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Original Research Article

Comparative efficacy study of brimonidine tartrate 0.15% and timolol maleate 0.5% ophthalmic solution (benzalkonium chloride free) versus Brimolol® (with benzalkonium chloride) following single ocular instillation in New Zealand white rabbits

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ABSTRACT

Background: Benzalkonium chloride (BKC) is the most used preservative in topical eye drops but it causes effects such as dry eye and trabecular meshwork degeneration. Polyhexamethylene biguanide (PHMB) is a polymeric biguanide is a less harmful preservative used in ophthalmic solutions. The objective of this study to compare the efficacy of PHMB preserved versus BKC preserved ophthalmic solutions containing brimonidine tartrate and timolol maleate on intraocular pressure (IOP) following single ocular instillation in New Zealand white (NZW) rabbits.

Methods: This study was conducted on 12 normotensive male NZW rabbits (2.9-3.6 kg) between 6-9 months of age. Animals received single ocular instillation of 35 µl ophthalmic solution containing brimonidine tartrate 0.15 %w/v and timolol maleate 0.5% w/v with PHMB as preservative (n=6, test) or BKC as preservative (n=6, reference) as per the randomization. Intraocular pressure (IOP) was measured before and 2, 4, 6, 8 and 24 hours after instillation using a pneumatonometer. Percentage change in IOP from pre-instillation values were calculated. Changes in IOP were analysed using the repeated-measures analysis of variance followed by Bonferroni post-test.

Results: Single ocular instillation of PHMB and BKC formulations show significant IOP reduction up to 6 hours as compared with baseline (p<0.05). Reduction in IOP was 35.8% and 32.0% at 2 hours with PHMB and BKC formulations respectively. No differences were observed between the test and reference groups for change in IOP from baseline.

Conclusions: PHMB preserved brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v ophthalmic solution was comparable to BKC preserved solution in normotensive NZW rabbits.

Keywords: Polyhexamethylene biguanide, Ophthalmic solution, Intraocular pressure, Brimonidine, Timolol

INTRODUCTION

Preservatives are a necessity in multi-dose ophthalmic solutions for stabilization and intraocular penetration and most preservatives act as detergents or by oxidative mechanisms because of which they can cause irritation

and side effects in the eye.¹ Benzalkonium chloride (BKC) is one of the most commonly used preservative in ophthalmology and is more toxic than other preservatives, such as polyhexamethylene biguanide (PHMB), sodium perborate, chlorite complex and oxychloro-complex.²

BKC toxicity depends on the amount administered daily, the duration of the treatment and its concentration in the administered solution. At each administration, its detergent effect disrupts the lipid layer of the tear film which cannot be regenerated and can no longer protect the aqueous layer of the tear film. The cornea is exposed, and eye dryness occurs. Also, BKC has a cellular toxicity, entailing a reduction in the amount of mucin, an additional reason for disrupting the tear film. BKC toxicity also manifests through apoptosis phenomena (free radical production) and/ or cellular necrosis, depending on the concentration.³

Since glaucoma patients are exposed to preservatives through multiple drops and/or long-term treatment, it is possible that BKC may have an even greater effect on the ocular surface.⁴

BKC disrupts the corneal epithelial barrier and helps in ophthalmic drug penetration. Therefore, there is a concern that BKC free formulations would not as effectively lower intraocular pressure. However, multiple studies have shown that this is not the case.⁴ For example, preservative-free timolol is equally effective in controlling intraocular pressure compared to various preserved formulations of timolol.⁴ Also, in another study preservative-free brimonidine was found to effectively reduce the intraocular pressure (IOP).⁵

PHMB is a safe and effective antimicrobial agent with cationic and amphipathic properties and has been widely used for wound dressings, water treatment, contact lens solutions, and disinfectants.⁶⁻⁹ PHMB compared to other agents has better antibacterial properties and also is biocompatible.¹⁰ It acts by targeting the cytoplasmic membrane and DNA. The PHMB polymer strands are incorporated into the bacterial cell wall which disrupts the membrane and reduces its permeability leading to complete loss of membrane functions. There is precipitation of intracellular constituents, and leakage of cytoplasmic contents which is lethal for the bacteria. Also binds to bacterial DNA, alter its transcription and causes lethal DNA damage.¹¹

Ophthalmic solutions containing brimonidine and timolol are commonly used for reducing IOP in patients with both open angle glaucoma and ocular hypertension.¹² The objective of this study was to compare the efficacy of PHMB preserved versus BKC preserved ophthalmic solutions containing brimonidine tartrate (0.15% w/v) and timolol maleate (0.5% w/v) on the IOP following single ocular instillation in normotensive New Zealand white rabbits.

METHODS

This prospective comparative efficacy study was carried out by the formulation development department of Sun Pharmaceutical Industries Limited in February 2019 at the biological research pharmacology facility, Tandalja,

Vadodara, Gujarat, India. The research protocol and study related documents were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and study was conducted as per the CPCSEA (committee for the purpose of control and supervision of experiments on animals) guidelines and recommendations regarding animal care and handling. Twelve normotensive male New Zealand white rabbits weighing 2.9 to 3.5 kg and between 6-9 months of age were selected for the study. Animals were identified by animal identification numbers based on cage card and ear marking. One rabbit was housed in one cage at ambient temperatures of 18-22 degree centigrade with 12 hours light-dark cycle at a relative humidity of 30-70 percent. Animals were fed on high fibre rabbit pellet feed ad libitum and filtered water ad libitum. All animals underwent veterinary health check for any abnormalities or conditions. All rabbits underwent screening for body weight and IOP. Pre-treatment measurements of IOP were obtained for left eye of each animal at 08:00 hours and 17:00 hours for two days preceding the test day (day-2 to day-1).

Animals were divided into two groups as per sequential randomization based on their serial numbers 1-12 for identification. Test group (n=6) received ocular instillation of ophthalmic solution containing brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v with PHMB (without BKC) as preservative (Sun Pharma, Batch No. 26202108SB014), whereas reference group (n=6) received single ocular instillation of ophthalmic solution containing brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v with BKC as preservative (Brimolol®, Sun Pharma, Batch No. HKT0499). All animals received a 35 µl single instillation in the left eye at 09:00 hours on the study day (day 0). The dose was selected based on literature of previous animal studies.

IOP was measured using a pneumatonometer model 30 classic™ (Reichert, USA). Baseline IOP was calculated as the mean IOP values up to 48 hours before the study dose instillation. During IOP measurements, each animal was restrained in restrained without sedation. The pneumatonometer probe was placed lightly on the cornea and allowed to rest for 10-15 seconds. The probe was placed entirely on the cornea in horizontal position and five consecutive readings were recorded, each with standard deviation value <1 which was displayed on the screen. The pneumatonometer probe filter was cleaned after each use by gently touching to cotton swab (immersed in normal saline) and just wiped with tissue paper. On the test day (day 0), IOP was measured at 9:00 hours followed immediately by the instillation of ophthalmic solutions (test or reference) using a micropipette. IOP readings were measured at 2, 4, 6, 8- and 24-hours post instillation.

All statistical analysis was carried with PRISM (GraphPad version 5.03, 10 December 2009) and p<0.05 was considered as statistically significant. Percentage change in IOP was calculated with respect to initial IOP

readings and changes in the IOP were analysed by using the one way repeated-measures analysis of variance (ANOVA) followed by Bonferroni post-test for individual values with their respective initial values. The IOP values were also analysed for differences using two-way Anova followed by Bonferroni post-tests (n=6) for comparison between the groups.

RESULTS

The two groups were similar with respect to the body weight, age and baseline IOP values. Table 1 shows the mean (standard error of mean- SEM) values for the IOP at baseline and post-instillation period in the two groups (test and reference). Single ocular instillation of test and reference ophthalmic solutions of brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v showed statistically significant reduction in the IOP at 2 hours and up to 6 hours compared with the baseline IOP in normotensive NZW rabbits. However, the differences in the IOP's between the test and reference products were not significant (p>0.05).

Table 1 also presents the mean and percent change in IOP from baseline in the two groups at 2, 4, 6, 8 and 24 hours post instillation. Maximum reduction in IOP observed

was 35.8% and 32% at 2 hours following single instillation test item and reference item respectively.

Table 1: Reduction in IOP (mmHg) in test and reference group at baseline and post-instillation period.

Variables	Test (SB014) without BKC PHMB preservative (n=6)	Brimolol (HKT0499) with BKC (n=6)
	Mean (SEM)	Mean (SEM)
Baseline (pre-instillation)	20.60 (0.78)	20.34 (0.58)
Change from baseline		
2 h	-7.4 (-35.8)*	-6.5 (-32.0)*
4 h	-6.1 (-29.8)*	-5.7 (-28.1)*
6 h	-3.4 (-16.7)*	-3.2 (-15.7)*
8 h	-1.5 (-7.4)	-1.6 (-7.7)
24 h	-0.3 (-1.3)	-0.5 (-2.7)

Negative values indicate reduction from baseline; *p<0.001 compared to baseline IOP value (one-way repeated measures analysis of variance); and no statistically significant differences were observed between the groups (two-way analysis of variance).

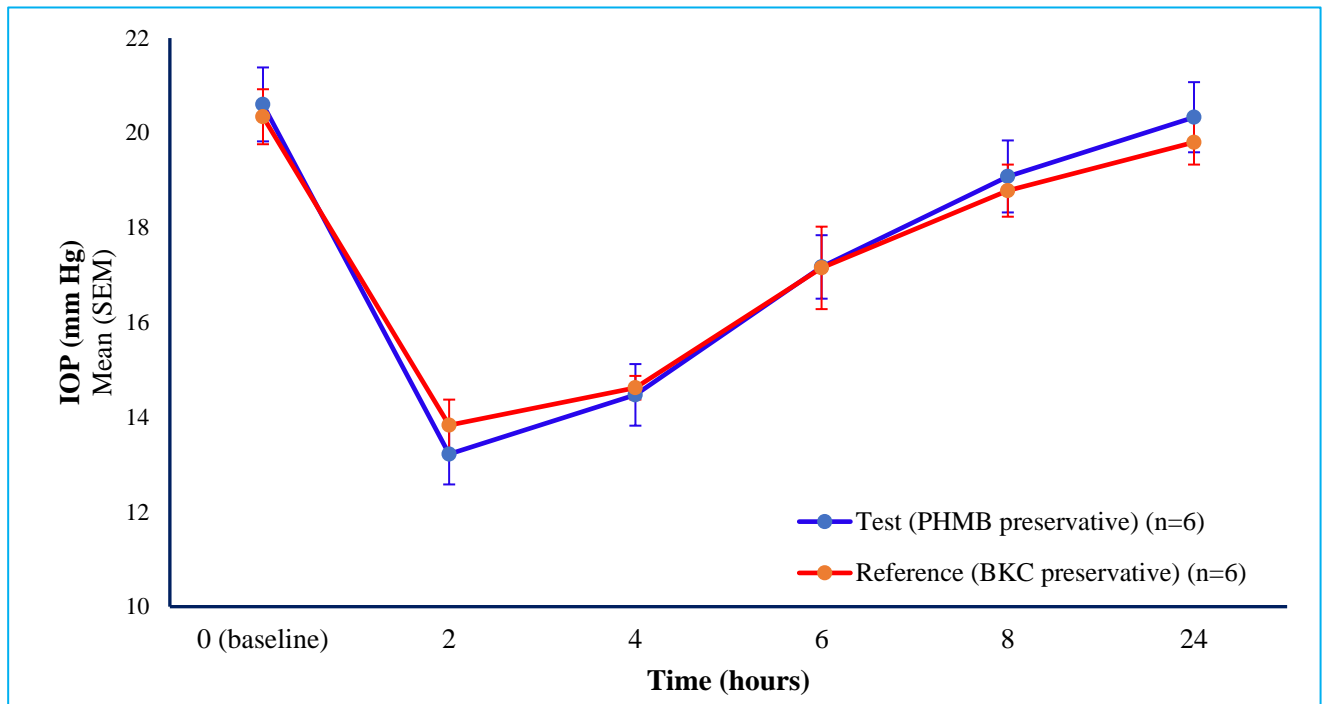


Figure 1: Mean IOP in test and reference group at baseline and post-instillation period.

DISCUSSION

This study compared the efficacy and safety of PHMB versus BKC for treatment of glaucoma. The results suggest that the efficacy of brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v ophthalmic solution - test item (26202108SB014) was comparable to reference

item Brimolol® (HKT0499) in normotensive NZW rabbits. There are several studies reporting the use of PHMB based formulations in treatment of infected wounds and other applications as disinfectant.^{13,14} However, there are no studies comparing PHMB formulations versus BKC formulations in treatment of glaucoma. In absence of any prior comparative studies,

this pre-clinical study is the first one which compared the PHMB based ophthalmic solutions for treatment of glaucoma with BKC based ophthalmic solutions.

Brimonidine and timolol based ophthalmic formulations are recommended for treatment of glaucoma and ocular hypertension.¹⁵ Brimonidine is an alpha 2 - adrenergic receptor agonist which is highly selective agent, lowers IOP similar to other agents and increases the uveoscleral outflow and reduction in aqueous humour production.¹⁶ In various animal models, brimonidine caused a reduction in IOP, but unlike apraclonidine, brimonidine did not cause mydriasis. It is an important addition to the field of ophthalmic preparations for glaucoma and other ocular conditions.¹⁵

The reduction in IOP with brimonidine is associated with a decrease in aqueous flow and an increase in uveoscleral outflow in humans.¹⁸ Timolol has become one of the first line therapies to reduce IOP in glaucoma since its FDA approval in 1978. It is a non-selective beta-adrenergic receptor blocker and is generally considered to have few systemic effects when administered in the eye.¹⁹

PHMB and BKC are both commonly used preservatives for ophthalmic and other topical preparations. PHMB is also useful as topical application in various bacterial infections and wound care.²⁰ Use of 0.2% polyhexamethylene biguanide dressing for infection of a superficial surgical incision site (ISSIS) after a laparoscopic cholecystectomy has reported to be beneficial.²¹

Ophthalmic formulations containing 0.2% BKC are shown to induce severe corneal epithelial defects, and 0.1% BKC once daily over 7 days induced punctate fluorescein staining without detriment to corneal smoothness.²² BKC treatment also modulated K₁₄ expression and distribution within the limbus in mice. In cultivated primary mouse corneo-limbal epithelial cells (CLEC's), BKC was found to trigger cell contraction and vacuolation, increased lactate dehydrogenase (LDH) release and elevated cell necrosis by almost four times.²² Toxicity to the ocular surface was not evident with PHMB when used for the treatment of *Acanthamoeba keratitis*.²³ Thus, PHMB probably is a better preservative compared to BKC for any topical formulations.

This study compared the efficacy of PHMB-preserved with BKC-preserved fixed dose combination of brimonidine tartrate 0.15% and timolol maleate 0.5% eye drops in NZW rabbits and showed similar IOP lowering effects suggesting that the efficacy is not dependent on BKC.

CONCLUSION

PHMB preserved brimonidine tartrate 0.15% w/v and timolol maleate 0.5% w/v ophthalmic solution was comparable to BKC preserved solution in normotensive

NZW rabbits. However, further investigation in clinical setting is required to substantiate these results.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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