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Original Article

The Effects of Topical Burow's and Castellani's Solutions on the Middle Ear Mucosa of Rats

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OBJECTIVE: The aim of this study is to investigate the early histopathologic effects of Burow's and Castellani's solutions on the middle ear mucosa of rats.

MATERIALS and METHODS: The study was conducted with 26 Wistar albino female rats. Gelfoam that was soaked in 4% Burow's solution was inserted into the middle ears of the rats in the Burow group (n=10); over 2 weeks, 0.1 mL Burow's solution was administered once a day through perforation into the middle ear. The same procedure was applied to the rats in the Castellani group (n=10) using classical Castellani's solution and to the rats in the control group (n=6) using physiological saline solution. At day 1 after the last administration, all groups were decapitated; their bullas were dissected. The bullas were histopathologically evaluated and graded with respect to increase in leukocytes with polymorphic nuclei, mononuclear cell infiltration, and fibrosis. The data obtained were statistically analyzed.

RESULTS: In the Burow group, the fibrosis scores were significantly higher than those in the control group (p=0.039), the scores of leukocytes with polymorphic nuclei were significantly higher than those in the control group (p=0.034), and the total scores were significantly higher than those in the control group (p=0.022).

CONCLUSION: We suggest Castellani's solution as a safe alternative in the treatment of otomycosis and external otitis in the presence of tympanic membrane perforation. However, because of the inflammatory changes it causes in the middle ear mucosa, we do not recommend the use of Burow's solution in the presence of tympanic membrane perforation.

KEYWORDS: Burow, Castellani, middle ear mucosa, rat, solution, topical drop

INTRODUCTION

The effectiveness and adverse effects of the magistral ear drops commonly used in the treatment of various external and middle ear diseases is a controversial topic of otorhinolaryngology. Burow's and Castellani's solutions, which are prescription medicines prepared by pharmacists, have long been used to control bacterial and fungal infections of the external or middle ear.

Burow's solution was developed by Karl August von Burow, a German doctor, as an ear drop [1]. The solution is a colorless and acidic mixture containing aluminum acetate and can be prepared at different densities [2]. In studies conducted using Burow's solution, 4%, 8%, and 13% concentrations are used frequently [3-5]. In our country, a ready-to-use preparation called Burow Galenik containing 4% aluminum acetate is available. In addition to the systemic toxic effects of aluminum acetate, its ototoxic effects have been reported in the literature [2, 3, 6-8].

Castellani's solution was developed in 1905 by Aldo Castellano, an Italian doctor [9]. Classical Castellani's solution contains 0.08 g boric acid, 0.4 g phenol, 0.04 g fuchsine, 0.8 g resorcinol, 0.4 mL acetone, 0.85 ml alcohol, and 10 ml distilled water [10]. Various modifications to the classical solution have been recommended [10-12]. Fuchsine has antifungal activity, and ethanol has antibacterial activity, whereas acetone makes the solution acidic. Because of the toxicity of phenol, acetone, and resorcinol, only topical administration is recommended [11, 13, 14].

The toxic effects on the inner ear of these solutions, which are effective against resistant bacteria and fungi and are also inexpensive and easy to apply, have been presented in various studies [2, 3, 7, 8]. However, no studies exist on the effects of applying the solutions directly on the middle ear mucosa [2].

Burow's and Castellani's solutions are frequently administered in otorhinolaryngology practices. However, in the literature, there is a limited number of studies about the effects of these agents on the middle ear mucosa. Our purpose with this study is to investigate

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the early histopathologic effects of Burow's and Castellani's solutions on the middle ear mucosa.

MATERIALS and METHODS

Animals

The study was conducted on 26 Wistar albino female rats aged 8–10 months and weighing up to 300–350 g; these rats were fed with standard pellet feed, could easily access water, and were maintained at room temperature (20±2°C) for 12 h of daylight/darkness. The rats were arbitrarily divided into three groups. There were 10 rats in the first and second groups each and 6 in the third group.

Inclusion and Exclusion Criteria

Healthy adult female rats aged 8–10 months without ear discharge or eruption were included in the study. In the event of post-procedural discharge or bleeding of the ear, anorexia, innutrition, or weight loss, the sick rat was excluded.

Burow's and Castellani's Solutions

4% Burow's solution (Burow's Solution; Galenik Pharmacy and Chemicals Warehouse, İzmir, Turkey) and classical Castellani's solution containing 0.08 g boric acid, 0.4 g phenol, 0.04 g fuchsine, 0.8 g resorcinol, 0.4 ml acetone, 0.85 mL alcohol, and 10 mL distilled water, prepared by Adnan Menderes University, Faculty of Medicine, Pharmacology Department, were used.

Anesthesia

In the first day of the study, general anesthesia was applied to all rats for the tympanic membrane perforation and before decapitation. As the general anesthetic, 7.5 mg/kg xylazine (Rompun; Bayer Ltd., Leverkusen, Germany) and 100 mg/kg ketamine (Ketalar; Eczacıbaşı, İstanbul, Turkey) were intraperitoneally administered.

Procedure

When the rats were under anesthesia, using a peak with a 0-degree nasal endoscope of 4-mm, tympanic membrane perforation was performed. The procedures applied to the groups following perforation were as follows:

Burow Group (n=10): Gelfoams (Surgispon ENT; Aegis Lifesciences, India) that were soaked in 4% Burow's solution were inserted into the middle ear, and over 2 weeks, 0.1 mL Burow's solution was administered through perforation to the middle ear once a day.

Castellani Group (n=10): Gelfoams that were soaked in Castellani's solution were inserted into the middle ear, and over 2 weeks, 0.1 mL Castellani's solution was administered through perforation to the middle ear once a day.

Control Group (CG) (n=6): Gelfoam that was soaked in physiological saline solution was inserted into the middle ear, and over 2 weeks, 0.1 mL physiological saline solution was administered through perforation to the middle ear once a day.

During the study, the rats were maintained at room temperature (20±2°C) for 12 h of daylight/darkness, fed with standard pellet feed and lettuce, and allowed to easily access water. Sheltering, care, the administration of medicines, and acquisition of the relevant tissue samples were performed in the laboratory of the Pharmacology Department of Adnan Menderes University. The approval of the local ethical board for animal experiments was obtained.

Pathological Examination

All groups were decapitated at day 1 after the last administration. The temporal bones at the processed side of the decapitated animals were removed and their bullas were dissected. The tissue samples obtained from the rats were fixed for 48 h in 10% formalin solution, then maintained in 10% nitric acid decalcification solution for 1 day. Decalcification and routine tissue monitoring were performed. The samples were embedded into paraffin blocks, and 3 micron thick sections were cut. After deparaffinization, the sections were stained with hematoxylin and eosin (H&E). The H&E stained glass slides were blindly evaluated by a pathologist uninformed about the groups. The H&E stained sections were evaluated by grading the increase in the leukocytes with polymorphic nuclei (PNL), mononuclear cell infiltration (MNH), and fibrosis relatively as (–), (+), (++), and (+++) [15, 16]. Accordingly, an increase in PNL was interpreted as indicative of acute inflammation, and MNH infiltration and fibrosis were interpreted as indicative of chronic inflammation.

Statistical Analysis

The statistical analysis of data was made using the Kruskal–Wallis test. The significance level was determined as $p<0.05$. The basic features of the data of the study were described using SPSS (SPSS 19, ADU licence number: 10241440; İstanbul, Turkey).

RESULTS

All 26 rats completed the study without any complication or ear infection. The histopathologic changes in the tympanic cavity mucosa of the rats were evaluated with respect to PNL increase, MNH infiltration, and fibrosis. These changes are shown in Figure 1 and Figure 2. The PNL increase, MNH infiltration, fibrosis, and total score points (median and 25–75 percentiles) of the study and control groups are given in Table 1.

In the Burow group, the fibrosis scores were significantly higher than those in the CG ($p=0.039$), the PNL increase scores were significantly

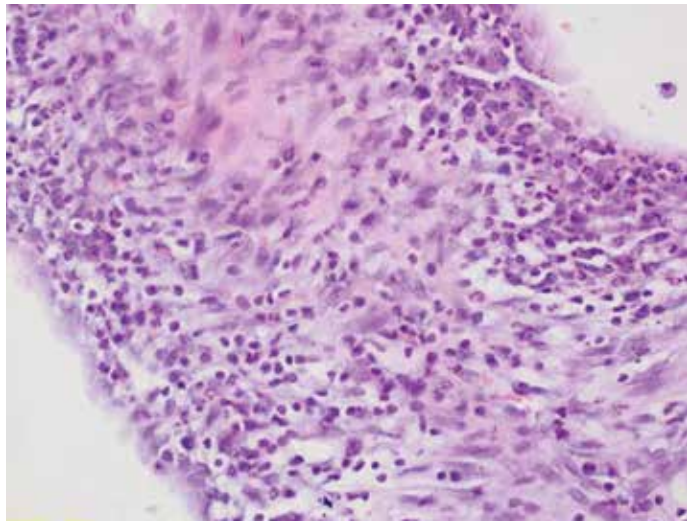

Figure 1. Histopathologic inflammation changes in the middle ear mucosa of rats in the Burrow group. (H&E, light microscope, 40×)

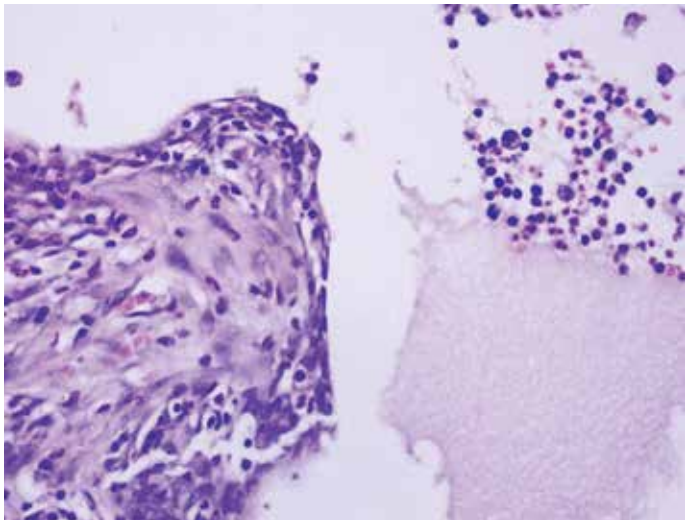

Figure 2. Histopathologic inflammation changes in the middle ear mucosa of rats in the Castellani group. (H&E, light microscope, 40×)

Table 1. The PNL increase, MNH infiltration, fibrosis, and total scores of the groups

	Burow	Castellani	CG	
Variables	**Median**	**Median**	**Median**	**p value**
PNL increase score	3.0 *	2.0	1.5	0.040
MNH infiltration score	3.0	3.0	2.0	p>0.05
Fibrosis score	2.0*	2.0	1.0	0.013
Total score	7.5*	7.0	5.5	0.005

The significance level was determined as p<0.05.
*Burow and CG were different.
CG: control group; PNL: polymorphic nuclei cell; MNH: mononuclear cell

higher than those in the CG (p=0.034), and the total scores were significantly higher than those in the CG (p=0.022). In the Burrow group, the MNH infiltration scores were not significantly higher than those in the CG (p=0.301).

In the Castellani group, the fibrosis scores, MNH infiltration scores, PNL increase scores, and total scores were not significantly higher than those in the CG (p=1.000, p=0.123, p=0.324, and p=0.059). Graphs of the statistically significant results are shown in Figures 3, 4, and 5.

DISCUSSION

Burow's solution is a medicine that can be locally administered in otitis externa, granular myringitis, chronic otitis, and post-operative mastoid cavities; it has also been demonstrated to be effective on resistant microorganisms [2, 17, 18]. Shimizu et al. [19] have reported the successful treatment of a patient with malign externa otitis using Burow's solution. Jinnouchi et al. [4] have reported the local treatment of 14 ears with persistent otorrhea using Burow's solution modified by fourfold dilution. However, other studies claim that Burow's solution has toxic effects on the inner ear [2, 3, 5]. Sugamura et al. [2] found that Burow's solution might be toxic to guinea pig cochlea [7, 8]. Oishi et al. [3] reported two sudden hearing loss cases caused by the use of Burow's solution. Contrastingly, Serin et al. [5] reported in their study that this solution has no toxic effects on guinea pig cochlea.

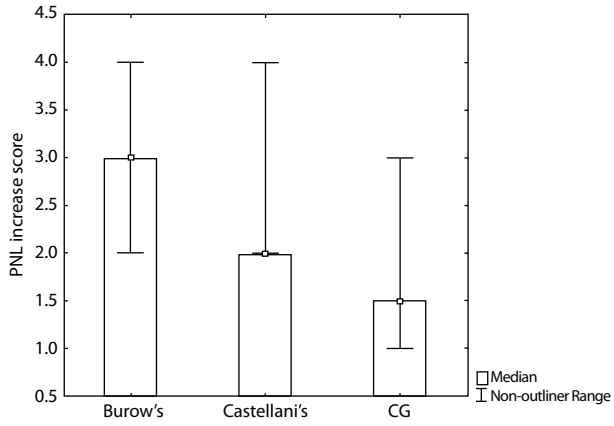

Figure 3. In the Burow group, the PNL increase scores were significantly higher than those in the CG

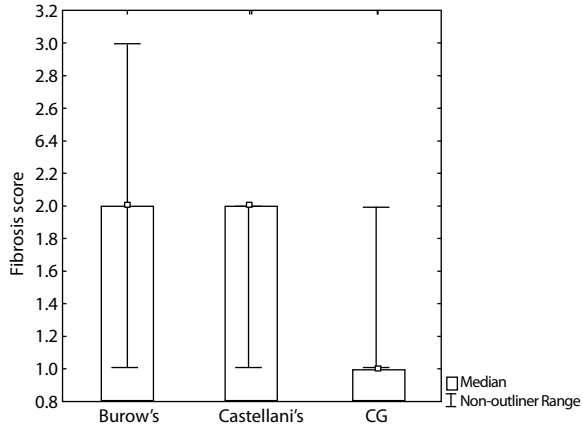

Figure 4. In the Burow group, the fibrosis scores were significantly higher than those in the CG

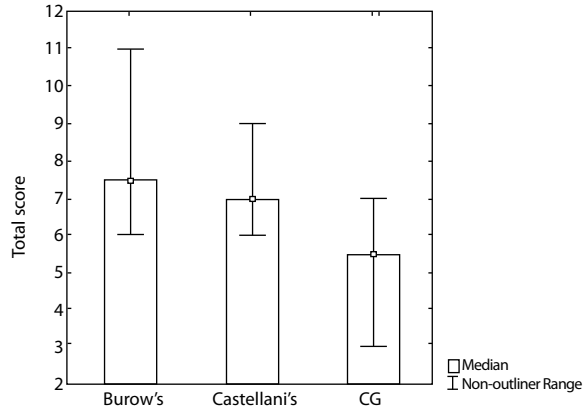

Figure 5. In the Burow group, the total scores were significantly higher than those in the CG

Although the effect of Burow's solution on the inner ear has been investigated, its effect on the middle ear mucosa is not yet known. The only related data we could find is the degeneration of the round window external epithelium that Suzuki et al. [7] identified in their study on the effects of Burow's solution on the cochlea. We determined in

the histopathologic examination of the middle ear mucosa of rats that Burow's solution causes acute and chronic inflammation.

The ototoxicity of Castellani's solution has been less investigated than that of Burow's solution [13]. Gültekin et al. [13] have demonstrated that Castellani's solution has no toxic effects on the functioning of outer hair cells. In the literature, no study researching the effect of Castellani's solution on the middle ear mucosa appears. As far as we know, our study is the first one investigating this subject. Based on the results of this study, Castellani's solution does not cause acute or chronic inflammation of the middle ear mucosa.

Burow's and Castellani's solutions are commonly used agents in otorhinolaryngology practice. However, our knowledge of the effects on the middle ear mucosa of these agents in the presence of a perforated tympanic membrane is limited. In light of the results of our study, we suggest that Castellani's solution is a safe alternative in the treatment of otomycosis and external otitis in the presence of tympanic membrane perforation. However, because of the inflammatory changes it causes in the middle ear mucosa, we do not recommend the use of Burow's solution in the presence of tympanic membrane perforation.

The limitation of this study is that this research cannot show whether the early period effects are reversible. In our study, early period effects on the middle ear of Burow's and Castellani's solutions were addressed. Therefore, this study does not provide insight into whether the inflammatory changes of the mucosa during the acute period are reversible. Further studies on the long term results of the inflammation caused by Burow's solution are needed.

The present study demonstrates that Castellani's solution does not cause acute or chronic inflammation of the middle ear mucosa, but that Burow's solution causes acute and chronic inflammation of the middle ear mucosa. Topical treatment of Castellani's solution is a safe alternative in the presence of tympanic membrane perforation.

Ethics Committee Approval: Ethics committee approval was received for this study from the animal ethics committee of Adnan Menderes University, 64583101/2013/072.

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