Turkish Archives of Otorhinolaryngology
Türk Otorinolarengoloji Arşivi

Turk Arch Otorhinolaryngol 2017; 55: 3-9

Effect of Topical Dexamethasone for Preventing Experimentally Induced Myringosclerosis

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Original Investigation

Abstract

Objective: We aimed to examine the effect of topical dexamethasone by otomicroscopic and histologic examinations for preventing myringosclerosis induced by myringotomy in rat tympanic membranes.

Methods: Twenty-one Sprague Dawley rats (42 ears) were randomly divided into the following three groups after otomicroscopic examinations: experimental surgical group (5 rats), control group (8 rats), and study group (8 rats). The rats of all the groups underwent myringotomy in both tympanic membranes. Other than myringotomy, no additional procedure was performed for the rats in the experimental surgical group. In the control group, 0.9% NaCl was applied to the ears, whereas in the study groups, topical dexamethasone was applied to the ears. These applications in the control and study groups were repeated for nine days. On the 10th day of the study, the rat ears of all groups underwent otomicroscopic and histologic examinations. The prevalence and process of myringosclerosis were evaluated by otomicroscopic examination, whereas inflammation, membrane thickness, and myringosclerosis intensity were evaluated by histologic examination.

Results: The growth of myringosclerosis with otomicroscopic examination was lesser in the study group in which topical dexamethasone was applied than the control and the experimental surgical groups. Moreover, it was observed that myringosclerosis effected fewer quadrants in the study group.

Histologic examinations revealed that inflammation was significantly lesser in the study group than in the experimental surgical and control groups. The average membrane thickness values were significantly lesser in the study group than in the experimental surgical group. With respect to myringosclerosis growth, no statistically significant difference was observed among all groups, whereas with respect to myringosclerosis intensity, the rat ears in the study group were less severely affected.

Conclusion: Thus, our study results suggest that applying topical dexamethasone after myringotomy has positive effects on limiting the intensity and prevalence of myringosclerosis.

Keywords: Myringotomy, dexamethasone, myringosclerosis, animal model

Introduction

Tympanosclerosis is the occurrence of ossification in the tympanic membrane, middle ear cavity, ossicular chain, and rarely in the mastoid bone, resulting from hyaline changes at submucosal level (1). The effect of tympanosclerosis on the tympanic membrane is called myringosclerosis (2, 3). Depending on the frequency of ventilation tube (VT) insertion due to otitis media with effusion (OME) in children, the incidence of myringosclerosis is high in pediatric population. The incidence of myringosclerosis in children who underwent VT insertion is 28%-61% (3, 4).

The exact pathogenesis of tympanosclerosis is still unknown. It is considered a special scar tissue or healed inflammation after recurrent otitis media (ROM). The second speculation is that it is the direct effect of hydrolytic enzymes in serous fluid due to lamina propria (2, 5-7). Another etiological factor is trauma. Any trauma, ranging from excessive tension to the fibers in the eardrum as the simplest form to tympanic membrane perforation as the heaviest form, can contribute to the development of myringosclerosis (8-10). After myringotomy and VT insertions, there may be intraepithelial bleeding that heals with fibrosis (11, 12). Studies indicate that bleeding into the ear canal may be associated with the development of myringosclerosis in the long term. According to these studies, minimizing the bleeding during myringotomy can reduce the development of myringosclerosis in the long term (13). In our study, we aimed to perform an automicroscopic and a histological examination to assess the effectiveness of topical dexametha-

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Received Date: 15.11.2016
Accepted Date: 27.01.2017

DOI: 10.5152/tao.2017.2077

sone in the prevention of myringosclerosis induced by experimental myringotomy of rat tympanic membranes.

Methods

For this study, 21 healthy male Sprague Dawley rats, aged 3-4 months, weighing 300-400 g were used. The rats were housed at a temperature of 16-21°C and 50% humidity and given pellet feed. Our study was conducted in compliance with the principles of the Declaration of Helsinki and it was approved by the local ethics committee for animal experiments (TÜHDEYEK-2009/006 protocol number).

Myringotomy was performed on rats under anesthesia with ketamine. Ketamine hydrochloride (40 mg/kg; Ketamine ampoule, Pfizer, İstanbul, Turkey) and xylazine hydrochloride (5 mg/kg; Rhompun ampoule, Bayer, İstanbul, Turkey) were intramuscularly administered. A total of 42 ears of 21 rats were otomicroscopically examined under anesthesia, and debris or plugs in the external auditory canal were removed. Normal tympanic membrane images were detected in all rats.

After otomicroscopic examinations, rats were divided into three groups as experimental surgery, control, and study groups. The experimental surgery group consisted of five rats, and the control and study groups of eight rats each. In all groups, two standard myringotomies, including anterior and posterior quadrants of ear membranes of both sides, were performed by attaching a dental needle to the end of an insulin injector piston using an ear speculum under an otomicroscope.

Experimental surgery group: No medication was administered to 10 ears of the five rats after myringotomy.

Control group: Following myringotomy, approximately 0.3 mL of 0.9% NaCl was instilled in 16 ears of the eight rats.

Study group: Following myringotomy, approximately 0.3 mL of (8 mg/2 mL) dexamethasone was instilled in 16 ears of the eight rats [dexamethasone 21-phosphate (Decort ampoule; Deva, İstanbul, Turkey)].

In the control and study group rats, NaCl and dexamethasone, respectively, were instilled with two more drops to the same ears without ketamine anesthesia by keeping the rats still with the help of an assistant on the second day of the study; this application was continued for 9 days.

Two rats from the control group and two rats from the study group were lost between the days 5 and 8. However, there was no animal loss in the experimental surgery group.

On day 10 of the study, the tympanic membranes of 17 rats (34 ears) in total, including five rats (10 ears) in the experimental surgery group, six rats (12 ears) in the control group, and six rats (12 ears) in the study group, were examined through otomicroscopy under anesthesia with ketamine.

The appearance of tympanic membranes and development of myringosclerosis were evaluated according to the distribution on four quadrants. Then, the rats were sacrificed with high-dose ketamine hydrochloride and xylazine hydrochloride injection. Following this, temporal bone dissection was conducted for future histological examinations.

Temporal bone dissection

Temporal bone dissection was performed with a technique providing rapid access to tympanic bulla and tympanic membranes. A horizontal incision was made in the occipital area and the skin was elevated forward. Temporalis muscle and periosteum on both sides were first elevated over the parietal bone, then over the temporal squama. By retracting the auricle to the lateral side, the cartilaginous external ear canal was separated from the tympanic ring with a sharp dissection. Continuing the elevation of the muscle tissues in the subperiosteal plane, the mastoid and tympanic parts (bulla) of the temporal bone were revealed. At the next stage, the mastoid fragment was dissected from the occipital bone, the squamous part was dissected from the parietal, frontal, palatine, and ethmoid bones; bulla was dissected from the occipital and sphenoid bones; the petrous part was dissected from the sphenoid bone. The temporal bone was also dissected from the surrounding muscle tissue and separated from the skull. After this stage, the tissues to be histologically examined were placed in 10% formaldehyde solution and left in the solution for 3 days. Thus, tympanic membranes were made visible from the sides of the middle and external ear. During the temporal bone dissection and removal of tympanic membranes, two tympanic membranes each from all three groups were excluded from the study because they were damaged. Eight tympanic membranes from the experimental surgery group, 10 from the control group, and 10 from the study group were thus included in the study for histological examination.

Histological evaluation

Four-micron slices from paraffin blocks were obtained in parallel with manubrium mallei according to the routine paraffin-processing protocol; after deparaffinization and rehydration, they were stained with hematoxylin and eosin stain, and the slices were assessed using the Zeiss Axioplan 2 imaging light microscope.

On histological examination, the tympanic membranes of the ears in all groups were assessed in terms of the presence/absence of inflammation, membrane thickness, and presence/absence of myringosclerosis. Besides, in the membranes in which myringosclerosis was detected, it was classified as mild, moderate, and severe myringosclerosis by the same pathologist according to the intensity of fibroblastic proliferation in the lamina propria and degree of sclerosis. In myringosclerosis rating, the classification of Mattsson et al. (14) was used and it was rated as light [MS (+)] if several single lesions existed in the lamina propria, moderate [MS (++)] if the lesions were single but tended to merge, and severe [MS (+++)] for large lesions.

The otomicroscopic examinations and procedures, temporal bone dissection, and histological assessments were conducted by experienced otorhinolaryngologists and pathologists who have been in the field for at least 15 years.

Statistical analysis was conducted using the Minitab package program (S0064 Minitab Release 13, license number: wep 1331, 00197, Coventry, UK). After searching for normal distribution consistency, Wilcoxon test was used for intra-group comparisons of non-parametric test values, and Mann-Whitney U test was used in the comparison of he groups in pairs. Comparison of more than two groups was made using Kruskal-Wallis test. Statistical significance level was accepted as $p<0.05$ with 95% confidence interval.

Figure 1 shows a tympanic membrane image of a rat belonging to the study group and considered to be within normal limits.

Results

Otomicroscopic findings

The otomicroscopic assessment of 17 rats (34 ears) in the three groups on day 10 of our study was performed in terms of the closure of the myringotomy sites, development of myringosclerosis, and the distribution of the myringosclerosis according to the quadrants.

Closure of the myringotomy sites

When closure of the myringotomy sites were examined, tympanic membranes in 11 (91.6%) of 12 ears in the study group (myringotomy+dexamethasone), 10 (83.3%) of 12 ears in the control group (myringotomy+0.9% NaCl), and 9 (90%) of 10 ears in the experimental surgery group (only myringotomy) were intact. No statistically significant difference was detected between the groups in terms of closure of myringotomy sites on day 10 ($p=0.595$).

Development of myringosclerosis

When the ears (34 ears) in all groups were assessed for the presence of myringosclerosis under otomicroscopy, development of myringosclerosis was detected in tympanic membranes in 7 (58.3%) of 12 ears in the study group, 11 (91.7%) of 12 ears in the control group, and all (100%) of the ears in the experimental surgery group. Statistical comparison of groups according to the development of otomicroscopic myringosclerosis is shown in Table 1.

Distribution of myringosclerosis according to the quadrants

When the distribution of myringosclerosis was examined under otomicroscopy, myringosclerosis was found to be less developed in the tympanic membranes of rats in the study group than those in the other two groups (Table 2). Under otomicroscopic assessment, development and distribution of myringosclerosis were not limited to the areas where myringotomy was performed and were mainly concentrated around the malleus and in the anterior tympanic membrane. Paired inter-group statistical comparison of the distribution of myringosclerosis according to the quadrants is shown in Table 3.

Histological findings

For histological examination, inflammation, membrane thickness, and myringosclerosis development were detected in a total of 28 tympanic membranes in all groups on day 10.

Inflammation

When the tympanic membranes of rats in all groups were histologically assessed, the presence of inflammation was detected in eight of the 10 ears in the study group (80%), all 10 ears (100%) in the control group, and in all eight ears (100%) in the experimental surgery group. Statistical comparison of inflammation findings in histologically paired groups is shown in Table 4.

Myringosclerotic plaques and intense inflammatory cells (foamy histiocytes and leukocytes) in the tympanic membrane of a rat from the experimental surgery group are shown in Figure 2. The common myringosclerotic plaques detected in the lamina propria of the tympanic membrane of a rat from the control group and especially the inflamed areas more intensely located on the mucosal surface of the tympanic membrane are shown in Figure 3. The accumulation of inflammatory cells (leukocytes and foamy histiocytes) on the mucosal surface of the tympanic membrane of a rat from the study group is shown in Figure 4.

Table 1. Statistical comparison among the groups according to the development of otomicroscopic myringosclerosis

Paired groups	p*
Experimental surgery group - Control Group	0.403
Control group - Study group	0.038
Study group - Experimental surgery group	0.021

*Mann-Whitney U test

Table 3. Statistical comparison of myringosclerosis distribution according to the quadrants in paired groups

Paired groups	p*
Experimental surgery group - Control Group	0.520
Control group - Study group	0.102
Study group - Experimental surgery group	0.044

*Mann-Whitney U test

Table 2. Distribution of myringosclerosis findings according to the quadrants under otomicroscopic assessment

	Distribution of myringosclerosis according to the quadrants				
Groups	**Not existing n: number of ears (%)**	**Quadrant 1 (+) n: number of ears (%)**	**Quadrant 2 (+) n: number of ears (%)**	**Quadrant 3 (+) n: number of ears (%)**	**Quadrant 4 (+) n: number of ears (%)**
Experimental surgery group (n=10 ear)	0 (0)	2 (20)	3 (30)	2 (20)	3 (30)
Control group (n=12 ear)	1 (8.3)	2 (16.6)	5 (41.6)	3 (25)	1 (8.3)
Study group (n=12 ear)	5 (41.6)	3 (25)	2 (16.6)	1 (8.3)	1 (8.3)

Tympanic membrane thickness

When all tympanic membrane thicknesses included in the histological study were assessed, the average thickness in the study group was found to be less than that in the experimental surgery and control groups. When the groups were compared in terms of membrane thickness, there were no statistical significant differences between the experimental surgery and control groups and between the study and control groups (p=0.205 and p=0.093). It was detected that there was a statistically significant difference between the study and experimental surgery groups (p=0.039) (Table 4).

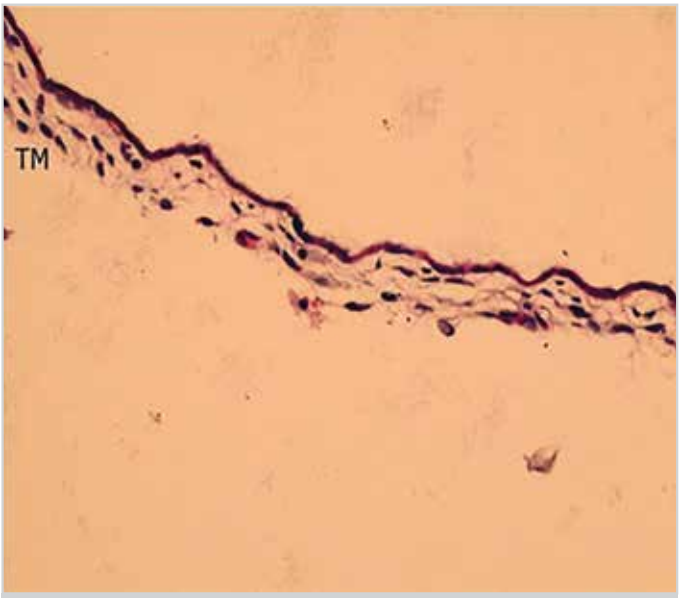

Figure 1. An image of a normal rat tympanic membrane from the study group (H&E ×200)
TM: tympanic membrane

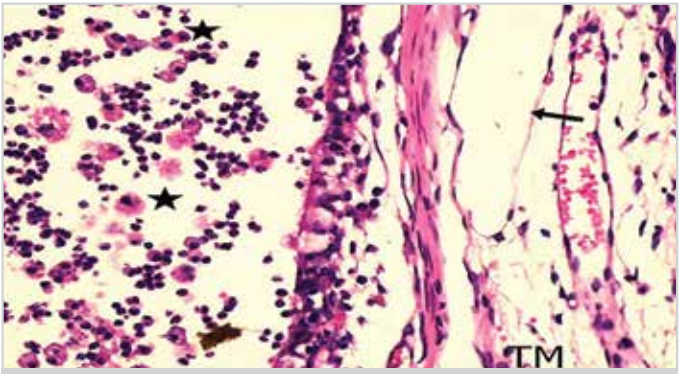

Figure 2. An image of a rat tympanic membrane from the experimental surgery group (H&E ×200)
↓: Myringosclerotic plaques; ★: inflammatory areas; TM: tympanic membrane

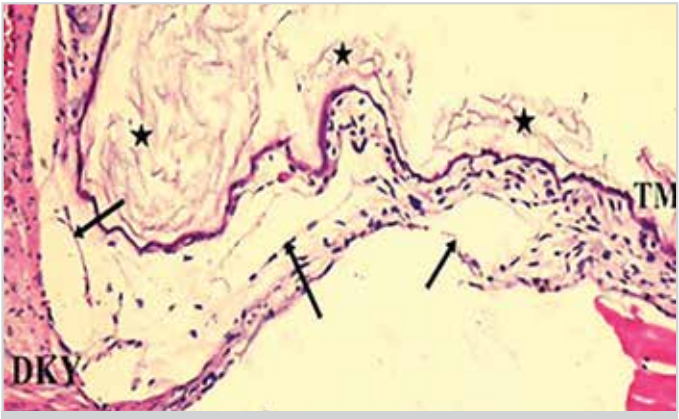

Figure 3. An image of a rat tympanic membrane from the control group (H&E ×200)
↓: Myringosclerotic plaques; ★: inflammatory areas; TM: tympanic membrane; EAC: external auditory canal

In the perforation and near the perforation area, the increase in membrane thickness and inflamed areas of a tympanic membrane of a rat from the experimental surgery group are shown in Figure 5. The increase in thickness of the tympanic membrane of a rat from the control group and the measurement method are shown in Figure 5.

Myringosclerosis

When the tympanic membranes of rats in all groups were histologically assessed in terms of the development of myringosclerosis, myringosclerosis was detected in eight (80%) of 10 ears in the study group, nine (90%) of 10 ears in the control group, and all 8 (100%) ears in the experimental surgery group (Table 5).

The histological assessment of myringosclerosis development on day 10 showed no significant difference among the study, experimental surgery, and control groups (p=0.005). The severity of

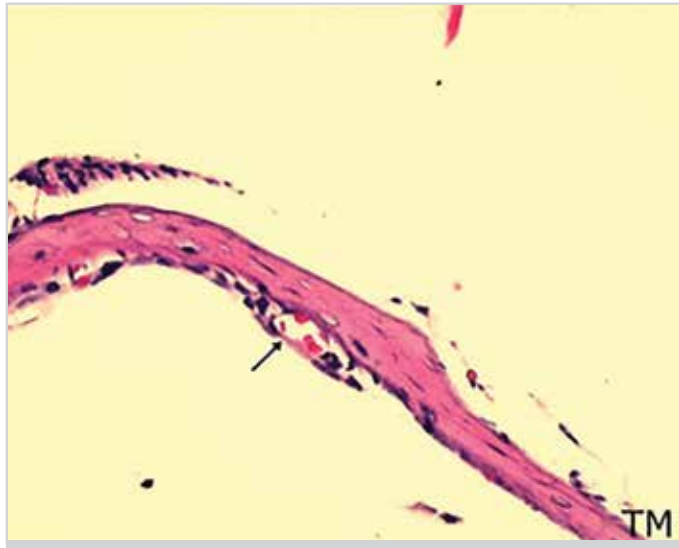

Figure 4. An image of a rat tympanic membrane from the study group
↓: inflammatory areas; TM: tympanic membrane

Table 4. Statistical comparison of inflammatory finding and membrane thickness in histologically paired groups

Paired groups	p* inflammatory finding	p* membrane thickness
Experimental surgery group - Control Group	1.0	0.205
Control group - Study group	0.048	0.093
Study group - Experimental surgery group	0.025	0.039

*Mann-Whitney U test

Table 5. Findings of myringosclerosis based on histological assessment

	Distribution according to the severity of myringosclerosis			
Groups	**MS (Not existing) n: number of ears (%)**	**MS (+) n: number of ears (%)**	**MS (++) n: number of ears (%)**	**MS (+++) n: number of ears (%)**
Experimental surgery group (n=8 ears)	0 (0)	1 (12.5)	3 (37.5)	4 (50)
Control group (n=10 ears)	1 (10)	2 (20)	4 (40)	3 (30)
Study group (n=10 ears)	2 (20)	5 (50)	3 (30)	0 (0)

MS: myringosclerosis; MS (+): mild myringosclerosis; MS (++): moderate myringosclerosis; MS (+++): severe myringosclerosis

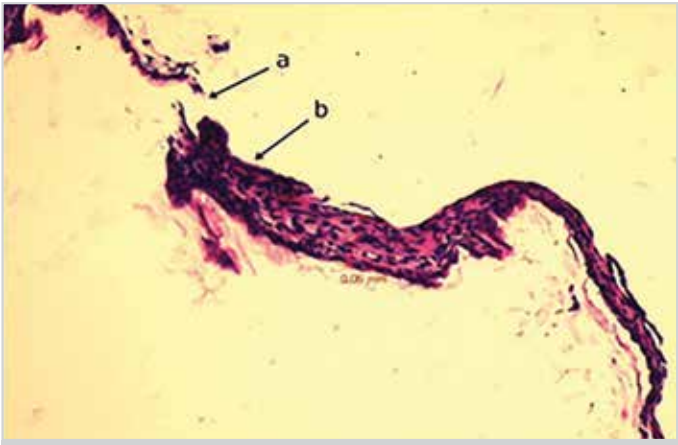

Figure 5. An image of a rat tympanic membrane from the experimental surgery group
a: perforation area; b: Inflamed and thickened areas

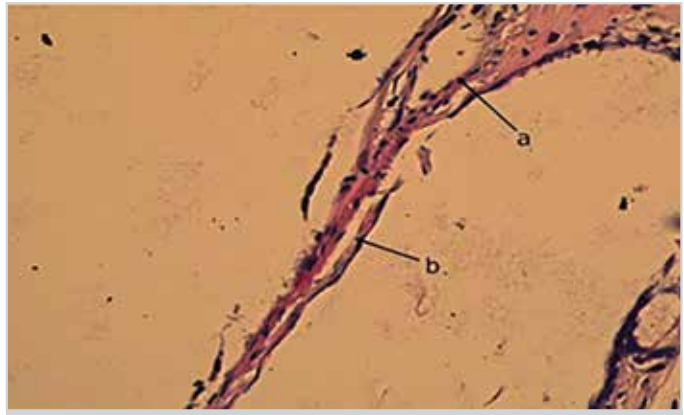

Figure 7. An image of a rat tympanic membrane from the experimental surgery group (H&E ×200)
↓: myringosclerotic plaques; a: MS (++) moderate myringosclerosis, b: MS (+) mild myringosclerosis; MS: myringosclerosis

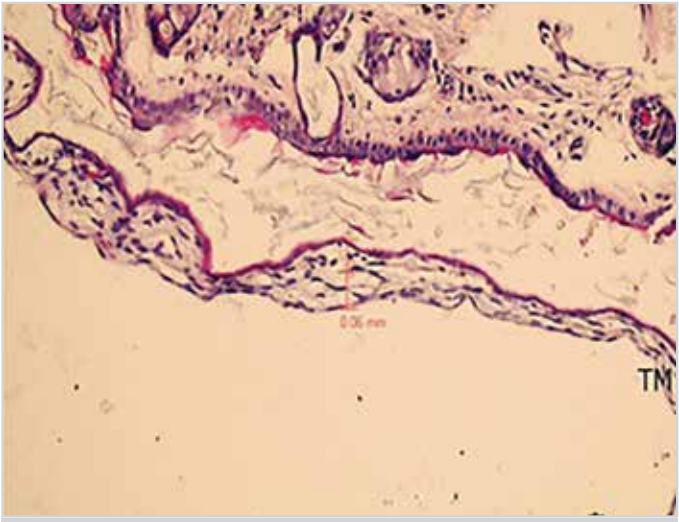

Figure 6. An image of a rat tympanic membrane from the control group (H&E ×200). Increase in membrane thickness is shown
TM: tympanic membrane

myringosclerosis was statistically low in the study group according to comparisons between the study and experimental surgery groups and between the study and control groups (p=0.005). On the other hand, there was no difference in myringosclerosis severity between the experimental surgery and control groups (p=0.290) (Table 6).

Table 6. Statistical comparison according to the severity of myringosclerosis in paired groups

Paired groups	**p***
Experimental surgery group - Control group	0.290
Control group - Study group	0.005
Study group - Experimental surgery group	0.005

*Mann-Whitney U test

Myringosclerotic plaques in the lamina propria in 1-2 areas on the tympanic membrane image of a rat from the experimental surgery group are shown (Figure 7). In the figure, the development of myringosclerosis is remarkable with the severity of MS (++) in the area shown with "a" and with the severity of MS (+) in the area shown with "b."

Discussion

The effect of tympanosclerosis only on the tympanic membrane is called myringosclerosis. Myringosclerosis is a common sequela of OME, ROM, chronic otitis media, and VT insertion (15, 16). Although many hypotheses on its origin were suggested, there is no precise information on its etiology and pathogenesis. However, there are factors that are blamed for tissue trauma, such as intratympanic hemorrhage, hyperoxygenation, foreign body reaction to VT, and autoimmune etiology (15, 16).

Myringosclerosis, a rare and important complication, is a rapidly developing process. Mattsson et al. (7) reported that sclerotic changes in the tympanic membrane developed within 9 hours of myringotomy and showed that a severe inflammatory response histologically occurred 12-24 hours after myringotomy in pars flaccida. Tympanosclerosis, which is histologically seen in 80% of cases with myringotomy, can only be seen in 40% of cases with otomicroscopy. This shows that, in fact, there is twice as much tympanosclerosis (at tissue level) in cases in which tympanosclerosis is detected by otomicroscopy.

In our study, we selected the trauma model (myringotomy) to generate myringosclerosis in light of the above-mentioned literature on myringosclerosis and tympanosclerosis, and we sacrificed the rats on day 10 to assess the clinical/ histological findings.

Although the etiology and pathogenesis of myringosclerosis are still unknown, studies show that this disease develops in three stages: the first phase, which is supposed to be reversible and characterized by collagen fiber damage caused by inflammatory processes; the repair phase, which is characterized by fibroblastic invasion, and the final and late phase, which is the irreversible phase and characterized by calcifications (15).

In general, studies related to myringosclerosis/tympanosclerosis were conducted to investigate the etiology of this disease, to assess myringosclerosis of the tympanic membrane based on histological and otomicroscopic findings, and investigate the protectiveness of therapeutic agents.

In our study, we planned to conduct both otomicroscopic and histological investigations on the possible preventive effect of topical dexamethasone on the development of myringosclerosis, considering that it will have an effect on the first phase defined in the pathogenesis of rat ears in which experimental myringosclerosis was formed.

In the literature, preventive treatments, especially with antioxidants, are commonly found in experimental myringosclerosis model studies. In the studies on myringotomized rats with different oxygen concentrations, fewer sclerotic lesions developed in animals living in a room environment than in rats subjected to myringotomy in a hyperoxic environment.

Mattsson et al. (14) reported that topically applied antioxidants such as copper, zinc, superoxide dismutase, catalase, and desferoxamine prevent or reduce the development of sclerotic lesions. In an experimental study, Spratley et al. (19) showed, under otomicroscopy, that the topical use of ascorbic acid, which is an antioxidant agent, prevents myringosclerosis in the perforated tympanic membranes of rats.

Özcan et al. (15) used N-acetyl cysteine in investigating antioxidant effects in tympanosclerosis and concluded that the extra use of topical N-acetyl cysteine on the ear in which VT was inserted can be useful in preventing the development of myringosclerosis. Ovesen et al. (20) suggested that N-acetyl cysteine reduces fibroblast proliferation and collagen release in fibroblast cultures. Furthermore, they indicated that N-acetyl cysteine reduces the thickness of the connective tissue layer in the middle ear.

Kazıkdaş et al. (21) observed in a study that myringosclerotic plaques were less common in subjects receiving alpha-tocopherol for the prevention of experimentally induced myringosclerosis.

When the otomicroscopic findings of our study were examined, the development of myringosclerosis was significantly higher in the control and experimental surgery groups than in the study group treated with topical dexamethasone. In addition, fewer quadrants in the tympanic membranes of rats in the study group were affected by myringosclerosis.

Polat et al. (22) concluded that inflammatory reactive oxygen species increased due to the immunological stimulation in the formation of tympanosclerosis after myringotomy and vitamin E reduced this effect. Selçuk et al. (23) emphasized that the application of topical calcium channel blocker in guinea pigs on experimental myringosclerosis, which they created with myringotomy and *Streptococcus pneumoniae* type 3 inoculation, helped prevent tympanosclerosis.

When the histological findings of our study were examined, inflammation of the rat tympanic membranes of the study group treated with topical dexamethasone was significantly less than that of the experimental surgery and control groups. The average tympanic membrane thickness of the study group is less than that of the other two groups. In addition, the fact that the formation and severity of myringosclerosis were low in the study group was remarkable.

In a study investigating the effect of dexamethasone+ciprofloxacin on the closure of myringotomy sites after experimentally generated OME, there were no statistically significant inflammations the in dexamethasone+ciprofloxacin group, but there was inflammation in 71.4% of cases in the control group. These results led to the conclusion that dexamethasone+ciprofloxacin treatment after myringotomy could remove the effects of early inflammation (24).

If corticosteroids are considered to eliminate the early histological effects of inflammation, such as capillary dilatation, vascular wall fibrin deposition, serodiapedesis, and local edema, the risk of tympanosclerosis caused by the corticosteroid treatment after myringotomy in OME is reduced. Besides, corticosteroids inhibit fibrosis, proliferation of capillaries, collagen accumulation, and scarification, which are late histological signs of inflammation, but long-term studies are needed for the beneficial effects of corticosteroids on tympanosclerosis.

Conclusion

In light of our study's otomicroscopic and histological findings, it can be suggested that topical dexamethasone administration may have a positive effect in preventing myringosclerosis formation. This effect may be related to the anti-inflammatory and immunosuppressive effects of dexamethasone.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Animal Experiments Local Ethics Committee (TÜHDEYEK-2009/006).

Informed Consent: Not required in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.Ü., M.K., R.Y., A.T., E.B.; Design - M.Ü., M.K., R.Y., A.T., E.B.; Supervision - M.Ü., M.K., A.T., Ş.A.; Resource - M.K., R.Y., Ş.A., E.B.; Materials - M.Ü., M.K.; Data Collection and/or Processing - E.B., M.K., R.Y.; Analysis and/or Interpretation - M.Ü., M.K., A.T., Ş.A.; Literature Search - M.Ü., M.K., R.Y., A.T.; Writing - M.K., A.T., Ş.A.; Critical Reviews - M.K., A.T., Ş.A.,R.Y.

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

Financial Disclosure: The authors declared that this study has received no financial support.

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