The Comparison of Bacterial Contamination and Antibacterial Efficacy of the Anti-Glaucomatous Eyedrops with and without Preservatives

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ABSTRACT

Purpose: To investigate the bacterial contamination risks and antimicrobial activities of the preservative-free and preservative-containing anti-glaucomatous ophthalmic solutions.

Materials and Methods: Ophthalmic solution bottles of preservative-free brimonidine 0.15% (D1), benzalkonium chloride-containing (BAK) brimonidine 0.15% (D2), purite-containing brimonidine 0.15% (D3) and BAK-containing timolol maleate dorzolamide fixed combination (D4) were included in this study in terms of microbial contamination risk. Moreover, microbial contamination of the two bottles [preservative-free brimonidine 0.15% (D7) and BAK-containing brimonidine 0.15% (D8)] was investigated after contacting their tips with the lower eyelid edge of a researcher. Every day twice a day for 60 days; the caps of the bottles were opened and they were closed after waiting for 20 sec. One drop was added from these six bottles 11 times during the study period (60 days) to the six separate and renewed blood-agar mediums. Microbial contamination was evaluated every visit by examining the blood agar mediums by the same microbiologist. In terms of antimicrobial efficacy; D1, D2, D3 and D4 were compared with the antibiotic containing ophthalmic solutions; moxifloxacin (D5) and tobramycin (D6) by using agar well diffusion method.

Results: No bacterial growth was observed in the mediums of D1, D2, D3, D4 and D8 bottles. The bacterial growth of methicillin-susceptible and resistant coagulase-negative staphylococci was observed in the medium of D7 bottle on some days. A large inhibition zone was seen around D5 and D6, whereas a smaller inhibition zone was detected around D2 and D4. No inhibition zone was detected around the D1 and D3 bottles.

Conclusions: Multi-dose preservative-free antiglaucomatous ophthalmic solutions have not any risk of bacterial contamination unless the tip of the bottle is contaminated.

Keywords: Benzalkonium chloride, Brimonidine, Glaucoma, Purite, Multi-dose non-preservative ophthalmic solutions.

INTRODUCTION

Chronic use of topical anti-glaucomatous drugs causes various forms of ocular surface problems including tear film instability, conjunctival inflammation, corneal epithelial apoptosis and subconjuntival fibrosis.[1] Although active components of the eye drops are the cause of these ocular surface problems in some cases, preservatives that are added to the multidose eye drops to prevent bacterial contamination is the main reason for most of the cases. These ocular surface problems and ocular discomfort results in long term decrease in patient compliance with the glaucoma treatment, may affect the patient's quality of life and increase the potential risk of failure for future glaucoma surgery.[2-4] In addition to prevent the bacterial contamination, some preservatives may increase the effectiveness of active components by increasing the passage through the cornea into the anterior chamber.[3,5,6]

Benzalkonium chloride (BAK) is a quaternary ammonium cationic surfactant and it is the most widely used preservative in ophthalmic solutions. Because of its non-specific nature, it is effective not only on the pathogens but also toxic to the human cells.[3] Another commonly

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used preservative, purite is a combination of chlorine dioxide, chlorite and chlorate that disrupts vital enzymes for cell function, causing oxidation of intracellular lipids and glutathions. It shows antimicrobial activity against bacteria, viruses and some fungi.[5] It is an effective oxidizer due to its tendency to produce free radicals and is rapidly degraded when exposed to the ocular surface.[5]

Preservative-free eye drops lack the side effects of preservatives and they are more compatible to the ocular surface. In order to prevent bacterial contamination, preservative-free eye drops must be manufactured either as single use vials or multi-dose bottles with special mechanisms that prevent bacterial contamination.[7]

In this study, we aimed to compare the bacterial contamination risks and antimicrobial activity of the preservative-free and preservative-containing anti-glaucomatous drugs.

MATERIALS AND METHODS

This prospective study was performed in accordance with the tenets of the Declaration of Helsinki and the study design and protocol was approved by the İstanbul Training and Research Hospital Ethics Committee (2011-KAEK-50).

Four different eye drop bottles were included in the study for microbial contamination risk; preservative-free brimonidine 0.15% (Brimogut; Bilim, Istanbul, Turkey) in Novelia multidose preservative-free system (Nemera, La Verpillière, France) (D1), BAK-containing brimonidine 0.15% (Brimogut; Bilim, Istanbul, Turkey) (D2), purite-containing brimonidine 0.15% (Alphagan P; Allergan, Irvine, CA) (D3), BAK-containing timolol maleate and dorzolamide fixed-combination (Oftomix, Bilim, Istanbul, Turkey) (D4). Moreover, microbial contamination of the two bottles [preservative-free brimonidine 0.15% (D7) and brimonidine 0.15% containing BAK (D8)] was investigated after contacting their tips with the lashing edge the lower eyelid of a researcher for mimicking the microbial contamination of the bottles in routine patient usage.

Every day twice a day for 60 days; the caps of the 6 eye drops bottles were opened and they were closed after waiting for 20 sec. A drop from each of these bottles were added on the 1st, 2nd, 3rd, 4th, 5th, 6th, 10th, 15th, 30th, 45th, 60th days to the six separate and renewed 5% sheep blood-agar mediums and incubated at 37 °C for 24-48 hours. In addition; one day later for each time point to see the effect of this intentional contamination on the remaining content of D7 and D8.

At the end of the incubation period, the colonies were defined by using classical microbiological methods such as colony morphology, gram staining, catalase and rapid latex agglutination test (Avipath®-Staph-Omega Diagnostic Ltd, UK). Methicillin susceptibility of Staphylococcus strains were performed by Kirby-Bauer disk diffusion method. Suspensions of *Staphylococcus* strains in 0.5 McFarland turbidity were prepared and inoculated on Mueller Hinton agar medium. The cefoxitin disc (Cefoxitin 30 µg –Becton Dickinson®, USA) was used and zone diameters around the cefoxitin discs were measured and evaluated according to EUCAST criteria after 24 hours of incubation at 37 °C.

For assessing the antimicrobial efficacy of drugs; D1, D2, D3, D4 were compared with antibiotic-containing two different drugs by using agar well diffusion method; moxifloxacin (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX, USA) (D5) and Tobramycin (Tobrased, Bilim, Istanbul, Turkey) (D6). A suspension of standard *Staphylococcus aureus* ATCC 29213 strains was prepared in 0.5 McFarland turbidity, and was spread to 4 different Mueller Hilton agar media, one drop from each 6 eye drop bottles was added to the wells opened on sterile media and incubated for 24 hours at 37 °C by agar well diffusion method. At the end of the incubation period, antimicrobial activity was evaluated according to the zone diameters around each drop.

RESULTS

Bacterial contamination: No bacterial contamination was observed in neither preservative-free D1 and nor preservative-containing D2, D3 and D4 in any time point for any of the mediums. Although bacterial growth was observed in intentionally contaminated preservative-free D7 on 5th, 15th, 30th, 45th and 60th days, no bacterial contamination was observed BAK-containing D8 in any time. However, none of the mediums which was inoculated one day after the tip contamination from D7 was positive (Table 1).

Antimicrobial activity: A large inhibition zone (36 mm and 28 mm) were detected around D5 and D6 which are antibiotics, whereas smaller inhibition zones (13 mm and 10 mm) was detected around the BAK-containing D2 and D4. The inhibition zone was not observed around the preservative-free D1 and the Purite-containing D3 (Figure 1).

Table 1. *Microbial contamination outcomes of eye drops.*

Day	D1	D2	D3	D4	D7	D8
1st day	No growth	No growth	No growth	No growth	No growth	No growth
2nd day	No growth	No growth	No growth	No growth	No growth	No growth
3rd day	No growth	No growth	No growth	No growth	No growth	No growth
4th day	No growth	No growth	No growth	No growth	No growth	No growth
5th day	No growth	No growth	No growth	No growth	2 colony MRCNS	No growth
6th day	No growth	No growth	No growth	No growth	No growth	No growth
10th day	No growth	No growth	No growth	No growth	No growth	No growth
15th day	No growth	No growth	No growth	No growth	2 colony MRCNS	No growth
16th day	-	-	-	-	No growth	No growth
30th day	No growth	No growth	No growth	No growth	4 colony MSCNS	No growth
31st day	-	-	-	-	No growth	No growth
45th day	No growth	No growth	No growth	No growth	2 colony MSCNS	No growth
46th day	-	-	-	-	No growth	No growth
60th day	No growth	No growth	No growth	No growth	1 colony MRCNS	No growth
61st day	-	-	-	-	No growth	No growth

MRCNS: Methicillin-Resistant Coagulase-Negative Staphylococci, **MSCNS:** Methicillin Susceptible Coagulase-Negative Staphylococci, **D1:** Preservative-free Brimonidine, **D2:** Brimonidine containing benzalkonium chloride, **D3:** Brimonidine 0.15% containing Purite, **D4:** Timolol maleate and Dorzolamide fixed combination containing benzalkonium chloride, **D7:** Preservative-free Brimonidine with tip contact, **D8:** Brimonidine containing benzalkonium chloride with tip contact,

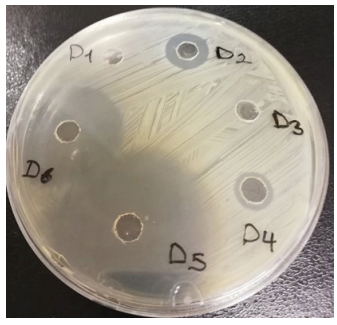

Figure 1. *Antimicrobial effects of eye drops on S.aureus ATCC 29213 strain by agar well diffusion method.*

DISCUSSION

Preservative-free artificial tear preparations are very popular and they have been widely using for the treatment of the dry eyes and other ocular surface diseases for a long time. Since the preservatives in eye drops cause many problems, preservative-free anti-glaucomatous ophthalmic solutions are becoming a good option for glaucoma treatment in recent years. In addition to the ocular surface problems, commonly used preservatives like BAK was shown to have intraocular toxic effects on ciliary body and trabecular system.[8]

Preservative-related side effects of eye drops are commonly seen in clinical practice and this situation is one of the most common causes of discontinuation of treatment.[9-11] Although the healthy ocular surface is relatively resistant to microbial contamination, direct exposure to large amounts of microorganisms can lead to infections that threaten vision by suppressing normal protective mechanisms.[11-14] In addition, many patients who need to use chronic medications have ocular surface pathologies that make them more susceptible to infection.[13] Contamination rates of eye drops in use have been reported in different rates in the literature as 0.07-35.8%.[9-15] After 1960, the use of preservatives in ophthalmic solutions became necessary as a result of the occurrence of some serious eye infections with multi-dose eye drops.[10]

In 2006, the Ophthalmic Compression Dispenser (OSD), a multi-dose device designed for preservative-free ophthalmic solutions, was developed.[16] The system uses improved valve sealing technology and sterile ventilation filtration to prevent microbial contamination of the product. During the instillation (squeezing the bottle), the pressure inside the system rises and the liquid is pushed through the fluid channel. When the pressure falls below a specified threshold, outlet valve closes immediately with an outward movement. This function prevents any backflow to the system and thus avoiding microbial contamination.[16,17]

In 2010, Nemera (La Verpillière, France) introduced the

Novelia® system which uses a similar technology as the OSD but with some important differences. Novelia system features a silicone tube-based valve mechanism, and the container is vented via air diffusion through a silicone membrane. Silver is added to the plastic material of the actuator, protection cap, and silicone valve to ensure microbial integrity.[18] Preservative-free brimonidine used in this study uses the Novelia system.

Increased awareness of the toxicity of ophthalmic preservatives has led to the development of preservative-free preparations or delivery systems.[16] In this way, as well as improving the quality of life of patients, better tolerability of eye drops provided. In this study, because the preservative-free brimonidine ophthalmic medication with Novelia technology was not contaminated for 60 days, we can say that it is advantageous when compared with ophthalmic solutions with the same active ingredient containing BAK or purite which have the risk of side effects and allergy.

The risk of microbial contamination increases as the exposure time increases after opening the cap of the classic ophthalmic drop bottles.[15,19] Our results showed that the preservative-free multidose brimonidine preparation (D1) showed no sign of bacterial contamination during the study period (60 days).

For commercially prepared topical ophthalmic solutions, contamination usually occurs during the process of instillation. The infection source is usually caused by dropper contact with fingers, eyelids or eyelashes during instillation.[19] Numerous studies have shown that the tip of the eye drop is the most frequently contaminated area.[10-15] Rahman et al. reported the rate of contamination of drop bottles as 8.4% and the mainly isolated bacteria were associated with normal skin flora (coagulase negative) or airborne gram-positive bacilli.[7] In our study, in order to investigate the microbial contamination, before installation on mediums, the tip of brimonidine ophthalmic solution bottles with and without preservative were contacted with the lower eyelids of the researcher himself. After this contamination, bacterial growth was only detected in the samples taken immediately after eye contact of the tip of the preservative-free multidose bottle (D7). Methicillin-resistant coagulase-negative staphylococci (MRCNS) and methicillin-sensitive coagulase-positive staphylococci (MSCNS) were isolated bacteria that might be associated with the skin flora of researcher. But when the inoculation is repeated from same bottle one day after intentional contamination, no growth was detected. This indicates that only the tip of the bottle contacting with eye is contaminated momentarily and the bacterium cannot contaminate the drug in the rest of the bottle. This result also indicates that the technological advances in preservative-free multidose bottles can prevent contamination of its contents even after contamination of bottle tips with the eyelash. The important point is, prevention of contamination of the remaining solution in the bottle, which shows the effectivity of the Novelia technology for prevention of contamination even after intentional contamination of the tip. These results are similar to earlier report.[7]

When the antimicrobial effects of drugs are examined; BAK-containing anti-glaucomatous drugs (D2 and D4) had less antibacterial activity than antibiotic-containing ophthalmic drugs (D5 and D6). The preservative-free brimonidine (D1) has been shown to have no antimicrobial activity as expected. In contrast to studies showing that the purite is a microbicidal stabilizing oxychloride complex with a broad spectrum of antimicrobial effect and low toxicity [5,10,11,20], in this study, no inhibition zone was observed in the purite-containing brimonidine (D3). We investigated the antimicrobial activity of purite only on standard Staphylococcus aureus ATCC 29213 strains, which can be the reason for discordance with the previous studies in literature.

The limited number of samples and mediums make difficult to generalization of the study results and this is the limitation of this study. There is a need for further studies that will reveal the real-life data showing the risks of microbial contamination by collecting eye drops of the patients with or without preservatives that are used for short or long term even if they are technological products like the current multidose dispensers.

As a result, when choosing medical treatment for glaucoma, it is important to understand not only the primary purpose of treatment, but also the contraindications and side effects of each drug used. In addition to efficacy in treatment selection, it is necessary to take into account the tolerability, related quality of life and treatment compliance. Switching to a preservative-free anti-glaucomatous multidose ophthalmic solution in patients who suffering ocular surface problems seems to be safe.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Human and animal rights: This article does not contain any studies with human participants or animals performed by any of the authors. Only, the tip of the bottle was contacted to the lower eyelid edge of a researcher (KO) for mimicking the microbial contamination of the bottles.

Informed consent: No informed consent was obtained from patients since this was a laboratory study.

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