Original Article

Effects of Benzalkonium Chloride on the Microanatomy of Corneal Stroma

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ABSTRACT

Benzalkonium chloride (BAC) is a commonly used preservative in topical ophthalmic preparations. It is a cytotoxic compound. The mechanism of action involves dissociation of bilaminar plasma membrane and its tendency to dissolve cholesterol, phospholipids and the proteins in the cell membrane. The indiscriminate use of eye drops containing BAC, quackery and self-medication may increase the incidence of corneal disorders particularly in those having a pre-existing corneal pathology. **Objective:** The present *in vivo* study was carried out to investigate the effects of BAC ons corneal stroma. **Methods:** Two different concentrations (0.02% and 0.0075%) of BAC solution comparable to those present in the commercially available eye drops were prepared in isotonic saline. Right eye of each animal was treated with BAC solution while left eye of the same animal served as a control treated with normal saline alone. **Results:** The analysis of the results revealed significant ($p<0.05$) histological changes in the corneal stroma. **Conclusion:** This study has provided the convincing evidence that BAC is toxic to the corneal stroma and is a factor contributing towards visual impairment. **Key Words:** Microanatomy, Stroma, Ulceration, Ophthalmic.

INTRODUCTION

The transparent nature of cornea and its importance in the visual pathway as the major refracting medium has intrigued researchers, and their studies have added extensively to the understanding of the corneal structure in health and disease[1]. Its anatomical position predisposes it to both physical and chemical damage that can affect its structure and transparency, resulting invariably to corneal blindness.

Corneal pathologies are often unreported, but are significant factors which produce corneal blindness and are responsible for 1.5-2.0 million new cases of monocular visual impairment every year.

Corneal ulceration, in developing countries, has only recently been recognized as a "silent epidemic"[2]. In the developing countries, as much as 90% of all cases of blindness are a direct result of corneal pathologies[3].

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Nearly every type of ophthalmic product, from artificial tears to contact lens solutions, contains some kind of preservative[4]. Some of the eye drops containing BAC are Alphagan, Alcain, Azopt, Betagan, Cosopt, Hypo Tears, Trusopt, Xalatan[4, 5]. The frequent use of eye drops, self-medication, increasing trend of optical and cosmetic use of soft contact lenses in young females, ophthalmic preparations freely available over the counter and those prescribed by the quacks, especially in the developing countries, may increase the number of visually handicapped persons in coming years. Furthermore, vast use of Benzalkonium chloride (BAC) in topical ophthalmic preparations as a preservative has also been reported to have toxic effects on ocular surface by many investigators[6,7,8]. The available evidence suggests that the damage caused by BAC to the cornea adversely affects its transparency, resulting in an increase in the prevalence of avoidable corneal blindness. Therefore, the hazards of BAC should not be taken lightly. The present investigations were planned to study the effects of BAC on the

microstructure of corneal stroma in experimental animals to compare these with the results of studies conducted in vitro.

Corneal Stroma: The corneal stroma (substantia propria) forms about 90% thickness of cornea and is composed of collagen fibers and cells. The characteristic transparency of cornea is related, in part, to the pattern of its collagenous fibrils arranged in arrays or lamellae which course parallel with the surface of the cornea [9]. According to Quantock *et al.*[10], the collagen fibrils which make up the stroma consist of parallel arrays of long collagen molecules held together by intermolecular bonds containing a mixture of collagen molecules (type I, III, and V). The lamellae frequently branch, sometimes remaining in the same layer and sometimes forming part of a contiguous layer. Thus the lamellae of the corneal stroma are not merely superimposed but form a truly intertwined structure[11, 12]. The fibrils within each lamella, as well as the lamellae themselves, are held together by a glycosaminoglycan matrix, rich in chondroitin sulphate, keratan sulphate and hyaluronic acid[13].

MATERIALS AND METHODS

Forty eight guinea pigs were used in this study. All experimental protocols were conducted in compliance with the requirements and approval of Ethical committee of the University of Health Sciences, Lahore. The animals were randomly divided into four groups (1, 2, 3, and 4). Each group contained six animals (12 corneas). BAC (Fluka, Germany) in different concentrations (Table 1) was used topically as eye drops. The topical solution of BAC in normal saline was instilled in the right eye while the left eye of each animal served as control receiving only the normal saline[14, 15].

Table 1: Dosage protocol of the study groups

Group	Dose Frequency[16] (as topical drops)	Concentration of BAC
1	Twice daily for four weeks	0.0075 %
2	Twice daily for four weeks.	0.02 %
3	Twice daily for eight weeks.	0.0075 %
4	Twice daily for eight weeks.	0.02 %

The animals in groups 1 and 2 were killed after four weeks, while those in 3 and 4 were killed after eight weeks. The eyes were enucleated and the following observations were made on the corneal stroma after preparing the slides:

a. Organization of corneal lamellae.
b. Oedema, if any.
c. Thickness, using Culling method of micrometry[17].

RESULTS

Histological Observations: Corneal stroma revealed oedematous changes in the BAC treated groups 2, 3, and 4. The corneal lamellae were separated from each other by numerous empty spaces which presumably appeared on account of accumulation of fluid in the corneal stroma (Fig. 1, B, and C). Continued treatment of cornea with BAC worsened these preliminary changes by disrupting the regular arrangement of corneal lamellae and increasing oedema of corneal stroma in group 4 (Fig. 1-D).

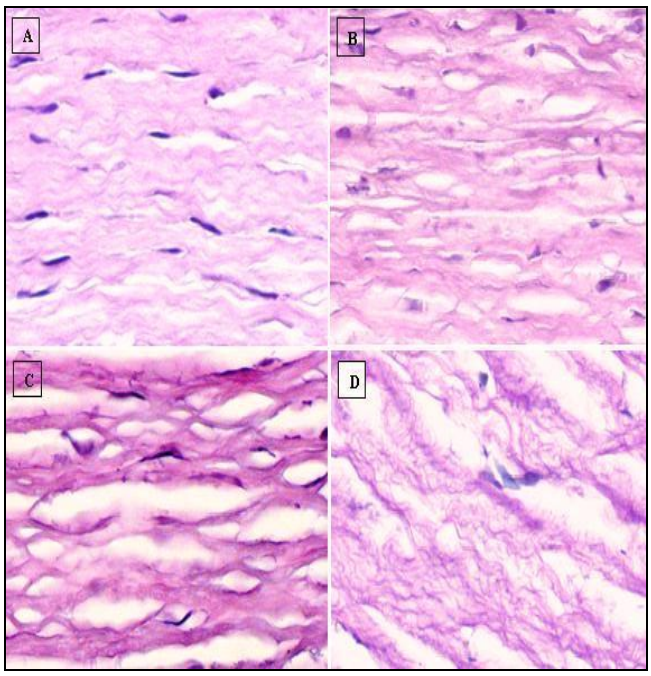

Fig. 1: Photomicrograph of guinea pig cornea (Gp. 2, 3, and 4) showing stromal oedema, seen as empty spaces. Mild oedema (A), Moderate oedema (B), Severe oedema (C), and Severe oedema with lamellar disruption (D). H and E stain. X 400.

STATISTICAL ANALYSIS

Thickness of corneal stroma in the BAC treated and control groups

The statistical analysis of the control and BAC treated groups 1, 2, 3, and 4 using independent sample t-test showed that the thickness of stroma was significantly increased ($p < 0.05$) in the treated groups 2 (μ = 339.933, S.E. = 15.464), 3 (μ = 345.366, S.E. = 13.764), and 4 (μ = 370.400, S.E. = 15.681) (Table. 2).

Table 2 Independent sample t-test comparison

Gp.	t-score	(df)	Significance (2-tailed)	Mean Difference	S. E
1	-1.933	10	0.082	-22.46	11.62
2	-3.609	10	0.005*	-59.13	16.38
3	-4.307	10	0.002*	-62.40	14.48
4	-4.985	10	0.001*	-81.36	16.32

* Significant

Thickness of corneal stroma among the BAC treated groups

Analysis of variance (ANOVA) showed that there was a significant increase in the thickness of stroma among the treated groups 1, 2, 3 and 4. Post-Hoc test, using the Tukey (HSD) showed that this difference was also significant ($p < 0.05$). There was a significant increase in the thickness of corneal stroma in treated group 4 as compared to 1 (Table.3, Fig. 2).

Table 3: Multiple Comparisons of Thickness of Stroma in Treated Groups

Comparison among groups		Mean Difference (I-J)	Std. Error (SE)	P-value
group (I)	group compared (J)			
1	2	-44.833	19.418	0.130
	3	-50.266	19.418	0.076
	4	-75.300	19.418	0.005*
2	1	44.833	19.418	0.130
	3	-5.433	19.418	0.992
	4	-30.466	19.418	0.418
3	1	50.266	19.418	0.076
	2	5.433	19.418	0.992
	4	-25.033	19.418	0.580
4	1	75.300	19.418	0.005*
	2	30.467	19.418	0.418
	3	25.033	19.418	0.580

* Significant

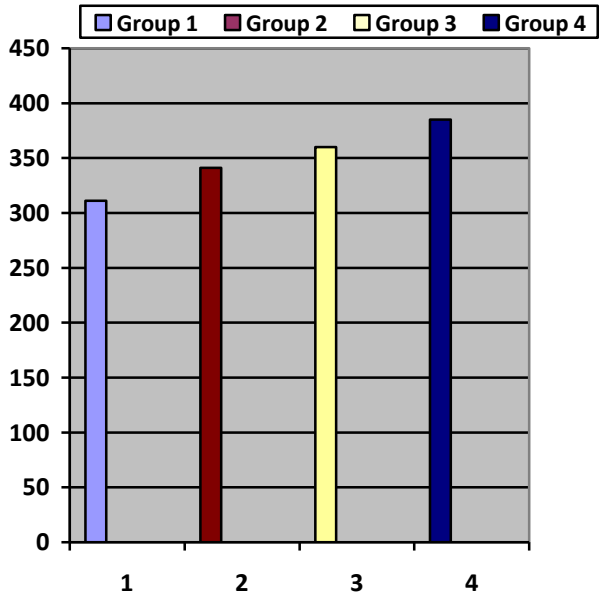

Fig. 2 Mean Thickness (µm) of Corneal Stroma in Treated Groups 1-4

DISCUSSION

BAC interferes with the growth, multiplication, and metabolism of microbial organisms; however, it has similar effects on eukaryotic cells, which accounts for its cytotoxicity[16].

This study was designed to investigate the changes in the stroma of cornea in the experimental animals treated with BAC topically. The central region of cornea was specifically focused in this study because this part of cornea is

not only directly affected by the instillation of eye drops containing BAC but also has clinical implications[18, 19, 20].

In the current study, stromal oedema was observed in long term treated group 2-4 leading to significant increase in the thickness of this layer (Fig. 1). It was seen in the experimental groups 2 ($p < 0.005$), 3 ($p < 0.002$), and 4 ($p < 0.001$) showing proportionate increase in the level of significance with concentration and duration of BAC. The corneal damage caused by chronic treatment with BAC has been reported to produce stromal oedema[21, 22]. Stromal oedema eventually leads to increased light scatter and decrease in refractive index[23]. The increase in stromal thickness, caused by oedema, produces haziness of cornea[23] and is an important factor contributing to visual impairment. Chen W and colleagues[24] also observed significant epithelial and stromal defects in all BAC-treated corneas of rabbit using the concentrations of 0.01, 0.05, and 0.1% concentrations in a confocal microscopic study. Sarkar J[25] and colleagues investigated the effects of topical application of BAC to the eye and concluded that BAC plays major role in corneal neurotoxicity, inflammation, and reduced aqueous tear production. They revealed that the corneal oedema is related to corneal inflammation which affects the corneal thickness.

The analysis of the results shows significant microscopic structural changes in the corneal stroma due to toxicity of BAC in our experimental model. This study provides convincing evidence that BAC is toxic to the cornea, causing structural changes in the corneal stroma ultimately leading to impairment of vision.

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