DOES THE INTRATYMPANIC APPLICATION OF MESNA PREVENT THE CHOLESTEATOMA? AN EXPERIMENTAL RATS STUDY

BİLAL SİZER[1], Aylin Gül[2], and Songül Karababa Demir[3]

[1]memorial diyarbakır hospital
[2]Medikal Park Hospital
[3]Mardin Public Hospital

January 16, 2021

Abstract

Purpose Studying the effect of Mesna on middle ear 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, their right ears being the control group and left ears being the experiment group. %50 propylene glycol, gentamicinsulfate and physiologic salt water were applied to the right ear and %50 propylene glycol, gentamicinsulfate 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 It was seen that cholesteatoma and fibrosis were less common in the experiment group in microscopic evaluation. A statistically significant decrease was observed when the average and maximum thicknesses of the tympanic membranes and the minimum thicknesses of the tympanic bulla of the control group and the experiment group were compared. ($p< 0.05$) Conclusion In the experimental cholesteatoma model created in rats, no statistical significance was observed, indicating that Mesna, which was applied intratympanically, completely prevented the formation of cholesteatoma. However, it was found that the prevalence of cholesteatoma formation was microscopically less in the experimental group.

DOES THE INTRATYMPANIC APPLICATION OF MESNA PREVENT THE CHOLESTEATOMA? AN EXPERIMENTAL RATS STUDY

Abstract

Purpose

Studying the effect of Mesna on middle ear 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, their right ears being the control group and left ears being the experiment group. %50 propylene glycol, gentamicinsulfate and physiologic salt water were applied to the right ear and %50 propylene glycol, gentamicinsulfate 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

It was seen that cholesteatoma and fibrosis were less common in the experiment group in microscopic evaluation. A statistically significant decrease was observed when the average and maximum thicknesses of

the tympanic membranes and the minimum thicknesses of the tympanic bulla of the control group and the experiment group were compared. ($p< 0.05$)

Conclusion

In the experimental cholesteatoma model created in rats, no statistical significance was observed, indicating that Mesna, which was applied intratympanically, completely prevented the formation of cholesteatoma. However, it was found that the prevalence of cholesteatoma formation was microscopically less in the experimental group.

Keywords: Mesna, cholesteatoma, fibrosis, otitis media, intratympanic

What's known?

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. Keratin is a protein that has disulfide bonds.

What's new?

Mesna can be used to treatment of cholesteatoma due to its mucolysis property.

Backgraund

Various studies have been published on the development of otitis media and cholesteatoma after the intratympanic application of chemicals on laboratory animals.[1-3] In the 1980's, it has been seen that the eye and ear drop called Cortisporin caused inflammatory changes and the formation of cholesteatoma in the middle ear.[4]It was shown that this effect of Cortisporin was due to the %10 propylene glycol used as a solvent. [4] In the following years propylene glycol has been used in experimental studies in the development of otitis media and cholesteatoma because of its inflammatory property for 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. Keratin is a protein that has disulfide bonds. Mesna can be used to ease the dissection of the tissue layers in the surgery of cholesteatoma due to its mucolysis property.[8] In the studies conducted, it has been reported that Mesna has no side effects or hazard on hearing subsequent to being applied to the middle ear cavity.[9-10] For this reason we planned to investigate the effect of Mesna on the 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.

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 ethical committee of our institution (document no. 2013/5).

Experimental Animals

16 healthy male Wistar albino rats weighing between 210-304 grams that were shown to have healthy outer ear canals and tympanic membrane in otoscopic examination were used in our study. All of the experimental animals were housed in appropriate cages under standard environmental conditions (room temperature 22°C-24°C, %50 relative humidity and 12 hour periods of light-dark). The animals could access water and 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 experiment group.

Propylene glycol was used to form cholesteatoma and inflammatory reaction in the middle ear mucosa. Mesna was used to inhibit the pathologic processes in the middle ear mucosa and gentamicin to inhibit the inflammatory process of 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 surgical microscope. Each ear had 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 gentamicinsulfate (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 gentamicinsulfate (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, but not sterile 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 cut into two by transverse cutting. Then they were dehydrated in baths of dereceli alcohol and tissue tracking procedure was applied, and later they were buried in paraffin. Cross sections with a thickness of 5 microns were taken. All cross sections were dyed with hematoxylin and eosin and then were examined with 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 (cholestearoma), thickness of the tympanic membrane, thickness of the tympanic bulla mucosa). The measurements of the thickness of the tympanic membrane and tympanic bulla were taken under a 10x magnifying objective.

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 abnormally distributed data Mann Whitney U tests were applied. $p < 0.05$ was considered statistically significant.

Results

The results were evaluated in two parts; as qualitative and quantitative results.

Qualitative results:

In the control group, the tympanic membrane was observed to be intact in 10 of the 16 ears, while it was not observed in 6. Inflamed cells were observed 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, and it was noted that almost all of the inflamed cells consisted of polymorphonuclear leukocytes. Cholesteatoma was seen in 8 ears. In 3 ears fibrinous-proteinosis material was observed.

In the experiment group the tympanic membrane was observed to be intact in 8 of the 16 ears, and it was not observed in 8. Cholesteatoma was seen in 7 of the ears (figure 1-2). Obvious inflammation and fibrosis

were not observed in the tympanic membrane and tympanic bulla mucosa.

No statistically significant difference between the two groups regarding the prevalence of cholesteatoma and the presence of fibrosis was observed ($p>0.05$). Meanwhile in the microscopic histopathological evaluation the prevalence of cholesteatoma and fibrosis were observed to be lower in the experiment group.

Quantitative results:

The tympanic bulla mucosa and tympanic membrane thicknesses of the experiment group and the control group were measured. These histological parameters are given in Table 1 and 2. The decrease in the minimum mucosal thickness of the tympanic bulla was evaluated as statistically significant ($p=0.019$). When the maximum and average thicknesses of the tympanic membrane were evaluated, the decrease in the experiment group was statistically significant ($p=0.008$, $p=0.011$).

Discussion

In the recent years, Mesna is being used in surgical procedures for tissue dissection because of its chemical properties.[12] It is being used in the practice of otolaryngology, especially to open the thickness between the tympanic membrane and the middle ear mucosa that occurs in the adhesive otitis media and atelectatic tympanic membranes.[8,13] Yılmaz et al conducted a study where they applied Mesna to the 42 ears of 39 patients diagnosed with retraction pockets fixed to the incudo stapedial joint, stapes or promontorium and adhesive otitis media.[8] As a result they reported that the use of Mesna is safe and eases the surgery, increasing surgical success. Kalcioğlu et al reported in a retrospective study that the use of Mesna increases surgical success, decreasing the need for second-look surgery.[13] We, in our clinic, 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. We usually use dental injectors and administer one dose. We wait approximately 4-6 minutes after administration.

When the studies in the literature are considered, it can be seen that different agents have been used to prevent the development of experimental cholesteatoma. In these studies, it has been reported that cyclophospamide, isotretinoin, hyaluronic acid and mitomicin – C have no inhibiting effect on the development of cholesteatoma, prednisolon, transretinoic acid, 5- fluorouracilin have been reported to stop the increase of cholesteatoma.[6,14-18] In our study we administered Mesna to provide the inhibition of the development cholesteatoma that occurred with the intratympanic injection of propylene glycol. According to the histopathological evaluation we observed that Mesna decreases the development of cholesteatoma.

According to the theory of epithelial migration, propylene glycol causes the formation of cholesteatoma.[4,17] However there is diversity amongs the studies regarding the prevalence of cholesteatoma formation and histopathological properties. In experimental studies it has been shown that proliferation of the epithelial basal layer of the tympanic membrane starts in the third week.[19,20] In the sixth week, the prevalence of the cholesteatoma caused by %90 propylene glycol (%90) is %87.5.[21] It has been shown in the tympanic bullas of the chinchilla type rats that a single application of %50 propylene glycol (%50) can form cholesteatoma after three weeks.[4] According to the information in the literature, the concentration of the mucosal irritant used to form experimental cholesteatoma and the duration of use are important. Therefore diversity is observed between the studies. We used %50 propylene glycol in our study and sacrificed the rats 45 days after the first administration. The cholesteatoma prevalence seen in the control group of our study, %50, was evaluated to be in concordance with the studies in the literature (%33–90).[5]

In our animal model study the decreases of the tympanic membrane thickness and the tympanic bulla mucosal thickness seen in the histopathological images between the control group and the experiment group were evaluated to be statistically significant. It was observed in the histopathological images that the cholesteatoma were mainly located at the tympanic bulla. Melo et al showed in their experimental study that epidermal invasion occurred from the tympanic membrane to the tympanic bulla in the control group and study group.[5]

In a study conducted on Winstar rats in which the effect of intratympanic single dose Mesna on cholestatoma

was investigated, it was emphasized that single dose intratympanic Mesna prevented the formation of cholestatoma.[22] In our study, despite the intratympanic Mesna application applied for 4 times, no statistical significance was found, indicating that the formation of cholestatoma was completely prevented. However, in microscopic examination, it was determined that Mesna reduced the occurrence prevalence of cholesteatoma and fibrosis.

Conclusion

According to the histopahtological results of our study on the experimental cholesteatoma model created on rats, the intratympanic administration of Mesna had a decreasing effect on the prevalence of cholesteatoma.

Acknowledgments: We thank Dr. Pınar Ergen for performing histopathological examination.

Conflicts of Interest: The authors declare no conflict of interest

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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

	ears(n)	**tympanic membrane thicknesses (μμ)** minimum	**tympanic membrane thicknesses (μμ)** maximum	**tympanic m** mean
Exp	11	0.3927	1.1409	0.7482
Ctrl	11	0.5045	3.1791	2.6745

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

	ears (n)	τψμπανις βυλλα μυϛοσα τηιϛκνεσσες (μμ) minimum	τψμπανις βυλλα μυϛοσα τηιϛκνεσσες (μμ) maximum	τψμπανις βυλλα μυϛοσα τηιϛκνεσσες (μμ) mean
Exp	12	2.2333	14.8733	12.6400
Ctrl	12	3.6208	22.7408	19.1200

References

1. Rüedi L. Cholesteatoma formation in the middle ear in animal experiments. Acta Otolaryngol 1959;50(3-6):233-42.
2. Hueb MM, Goycoolea MY, Muchow D, Duvall AJ, Paparella MM, Sheridan C. In search of missing links in otology. III. Development of a new animal model for cholesteatoma. Laryngoscope 1993;103:774-784
3. Piltcher OB, Swarts JD, Magnuson K, Alper CM, Doyle WJ, Hebda PA. A rat model of otitis media with effusion caused by eustachian tube obstruction with and without Streptococcus pneumoniae infection: methods and disease course. Otolaryngol Head Neck Surg 2002;126(5):490-8.
4. Masaki M, Wright CG, Lee DH, Meyerhoff WL. Effects of Otic Drops on Chinchilla Tympanic Membrane. Arch Otolaryngol Head Neck Surg 1988;114:1007-1011
5. Melo AA, Caldas Neto SS, Leão FS, Campos AJ. Effect of intratympanic mitomycin C on the development of cholesteatoma and otitis media in rats. J Laryngol Otol 2013;127: 359-363
6. Sennaroglu L, Ozkul A, Gedikoglu G, Turan E. Effect of intratympanic steroid application on the development of experimental cholesteatoma.Laryngoscope 1998;108:543-7
7. Antunes ML, Fukuda Y, Penido Nde O, Ferreira R. Effect of trans-retinoic acid in the inhibition of cholesteatoma in guinea pigs. Rev Bras Otorrinolaringol 2008;74(1):53-60
8. Yilmaz M, Goksu N, Bayramoglu I, Bayazit YA. Practical use of MESNA in atelectatic ears and

adhesive otitis media. ORL J Otorhinolaryngol Relat Spec 2006; 68:195-8.
9. Vincenti V, Mondain M, Pasanisi E, Piazza F, Puel JL, Bacciu S, et al. Cochlear effects of MESNA application into the middle ear. Ann N Y Acad Sci 1999;884:425-432
10. Van MP, Timmermans JP, Claes J, Scheuermann DW, Wuyts FL, Van de Heyning PH. Single ototopical application of MESNA has no ototoxic effects on guinea pig cochlear hair cells: a morphological study. Acta Otolaryngol 1999;119:685-9
11. Institute of Laboratory Animal Research. Commission on Life Sciences. National Research Council. The guide for the care and use of laboratory animals. 7th ed. Washington DC: National Academies Press; 1996
12. Casale M, Di Martino A, Salvinelli F, Trombetta M, Denaro V. Mesna for chemically assisted tissue dissection. Expert Opin Investig Drugs 2010;19: 699-707
13. Kalcioglu MT, Cicek MT, Bayindir T, Ozdamar OI. Effectiveness of mesna on the success of cholesteatoma surgery. Am J Otolaryngol Head Neck Surg 2014;35: 357-361
14. Pownell PH, Wright CG, Robinson KS, Meyerhoff WL. The effect of cyclophosphamide on development of experimental cholesteatoma. Arch Otolaryngol Head Neck Surg 1994;120:1114-1116
15. Jove MA, Vassalli L, Raslan W, Applebaum EL. The effect of isotretinoin on propylene glycol-induced cholesteatoma in chinchilla middle ears. Am J Otolaryngol1990;11: 5-9
16. White SJ, Wright CG, Robinson KS, Meyerhoff WL. Effect of topical hyaluronic acid on experimental cholesteatoma. Am J Otolaryngol 1995;16: 312-8
17. Antunes ML, Fukuda Y, Penido Nde O, Ferreira R. Effect of transretinoic acid in the inhibition of cholesteatoma in guinea pigs. Braz J Otorhinolaryngol 2008;74: 53-60
18. Wright CG, Bird LL, Meyerhoff WL. Effect of 5-fluorouracil in cholesteatoma development in an animal model. Am J Otolaryngol 1991; 12:133-138
19. Masaki M, Wright CG, Lee DH, Meyerhoff WL. Experimental cholesteatoma: epidermal ingrowth through tympanic membrane following middle ear applications of propylene glycol. Acta Otolaryngol (Stockh)1989;108:113-121
20. Huang CC, Shi GS, Yi ZX. Experimental Induction of middle ear cholesteatoma in rats. Am J Otolaryngol 1988; 9: 165-172
21. Vassalli L, Harris DM, Gradini R, Applebaum EL. Propylene Glycol-Induced Cholesteatoma in Chinchilla Middle Ears. Am J Otolaryngol 1988; 9: 180-188
22. Ismi O, Karabulut YY, Bal KK, Vayisoglu Y, Unal M. Single dose intratympanic mesna application inhibits propylene glycol induced cholesteatoma formation. J Laryngol Otol 2017;131(3):215.