



Original Article

# The Effects of MESNA on the Facial Nerve, an Experimental Animal Study

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**OBJECTIVE:** MESNA (Sodium-2-mercaptoethanesulfonate) is a mucolytic substance that is used for chemically assisted tissue dissection in various surgical operations. The aim of this study was to address the issue of possible neurotoxicity from topical administration of MESNA solution on the facial nerve. We used different concentrations of MESNA solution and evaluated their effects on facial nerve by histopathological and functional analysis.

**MATERIALS and METHODS:** These groups were the saline administered group (control) (3 rats, 6 facial nerves), the 25% MESNA solution group (3 rats, 6 facial nerves), and the 100% MESNA solution group (3 rats, 6 facial nerves). Under general anesthesia (ketamine 150 mg/kg, xylocaine 4 mg/kg), the bilateral facial nerves of rats were dissected. The saline, 25% MESNA, and 100% MESNA solutions. Facial nerve functions of the rats were evaluated using mustache whisker and blink reflex scores at day 20 days. On day 20, the rats were sacrificed and the buccal and marginal mandibular branches of the facial nerve were removed. The specimens were examined in terms of inflammation, granulation tissue, and foreign body reaction formation around the nerve. The functional and histopathological changes on facial nerves were compared between groups.

**RESULTS:** Mustache and blink reflex scores of the rats were 5 (normal) in both the control and study groups. There were no statistically significant differences between the three groups in terms of facial nerve functions ( $p=1.00$ ). On histopathologic examination, the 25% and 100% MESNA groups had significantly more inflammation compared with the control group ( $p=0.038$  and  $p=0.007$ , respectively). There were no statistically significant differences between the 25% and 100% MESNA groups in term of inflammation ( $p>0.05$ ). There were no statistically significant differences between the three groups in terms of foreign body reaction formation ( $p>0.05$ ).

**CONCLUSION:** Topical administration of MESNA solution onto the facial nerve causes increased inflammation in both the 25% and 100% concentrations. Nevertheless, it does not cause any facial nerve dysfunction.

**KEYWORDS:** MESNA, facial nerve, toxicity

## INTRODUCTION

Sodium-2-mercaptoethanesulfonate (MESNA) is a synthetic sulfur compound and belongs to a class of thiol compounds that induces mucolysis by destroying the disulfide bonds of the mucous polypeptide chains<sup>[1,2]</sup>. It can be used as a mucolytic agent for pulmonary disorders and as a protective agent against the toxicity of some chemotherapeutic agents. MESNA was also shown to inhibit the development of bladder tumors in rats by increasing the kidney levels of free thiol<sup>[3]</sup>. MESNA was shown to prevent renal oxidative damage in rats treated with ferric nitrilotriacetate<sup>[4]</sup>. MESNA has been used for tissue dissection in various surgical procedures due to its chemical properties<sup>[5]</sup>. The first clinical applications of MESNA were in the field of ENT surgery<sup>[6-8]</sup>.

Adhesions between the different layers of tissues are rich in disulfide bonds. Starting from the hypothesis that adhesions between the healthy and pathologic tissues are rich in disulfide bonds, Zini et al.<sup>[6]</sup> developed a research project entitled "Chemically Assisted Tissue Dissection" and used MESNA as a chemical dissection material. They patented the use of MESNA, and claimed its use in various surgical applications, including head and neck, neurological, maxillofacial, cardiovascular, urological, orthopedic, plastic, ophthalmic, gynecological, base of skull, thoracic, and dental surgery<sup>[6]</sup>. They associated compared the chemical dissection to mechanical technique<sup>[9]</sup>.

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As used in other surgical areas, MESNA can be used during middle ear surgery, such as in the case of cholesteatoma or atelectatic ears, when dissecting the tissue layers [10]. The matrix of cholesteatoma is mainly composed of keratin, a protein rich in disulfide bonds. These bonds can be disrupted by MESNA. Thus, the surgeon can safely dissect the matrix of cholesteatoma from the intact ossicular chain, labyrinthine fistula, exposed facial nerve, and bony defect of the tegmen tympani and mastoideum without damaging these vital structures.

It has been reported that there is no side effect or hazard of middle ear application of MESNA on hearing. But the effects of topical application of MESNA on the facial nerve are still unknown. In our study, we applied different concentrations of MESNA solution directly onto the facial nerve and determined the histopathologic and functional effects of MESNA on the facial nerve. To our knowledge, this is the first study to investigate the effect of topical application of MESNA on the structure and function of the facial nerve.

### MATERIALS and METHODS

This study was performed in the Ankara Training and Research Hospital Laboratory of Animal Experiments. The study protocol was approved by the Ankara Education and Research Hospital's Institutional Ethical Committee (September 4, 2016, decree no: 2016-15-111). The principles of animal care and use according to the Declaration of Helsinki were strictly followed.

### Animals and Study Groups

Nine Wistar albino rats were included in the study. Three rats were used as the control group, three rats were used in the 25% MESNA group, and three rats were used in the 100% MESNA group. In each group the facial nerves were dissected bilaterally. All rats completed the study.

### Surgical Procedure

The rats were administered general anesthesia with 40 mg/kg of intramuscular ketamine HCl (Ketalar ampoule®; Pfizer, İstanbul, Turkey) and 5 mg/kg intramuscular xylazine HCl (Rhompun ampoule®; Bayer, İstanbul, Turkey). A preauricular 2-cm incision was made on the both sides of all rats. The parotid gland was removed, and the trunk of the facial nerve was identified as it emerged from the stylomastoid foramen into the soft tissues of the neck. The facial nerve was circumferentially dissected from the surrounding soft tissues. The buccal and marginal mandibular branches of the facial nerve were identified (Figure 1). In the control group (3 rats, 6 nerves), normal saline was applied on the bilateral facial nerves. In the study group, 25% MESNA (Ureomitexan, MESNA, Baxter oncology, Germany) group (25% MESNA and 75% normal saline by volume) (3 rats, 6 nerves) and the 100% MESNA group (3 rats, 6 nerves), the solutions were applied on the facial nerves. The wounds were properly closed without washing the solution after waiting at least 5 min. On day 20, the facial nerve functions of the rats were examined with mustache and blink reflex scores (Table 1) [11]. After the examination, the rats were sacrificed and approximately 3 cm of the buccal and marginal mandibular branches of the facial nerve were excised, starting from the trunk of the facial nerve. The tissue samples were fixed in 10% formalin for at least 24 hours and embedded in paraffin blocks. Sections of 5µm were obtained using a microtome and placed on glass slides. Tissue samples were stained with hematoxylin & eosin. The sections were examined by the same pathologist under a light microscope, and photomicrographs were taken at 200x mag-



Figure 1. Incision and identification of the branches of the facial nerve (white arrow). The black arrow shows the parotid gland.

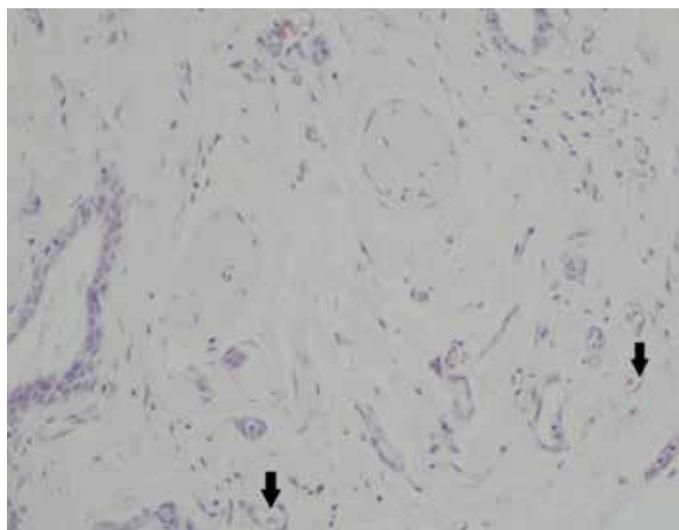


Figure 2. Histopathologic view of 1 or 2 eosinophils (black arrows) in the perineural area\* in the 25% MESNA group, H&E, 200x magnification.

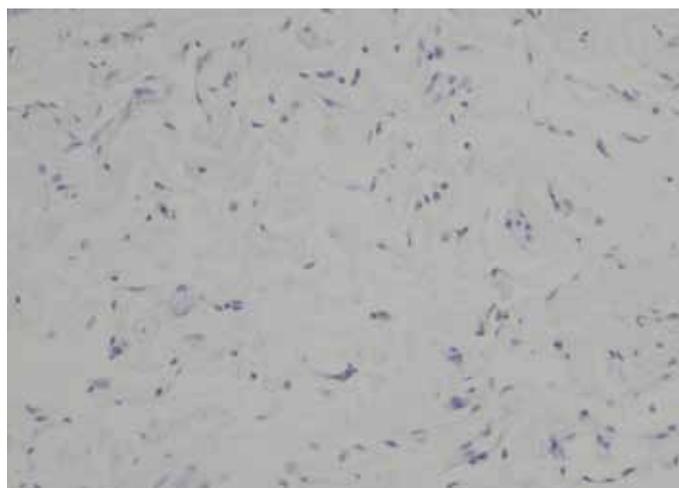


Figure 3. Histopathologic view of a specimen in the control group showing the absence of inflammatory cells in the perineural area\*, H&E, 200x magnification.

**Table 1.** Evaluation of mustache and blink reflexes in rats

Vibrissae observation scale		
Score	Movement	Position
1	No movement	Posterior
2	Light tremor	Posterior
3	Greater tremor	Posterior
4	Normal movement	Posterior
5	Normal movement	Anterior
Scale of eye closing and blinking reflex observation		
Score	Movement	
1	No movement	
2	Contraction/No closure	
3	50% closure	
4	75% closure	
5	Complete closure	

**Table 2.** Histopathologic findings in control and study groups in terms of inflammation

	Inflammation			<sup>a</sup> p
	Absent (4)	1-2 Eosinophils (6)	Mild (8)	
25% MESNA	0	3	3	
100% MESNA	0	1	5	
Control	4	2	0	
25% MESNA - 100% MESNA		<sup>b</sup> 0.783		
25% MESNA- Control		<sup>b</sup> 0.003*		<sup>a</sup> 0.001*
100% MESNA- Control		<sup>b</sup> 0.001*		

<sup>a</sup>One-way ANOVA Test; <sup>b</sup>Bonferroni Test; \*p<0.05

**Table 3.** Histopathologic findings in control and study groups in term of granulation

	Granulation		<sup>a</sup> p
	Absent (14)	Mild Vascularization Increase (4)	
25% MESNA	6	0	
100% MESNA	2	4	
Control	6	0	
25% MESNA - 100% MESNA		<sup>b</sup> 0.005	
25% MESNA- Control		<sup>b</sup> 1.000	<sup>a</sup> 0.002*
100% MESNA - Control		<sup>b</sup> 0.005	

<sup>a</sup>One-way ANOVA Test; <sup>b</sup>Bonferroni Test

nification. Facial nerves of the rats were histopathologically examined in terms of inflammation, formation of granulation tissue, and foreign body reaction and scored as follows:

- Foreign body reaction: 0=absent, 1=present
- Inflammation: 0=absent, 1=presence of 1 or 2 eosinophils, 2=mild inflammation
- Granulation: 0=absent, 1=mild increase of vascularization, 2=presence of granulation

**Table 4.** Histopathologic findings in control and study groups in term of foreign body reaction

	Foreign Body Reaction		<sup>a</sup> p
	Absent (17)	Present (1)	
25% MESNA	5	1	
100% MESNA	6	0	
Control	6	0	
25% MESNA - 100% MESNA		<sup>b</sup> 0.719	
25% MESNA- Control		<sup>b</sup> 0.719	<sup>a</sup> 0.391
100% MESNA - Control		<sup>b</sup> 1.000	

<sup>a</sup>One-way ANOVA Test; <sup>b</sup>Bonferroni Test

### Statistical Analysis

Statistical Package for the Social Sciences version 17.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. Results were analyzed using one-way ANOVA test and followed by Bonferroni post-hoc test. The significance was set at p<0.05.

### RESULTS

Clinical examination revealed normal mustache and blink reflex scores (score 5) in all rats. Histological examination revealed inflammation findings in two nerves from the control group and in all six nerves from both MESNA groups. In the 25% MESNA group, there were 1 or 2 eosinophils in three nerves and mild inflammation in the remaining three nerves. In the 100% MESNA group, there were 1 or 2 eosinophils in one nerve (Figure 2) and mild inflammation in the remaining five nerves. The 25% and 100% MESNA subgroups had significantly more inflammation compared to the control group (p=0.003 and p=0.001 for the 25% and 100% MESNA subgroups, respectively) (Table 2). There was no statistically significant difference between the 25% and 100% MESNA subgroups in terms of inflammation (p=0.783) (Table 2). Figure 3 shows the absence of inflammation in a sample in the control group.

Histological examination revealed granulation tissue formation in four nerves in the 100% MESNA group. There was a mild increase in vascularization in these specimens. There were no granulation tissue reaction findings in the control or 25%MESNA groups. There was significantly more granulation tissue formation between the 100% MESNA group compared to the 25% MESNA group (p=0.005). There was significantly more granulation tissue formation in the 100% MESNA group compared to the control group (p=0.005) (Table 3).

Histological examination revealed foreign body reaction findings in one nerve in the 25% MESNA group. There were no granulation tissue reaction findings in the control or 100% MESNA groups. There was no statistically significant difference between the 25% and 100% MESNA subgroups (p=0.719) or between the 25% MESNA and control groups (p=0.719) in terms of foreign body reaction (Table 4).

### DISCUSSION

In this study, we applied MESNA on the facial nerve in rats. The MESNA solution caused significantly more inflammation around the facial nerve when compared to the control group. Nevertheless, it did not cause any facial nerve dysfunction, even at a 100% concentration.

In cholesteatoma surgery, complete and accurate removal of the matrix is essential to minimize the likelihood of leaving in place epidermal debris that might grow into a residual cholesteatoma. However, sometimes cholesteatoma matrix can infiltrate the mastoid cavity or replace the middle ear mucosa, and it might be challenging to completely remove cholesteatoma matrix from the middle ear cavity [12]. In case of an intact ossicular chain, bony labyrinth defect, epidermization of the facial nerve, or bony defects of the middle and posterior cranial fossa, gentle dissection is required to avoid iatrogenic complications like sensorineural or conductive hearing loss, facial paralysis, or cerebrospinal fluid leaks [13]. These complications usually occur while performing mechanical dissection of the cholesteatoma matrix from the underlying structures. Yilmaz et al. [10] reported that MESNA was successful in atelectatic ears and adhesive otitis media because it made the operation easier and safer by allowing elevation of the tympanic membrane through its mechanical and chemical actions. Kalcioğlu et al. [14] investigated the use of 20% MESNA in chronic otitis with cholesteatoma and reported that it could be helpful for eliminating the disease and increasing surgical success. In that study, residual cholesteatoma rates were significantly higher when MESNA was not applied.

Any chemical agent applied into the middle ear can enter the inner ear via the round window membrane, and it may cause toxic effects in the cochlea [15]. In the literature, few experimental studies have investigated the ototoxic effect of MESNA. Vincenti et al. [16] investigate dinner ear toxicity of MESNA using transmission electron microscopy, scanning electron microscopy, and auditory brainstem response testing. That study did not reveal any audiological effects of MESNA or changes in cochlear morphology after its application [16]. Similarly, Van Spaendonck et al. [17] did not observe any toxic effect of ototopical application of MESNA.

The exposed facial nerve is the most vulnerable structure during middle ear surgery. The facial nerve travels through the temporal bone in a bony canal called the facial or fallopian canal. This canal protects the facial nerve from iatrogenic trauma, direct pressure of masses, enzymatic activation of cholesteatoma, inflammation, and toxic agents applied to the middle ear. In an anatomical study, Baxter found that 57% of people have congenital fallopian canal dehiscence in the tympanic segment [18].

Acquired bony dehiscences are usually associated with chronic otitis media with cholesteatoma. The incidence of facial canal dehiscence in chronic otitis media with cholesteatoma has been reported to be around 33.3% during initial surgery [19]. The application of any chemical agent on the facial nerve might lead to neurotoxicity when facial canal dehiscence is present.

There are various studies investigating the effects of MESNA on neural tissues. In one study, repeated intraperitoneal injection of high dose (> 300mg/kg) MESNA induced spinal activity and contralateral activity of the trigeminoreticular areas of the brainstem-spinal cord junction in rats [20]. In an animal study, a single dose of 150 mg/kg MESNA was shown to decrease the apoptotic activity in the spinal cord following ischemia/reperfusion injury [21]. These studies showed that systemic administration of MESNA had neuroprotective effects on neural tissues. There is only one study investigating the effects of

topical MESNA application on neural tissue. In that study, 50% and 100% MESNA solutions were applied to the subarachnoid space over the brain tissue, and effects of MESNA solutions were assessed by means of light microscope for both concentrations. That study did not reveal any neurotoxic effects of MESNA that would indicate toxicity in neural tissues [22].

The locations of facial nerve injuries influence the recovery times for the return of facial nerve paralysis. An intratemporal crush of the facial nerve leads to significantly delayed functional recovery and decreased motor nerve conduction as compared to an extratemporal crush [23]. From this, it is conceivable that MESNA might lead to different effects on the different parts of the facial nerve. In our study, we applied MESNA on the extratemporal part (the buccal and marginal mandibular branches) of the facial nerve. Our future direction will aim to investigate the effect of this agent on the intratemporal part of the facial nerve.

To our knowledge, no studies to date have investigated the effect of MESNA on the facial nerve. This study sheds light on the effect of MESNA application on the facial nerve in rats. Our results showed that administration of MESNA causes increased inflammation around the neural tissue without any loss of facial nerve functions in the context of a 20-day follow-up. However, we cannot disregard the inflammatory reaction found that might evolve later and cause facial nerve dysfunction. This is a limitation of our study. Therefore, further studies are needed to reveal the long-term effects of MESNA on the facial nerve and its functions. Another limitation of this study is the lack of electrophysiological examination to investigate subclinical toxicity of MESNA on the neural tissues.

## CONCLUSION

According to the results of our study, MESNA did not cause any toxic effect on the facial nerve in rats in a short time period. However, long-term follow-up studies including electrophysiological examination with a larger number of animals and human studies are needed.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Ankara Training and Research Hospital.

**Informed Consent:** N/A

**Peer-review:** Externally peer-reviewed.

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