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Does intratympanic Mesna application prevent cholesteatoma? An experimental study on rats

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Ethics Committee Approval
The study protocol was approved by the ethics committee of Dicle University Animal Experiments Local Ethics Committee with the decision no. 2013/5.
The study was conducted in line with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health, Commission on Life Sciences, and National Research Council.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Cholesteatoma is an invasive and destructive disease, responsible for most of the complications related to chronic otitis media. The only effective treatment is surgical excision. Researching medical treatment alternatives can bring a new perspective to the treatment of this disease. This study aimed to investigate the effect of Mesna on otitis media and cholesteatoma induced by propylene glycol on an experimental animal model.
Methods: The study was designed to consist of sixteen Wistar albino rats, with their right ears being the control group and the left ears being the experiment group. Fifty percent propylene glycol, gentamicin sulfate, and physiologic saltwater were administered to the right ear, and 50% propylene glycol, gentamicin sulfate, and 20% Mesna were administered to the left ear through intratympanic injections on days 1, 3, 8, 15, and 21. The rats were sacrificed 45 days after the first injection and underwent histopathological examination.
Results: Cholesteatoma and fibrosis were less common in the experimental group. In the study group, the average and maximum thicknesses of the tympanic membranes (P=0.008) and the minimum thicknesses of the tympanic bulla (P=0.019) were significantly less than those of the control group.
Conclusion: In the experimental cholesteatoma model created in rats, Mesna, administered intratympanically, was seen to completely prevent the formation of cholesteatoma. However, histopathological examination revealed that although present, cholesteatoma formation and fibrosis were significantly less in the experimental group.

Keywords: Mesna, cholesteatoma, Fibrosis, Otitis media, Intratympanic

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Introduction

Various studies were published on the development of otitis media and cholesteatoma after the intratympanic application of chemicals on laboratory animals [1-3]. In the 1980s, the eye and ear drop called Cortisporin was observed to cause inflammatory changes and cholesteatoma in the middle ear because of the 10% propylene glycol used as a solvent [4]. In the following years, propylene glycol was used in experimental studies on otitis media and cholesteatoma because of its inflammatory effect on the ear [5-7]. Sodium 2-mercaptoethanesulfonate (C2 H 5 NaO 3S 2, Mesna) is a synthetic sulfur compound that carries a thiol group. It breaks the disulfide bonds in a polypeptide chain with mucolysis. The matrix of the cholesteatoma or squamous epithelial is made of keratin, a protein with disulfide bonds. Mesna can be used to ease the dissection of the tissue layers in the surgery of cholesteatoma due to its mucolytic properties [8]. Studies reported that the administration of Mesna to the middle ear cavity does not affect hearing [9, 10]. For this reason, we planned to investigate the effect of Mesna on cholesteatoma and otitis media created in the middle ear cavity of experimental animals by propylene glycol. We aimed to show the presence of keratinized epithelium in the middle ear, the inflammatory changes in the middle ear mucosa, and the changes in the morphology of the tympanic membrane through histopathological evaluation.

Materials and methods

The study was conducted in line with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health, Commission on Life Sciences, and National Research Council [11]. The study protocol was approved by the Dicle University Animal Experiments Local Ethics Committee (10/10/2013/5)

Experimental animals

Sixteen healthy male Wistar albino rats weighing between 210-304 grams with healthy outer ear canals and tympanic membrane in otoscopic examination were used in our study. All experimental animals were housed in appropriate cages under standard environmental conditions (room temperature 22^{o}C-24^{o}C, 50% relative humidity, and 12-hour periods of light-dark). The animals could access water and a traditional laboratory diet until they were sacrificed.

Experimental design

The study was designed to have the right ears of the rats as the control group and the left ears as the experimental group.

Propylene glycol was used to form a cholesteatoma and an inflammatory reaction in the middle ear mucosa. Mesna was used to inhibit the pathologic processes in the middle ear mucosa and gentamicin was utilized to inhibit the inflammatory process caused by sulfate. Intratympanic injections were administered to all rats on the pars tensa region of the tympanic membrane on days 1, 3, 8, 15, and 21 under a surgical microscope. Each ear underwent 5 administrations in total. The rats were sacrificed 45 days after the first injection.

Solutions used in the control group (right ear): 0.2 ml 50% propylene glycol, 0.1 ml gentamicin sulfate (40 mg/ ml) and 0.1 ml physiologic salt water (0.9%).

Solutions used in the experimental group (left ear): 0.2 ml 50% propylene glycol, 0.1 ml gentamicin sulfate (40 mg/ ml) and 0.1 ml 20% Mesna (100mg/ ml).

Anesthesia

All rats were anesthetized with intramuscular 60 mg/kg ketamine hydrochloride and 10mg/kg 2% xylazine hydrochloride.

Tissue preparation and histopathological examination

All procedures were conducted under hygienic, albeit nonsterile conditions. The animals were sacrificed after anesthesia and the tympanic membrane and tympanic bulla were removed with microdissection. The specimens were fixed for 24 hours in 10% formaldehyde solution. Then they were decalcified for one week in a 10% formic acid solution. After the fixation and decalcification procedures, the specimens were transversely cut into two, dehydrated in baths of alcohol and a tissue tracking procedure was implemented. Then, they were buried in paraffin. Cross-sections with a thickness of 5 microns were taken, which were dyed with hematoxylin and eosin and examined under a light microscope (Zeiss Axiophot Axioplan, Germany) by a single expert pathologist. In the examination, the tympanic membrane and the middle ear mucosa were evaluated according to various pre-determined histopathological properties (presence of inflammatory cells, presence of fibrosis, presence of keratinized epithelium in the middle ear (cholesteatoma), thickness of the tympanic membrane, thickness of the tympanic bulla mucosa). The thicknesses of the tympanic membrane and tympanic bulla were measured under a 10x magnification.

Statistical analysis

Data analyses were performed with the Statistical Package for the Social Sciences (SPSS for Windows, version 15.0) software. Fisher's exact tests were used in the investigation of the relationships between parameters. For nonparametric data, Mann Whitney *U* test was performed. $P<0.05$ was considered statistically significant.

Results

The results were evaluated qualitatively and quantitatively.

Qualitative results

In the control group, the tympanic membrane was intact in 10 of the 16 ears, and not intact in 6. Inflamed cells were present in the tympanic membrane of 1 ear and the tympanic bulla mucosa of 2 ears. A small number of inflamed cells were present in the tympanic membrane, while a significant amount of inflamed cells were observed in the tympanic bulla mucosa. It was noted that almost all inflamed cells consisted of polymorphonuclear leukocytes. Cholesteatoma was seen in 8 ears, and fibrinous-proteinosis material was observed in 3.

In the experimental group, the tympanic membrane was intact in 8 of the 16 ears, and not intact in 8. Cholesteatoma was seen in 7 ears (Figures 1-2). Obvious inflammation and fibrosis were not observed in the tympanic membrane and tympanic bulla mucosa.

Figure 1: Development of keratinized epithelium and cholesteatoma in the middle ear in the control group. Thin arrow = tympanic membrane, disc = keratinized stratified squamous epithelium (i.e. cholesteatoma), thick arrow = tympanic bulla mucosa (H & E; × 10)

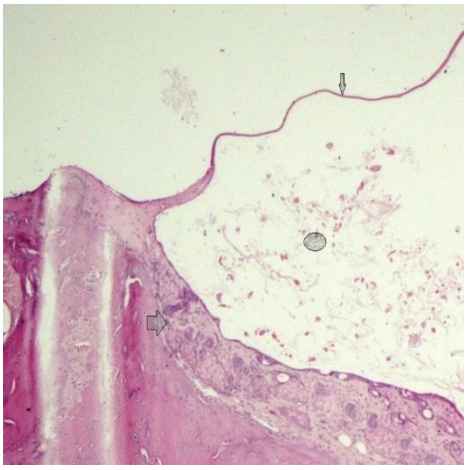
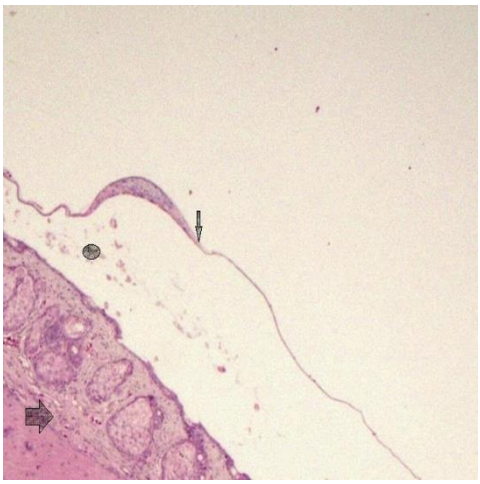


Figure 2: Development of keratinized epithelium and cholesteatoma in the middle ear in the experimental group. Thin arrow = tympanic membrane, disc = keratinized stratified squamous epithelium (i.e., cholesteatoma), thick arrow = tympanic bulla mucosa (H & E; × 10)



The two groups were similar regarding the prevalence of cholesteatoma and fibrosis (P=0.08). Meanwhile, microscopic histopathological evaluation revealed that the prevalence of cholesteatoma and fibrosis was lower in the experimental group.

Quantitative results

The tympanic bulla mucosa and tympanic membrane thicknesses of the experiment group and the control group were measured (Tables 1 and 2). The minimum mucosal thickness of the tympanic bulla (P=0.019), as well as the maximum and average thicknesses of the tympanic membrane were significantly less in the experimental group compared to the control group (P=0.008, P=0.011, respectively).

Table 1: Statistical analysis of groups according to the thickness of the tympanic membrane

Groups	Ears(n)	Tympanic membrane thicknesses(μm)		
		Minimum	Maximum	Mean
Experiment	11	0.3927	1.1409	0.7482
Control	11	0.5045	3.1791	2.6745

Table 2: Statistical analysis of groups according to the tympanic bulla mucosal thickness

Groups	Ears(n)	Tympanic bulla mucosa thicknesses (μm)		
		Minimum	Maximum	Mean
Experiment	12	2.2333	14.8733	12.6400
Control	12	3.6208	22.7408	19.1200

Discussion

In the recent years, Mesna is being used in surgical procedures for tissue dissection because of its chemical properties [12]. In the practice of otolaryngology, it aids in dissecting the thickness between the tympanic membrane and the middle ear mucosa in adhesive otitis media and atelectatic tympanic membranes [8, 13]. Yılmaz et al. [8] conducted a study where they administered Mesna to 42 ears of 39 patients with retraction pockets fixed to the incudostapedial joint, stapes, or promontorium who had adhesive otitis media and reported that the use of Mesna is safe and eases the surgery, increasing surgical success. In their retrospective study, Kalcioğlu et al. [13] reported that the use of Mesna increases surgical success, decreasing the need for second-look surgery. In our clinic, we usually use 20% Mesna in the surgery of adhesive otitis media. We administer Mesna from the non-retracted region of the tympanic membrane or the antrum to the middle ear cavity and usually use dental injectors to administer one dose. We wait for approximately 4-6 minutes after administration.

Different agents have been used to prevent the development of experimental cholesteatoma. Various studies report that cyclophosphamide, isotretinoin, hyaluronic acid, and mitomycin–C have no inhibiting effect on the development of cholesteatoma. Prednisolone, trans-retinoic acid, 5- fluorouracil have been reported to stop the increase of cholesteatoma [6, 14-17]. In our study, we administered Mesna to inhibit the cholesteatoma that occurred with the intratympanic injection of propylene glycol, and according to the histopathological evaluation, it succeeded.

Based on the theory of epithelial migration, propylene glycol causes cholesteatoma [4, 7]. However, the studies regarding the prevalence of cholesteatoma formation and histopathological properties report varying results. Experimental studies showed that proliferation of the epithelial basal layer of the tympanic membrane starts in the third week [18, 19]. In the sixth week, the prevalence of cholesteatoma caused by 90% propylene glycol (90%) is 87.5% [20]. In the tympanic bullae of chinchilla-type rats, a single application of 50% propylene glycol can form a cholesteatoma after three weeks [4]. The concentration of the mucosal irritant used to form experimental cholesteatoma and the duration of use are important. We used 50% propylene glycol in our study and sacrificed the rats 45 days after the first administration. The cholesteatoma prevalence of 50% in our control group was in concordance with the studies in the literature (33–90%) [5]. The cholesteatoma was mainly located at the tympanic bulla. Melo et al. [5] showed that epidermal invasion extends from the tympanic membrane to the tympanic bulla.

A study conducted on Wistar rats emphasized that single dose intratympanic Mesna prevented the formation of cholesteatoma [21]. In our study, despite the intratympanic Mesna administration five times, no significance was found, indicating that the cholesteatoma was completely prevented. However, in microscopic examination, Mesna was observed to reduce the prevalence of cholesteatoma and fibrosis.

Limitations

Ours is a single-center study which was conducted with a limited number of Wistar albino rats. Further studies with more subjects in which appropriate laboratory conditions are provided are needed to clarify the effect of Mesna on cholesteatoma. The researchers can investigate the most effective dose, or the optimal number of administrations.

Conclusion

According to the histopathological results of our study, the intratympanic administration of Mesna decreased the prevalence of cholesteatoma.

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