Histopathological examination

The 10% neutralized formalin fixed samples were embedded in paraffin blocks, and sections of four microns were taken and deparaffinized in the oven at 70C for 30 minutes, then kept in xylol for 10 minutes. Hematoxylin-eosin (HE) staining was done with an automatic procedure using an automatic staining and cover slipping device. Sections stained with HE were examined under a light microscope (Olympus BX50).

Under microscopic examination, samples were evaluated for the presence and severity of inflammation, subepithelial thickening, vascular proliferation, hemosiderin loaded macrophage, and fibrosis. Semiquantitative tissue scoring was used for each sample in terms of each of these findings: a score of 0 for no finding, 1 for mild signs, 2 for moderate signs, and 3 for severe signs. Detailed explanation of this scoring system: Score 0 is for normal histologic appearance for all parameters. Mild inflammation (Score 1) is that the inflammatory cell count of less than 5 cells/field, at 200x magnification. Moderate inflammation (Score 2) is that the cell count of more than 5 cells/field but less than 10 cells/field, and severe inflammation (Score 3) is that the cell count of more than 10 cells/field. When the

submucosal thickness had increased less than 10%, it has graded as score 1, when it had increased by greater than 10%, but less than 25% graded as score 2 and when it had increased by 25% or greater graded score 3. When the vascular lumen seen in one area had increased less than 10%, the vascular proliferation is graded score 1, when the vascular lumen seen in one area had increased by more than 10%, but less than 25% is graded score 2 and when it had increased by 25% or more is graded score 3. Hemosiderin-loaded cells and foreign body reactions were evaluated as present or absent.

Statistical analysis

Statistical evaluation was done with the IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) package program. The normal distribution test was evaluated with the Kolmogorov–Smirnov Test. Numerical variables were given as mean ± standard deviation and median (25^{th}–75^{th} percentile) and frequency (percentages). Fisher›s exact Chi-square test and Monte–Carlo Chi-square test were used for categorical variables to evaluate the differences between groups. $P < 0.05$ was considered sufficient for statistical significance in two-sided tests.

Results

No difficulty was observed in returning the experimental animals to normal life after the Mesna application. There was no change in food and water consumption. The study was completed with 31 rats (57 ears). No infections were observed during the microscopic examination performed before the mucosa samples were taken. It was observed that tympanic membranes were intact. Histopathological evaluation was performed on a total of 57 middle ear mucosa samples. Early-stage histopathological changes are summarized in Table 1, and late-stage changes are summarized in Table 2.

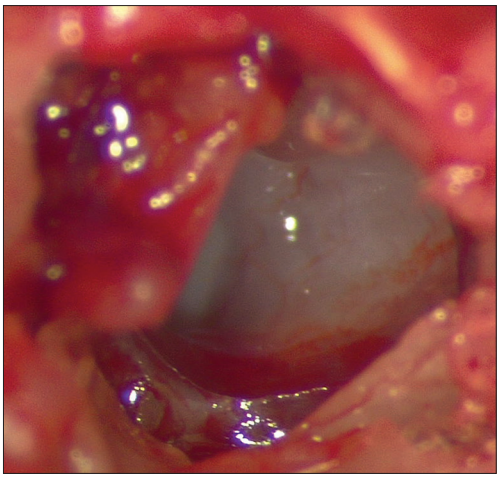

Figure 1: Appearance of the middle ear mucosa after the tympanic membrane is elevated

Inflammation

Inflammatory changes were evaluated at four degrees: absent, mild, moderate, and severe. In the comparison of the groups in which inflammatory changes in the middle ear mucosa were evaluated in the early period, severe inflammation was observed in six (75%) of the eight samples in Group 4 in which 100% Mesna was applied, and a statistically significant difference was observed ($P = 0.004$) [Figures 2 and 3].

Table 1: Early-stage histopathological changes

	Inflamation	Fibrosis	Subepithelial Thickening	Vascular Proliferation
Group 1				
Absent	4/8	6/8	5/8	6/8
Mild	-	1/8	2/8	2/8
Moderate	1/8	1/8	1/8	-
Severe	3/8	-	-	-
Group 2				
Absent	1/7	4/7	6/7	6/7
Mild	4/7	3/7	1/7	1/7
Moderate	2/7	-	-	-
Severe	-	-	-	-
Group 3				
Absent	1/7	5/7	6/7	1/7
Mild	1/7	1/7	-	5/7
Moderate	4/7	1/7	1/7	1/7
Severe	1/7	-	-	-
Group 4				
Absent	-	5/8	5/8	2/8
Mild	1/8	3/8	3/8	6/8
Moderate	1/8	-	-	-
Severe	6/8	-	-	-
P	0.004	0.657	0.591	0.014

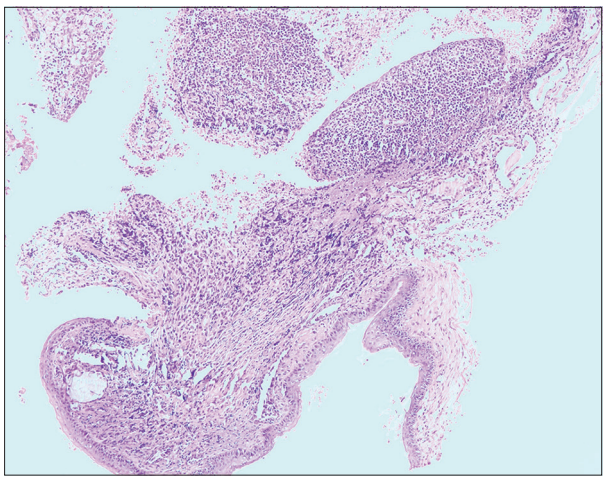

Figure 2: Inflamed mucosa sample with intense inflammatory cell infiltration and mucosal thickening in Group 4 (HE, light microscope, X100)

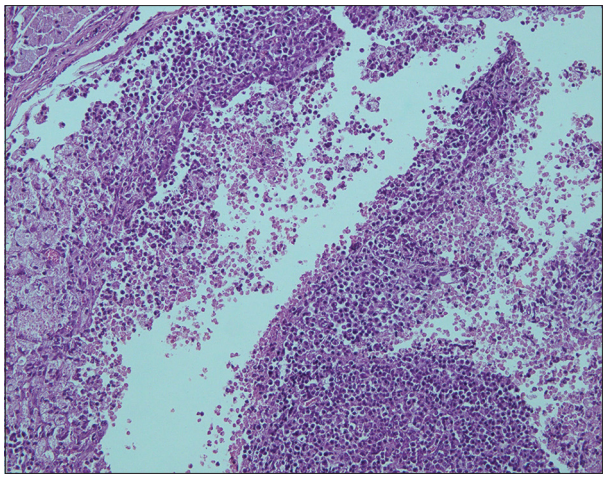

Figure 3: Polymorph nucleus neutrophil leukocytes, lymphocytes, and plasma cells that cause inflammation in Group 4 (HE, light microscope, X200)

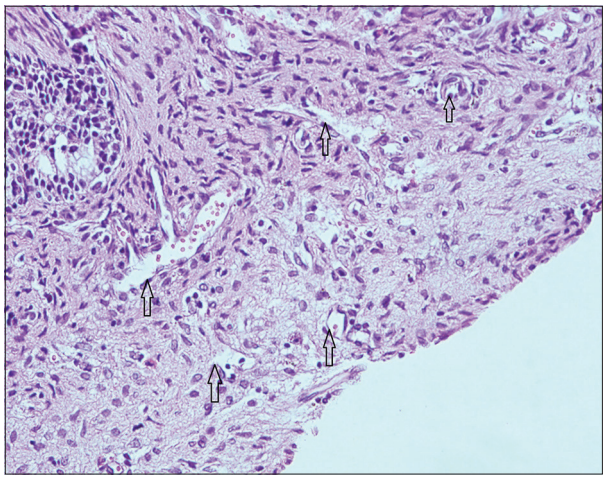

Figure 4: Vascular proliferation in Group 4 (arrow: vascular lumen sections) (HE, light microscope, X400)

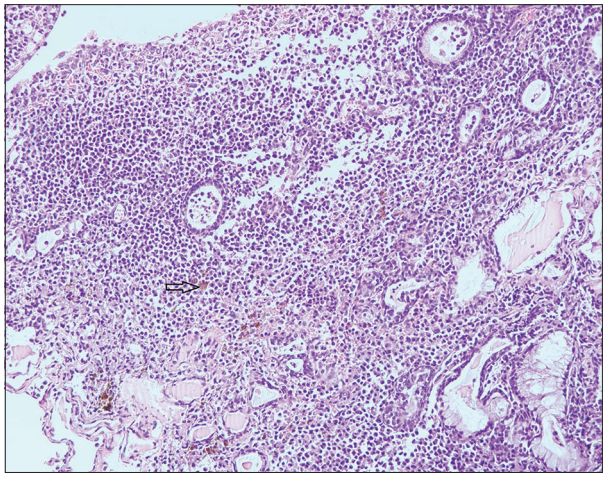

Figure 5: Hemosiderin-loaded macrophages, which are shown by the arrow at the site of inflammation in Group 4 (HE, light microscope, X200)

No significant difference was observed between the groups in the comparison of the groups in which inflammatory changes were evaluated in the late period (P = 0.212).

Table 2: Late-stage histopathological changes

	Inflamation	Fibrosis	Subepithelial Thickening	Vascular Proliferation
Group 5				
Absent	-	4/7	4/7	3/7
Mild	6/7	-	2/7	3/7
Moderate	-	3/7	1/7	1/7
Severe	1/7	-	-	-
Group 6				
Absent	-	3/7	5/7	5/7
Mild	5/7	4/7	2/7	2/7
Moderate	2/7	-	-	-
Severe	-	-	-	-
Group 7				
Absent	1/7	3/7	4/7	3/7
Mild	4/7	3/7	3/7	1/7
Moderate	2/7	1/7	-	3/7
Severe	-	-	-	-
Group 8				
Absent	-	2/6	1/6	1/6
Mild	4/6	2/6	3/6	4/6
Moderate	-	2/6	2/6	1/6
Severe	2/6	-	-	-
P	0.212	0.300	0.346	0.219

Fibrosis

Fibratory cell changes were evaluated at four degrees: absent, mild, moderate, and severe. When comparing the groups in which fibrosis changes in the middle ear mucosa were evaluated in the early period, there was no significant difference between the groups (P = 0.657).

In the comparison of the groups in which fibrosis changes in the middle ear mucosa were evaluated in the late period, there was no significant difference between the groups (P = 0.300).

Subepithelial thickening

Subepithelial thickening was evaluated at four degrees: absent, mild, moderate, and severe. In the comparison of the groups in which subepithelial thickening changes in the middle ear mucosa were evaluated in the early period, no significant difference was observed between the groups (P = 0.591).

In the comparison of the groups in which subepithelial thickening changes in the middle ear mucosa in the late period were evaluated, no significant difference was observed between the groups (P = 0.346).

Vascular proliferation

Vascular proliferation was evaluated at four degrees: absent, mild, moderate, and severe. In the comparison of the groups in which vascular proliferation changes in the middle ear mucosa were evaluated in the early period, a significant difference was observed in the fourth group, in which 100% Mesna was applied compared with the other groups ($P = 0.014$) [Figure 4].

In the comparison of the groups in which vascular proliferation changes in the middle ear mucosa in the late period were evaluated, no significant difference was observed between the groups ($P = 0.219$).

Pigment-loaded cells

Cells containing brown pigment were evaluated as present/absent. Comparing the groups in which pigment-loaded cells in the middle ear mucosa were evaluated in the early period, no significant difference was observed between the groups ($P = 0.148$). Hemosiderin-loaded macrophages in the area of inflammation in group 4 are shown in Figure 5.

In the comparison of the groups in which pigment-loaded cells in the middle ear mucosa were evaluated in the late period, no significant difference was observed between the groups ($P = 0.355$).

Foreign body reaction

Giant cells, granuloma formation, cholesterol clefts, and histiocyte clusters were examined. However, no finding that could be considered as a foreign body reaction was observed in any of the samples.

Discussion

Mesna is used in various surgeries due to its chemical dissection feature of breaking the disulfide bonds.[3-5] However, experimental studies of its use in new surgical fields are also being carried out.[15,16] Otorhinolaryngology is an area where Mesna is used quite frequently, most commonly in otological surgeries. The first experimental study on this subject was conducted by Vincenti *et al*.[13] They reported that 10–20% concentrations of Mesna do not have an ototoxic effect on the cochlea of guinea pigs. Currently, the preferred usage concentration for topical application of Mesna in otological surgery is 10–20%.[8,9,11]

In ear surgeries, topical Mesna application is used to provide membrane lateralization in adhesive cases and chemical dissection in cases of cholesteatoma. Yılmaz *et al*.[8] used 20% concentration of Mesna in 42 ears of 39 cases with adhesive otitis media and/or retraction pockets features, and they concluded that it facilitates tympanic membrane elevation, thus making the surgery safer and easier to apply.[8] They reported that there was no sensorineural hearing loss in any of the patients during their follow-up.

The primary purpose of surgery for cholesteatoma is to eradicate the disease. Postoperative residual cholesteatoma is one of the main problems. In order to reduce this situation, various innovations such as endoscopy, potassium titanyl phosphate laser, and immunofluorescence examination have been used.[17-19] In addition, materials that facilitate dissection by breaking the disulfide bonds in the structure of cholesteatoma have also been used. Kluyskens *et al*.[20] used N-acetyl cysteine powder in 29 cases of cholesteatoma to break these bonds in keratin.[20] The follow-up period specified in this study was only 6 months, and cholesteatoma was encountered in two patients (6.9%).[20] It is known that recurrent and residual cholesteatomas can be seen for more than 6 months.

Vincenti *et al*.[11] used Mesna at a 10% concentration for chemical dissection.[11] In this study, 16.7% of residual disease was observed in 108 cases where Mesna was used. However, 24.5% of residual disease was observed in 106 cases where Mesna was not used.

Kalcıoglu *et al*.[9] used 20% Mesna for chemical dissection in cholesteatoma surgery.[9] In this study, in which patients were followed for at least 1 year, the rates of residual cholesteatoma were 6.5% in patients where Mesna was used and 17.9% in the control group.

Propylene glycol is one of the methods of experimentally inducing cholesteatoma in rats. Ismi *et al*.[21] reported that a single dose of a 10% Mesna application reduces the rate of cholesteatoma formation in this experimental cholesteatoma model compared with a saline injection.[21] It is thought that this effect may increase with the use of Mesna in high concentrations.

Mesna used at higher concentrations can provide faster and more effective chemical dissection. Dogan *et al*.[14] reported that high concentrations had no audiologically or histopathologically toxic effects on the inner ear.[14] Since there is no study revealing the effect of Mesna on middle ear mucosa, this study examined the effects of topical Mesna applications in different concentrations on middle ear mucosa in both the early and late stages.

Severe postoperative fibrosis and inflammation negatively affect the success of otological surgery.[22,23] Postoperative fibrosis can cause adhesion. This situation can also lead to hearing loss, membrane retraction, and graft loss.[24] For this reason, it is important that the substance to be applied to the middle ear does not cause fibrosis. Inflammation can occur in the middle

ear mucosa as an immune response of the body against foreign substances. The presence of inflammation may also adversely affect postoperative wound healing and cause the otological surgical failures mentioned above.

In the present study, no significant differences were observed in all concentrations of Mesna in the early and late stages in terms of fibrosis. However, when considering the early results, it was observed that there was significant severe inflammation in the 100% Mesna group. In addition, a significant increase in vascular proliferation was observed in the same group and in the early period. Although it is observed that these effects diminish in the long term and there is no statistically significant difference between the other groups, it is thought that the severe inflammation observed in the early stage may negatively affect the success of otologic surgery. In high-dose Mesna applications, measures to reduce possible early-stage inflammation, such as adding anti-inflammatory agents, may be helpful in preventing this negative effect. In experimental studies, corticosteroids have been shown to reduce inflammation and fibrosis caused by absorbable sponges.[24,25] Future studies on the control of early-stage inflammation in high dose applications of Mesna will likely provide useful information regarding maximum effect and minimum side effects.

One of the limitations of our study is that this research cannot show whether the early-period inflammatory effects are reversible with anti-inflammatory drugs. The limited number of cases because it was a study on animals can be considered another limitation.

Conclusion

A 25–50% concentration of Mesna seems to be safe to use in otological surgeries due to its effect on the middle ear mucosa. More studies are needed on the safe usage of 100% concentration of Mesna. In addition, future studies on different and high-dose Mesna applications in combination with anti-inflammatory agents will contribute to the control of possible early-stage inflammation.

Ethic statement

This experimental research was carried out with the approval of the Kocaeli University Animal Experiments Local Ethics Committee (KOU 1/1-2018)

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Conflicts of interest

There are no conflicts of interest.

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