

Effect of topical dexamethasone and ciprofloxacin on bacterial flora of healthy conjunctiva

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CLINICAL STUDY

Abstract

Purpose To investigate the effects of topical dexamethasone and ciprofloxacin, alone and in combination, on the colony counts of conjunctival bacterial flora of healthy subjects.

Methods A total of 100 healthy subjects were randomly divided into four groups, consisting of 25 subjects each. Group 1 was treated with dexamethasone phosphate 0.1% (Maxidex). Group 2 was treated with ciprofloxacin 0.3% (Ciloxan) and Group 3 with a combination of these drugs. Group 4 received artificial tear solution (Tears Naturale) and served as control. The drops were administered one drop every 1 min. A pretreatment culture and recultures at 30, 90, and 180 min after application of final drops were performed on all subjects. The bacterial growth was evaluated in a quantitative manner. All cultures were performed in a double-masked fashion. ANOVA test was used for statistical analyses.

Results While there was no significant difference among mean number of pretreatment colonies between the groups ($P=0.85$); the differences at 30, 90, and 180 min were statistically significant ($P<0.001$). During the study there was no statistically significant change in colony counts in Groups 1 and 4 ($P=0.66$ and 0.55 , respectively). The colony count numbers decreased significantly at 30 and 90 min in Group 2 ($P<0.001$ and $P=0.004$) and at 30 min in Group 3 ($P=0.002$).

Conclusion Topically applied dexamethasone did not cause a significant change in colony counts of the healthy conjunctiva. Both ciprofloxacin and a combination of ciprofloxacin and dexamethasone were effective in the reduction of bacterial ocular flora.

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Keywords: Conjunctival flora; ciprofloxacin; dexamethasone; healthy conjunctiva

Introduction

Topical corticosteroids are widely used in ophthalmology, alone and in combination with antibiotics. Corticosteroids inhibit the macrophage and neutrophil migration, production of potent chemical mediators, function of immunocompetent cells, and reduce capillary permeability and vasodilatation. It is postulated that this increased spectrum of activity is responsible for increased ocular infection.^{1,2}

In this study, we investigated the short-term effects of topical dexamethasone 0.1% and ciprofloxacin 0.3%, alone and in combination, on the colony counts of conjunctival bacterial flora of healthy subjects. To the best of our knowledge, the effect of topical corticosteroids on ocular bacterial flora has not been investigated. Additionally, it may be important to know whether combined administration of corticosteroid and antibiotic eye drops changes the efficacy of topical antibiotic use.

Materials and methods

A total of 100 (one hundred) healthy subjects (55 men and 45 women) were randomly divided into four groups, consisting of 25 subjects each. A nurse randomly assigned the subjects into the groups. The right eye of each subject was included in the study. The eyes in Group 1 (dexamethasone group) were treated with three drops of dexamethasone phosphate 0.1% (Maxidex, Alcon). The eyes in Group 2 (ciprofloxacin group) were treated with three drops of ciprofloxacin 0.3% (Ciloxan, Alcon) and Group 3 (combination group) with a combination of these drugs, three drops of

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ciprofloxacin 0.3% followed by three drops of dexamethasone phosphate 0.1%. Group 4 (control group) received an artificial tear solution (Tears Naturale, Alcon), which has the same preservative (benzalkonium chloride) as the other two eye drops and served as control. The drops were administered one drop every 1 min. All drops were instilled in to the inferior cul-de-sac.

Before enrolment in the study, patients underwent an ophthalmologic examination. To be eligible, subjects were required to be older than 20 years and to have healthy eyes except from lens opacities. Those who had clinical evidence of inflammatory ocular pathologic features, adnexal or systemic infection, blepharitis, conjunctivitis, previous ocular surgery, evidence of systemic antibiotic or corticosteroid treatment 2 months before the study, or use of topical ocular medications 3 weeks before the study were excluded. Written informed consent was obtained from each subject. All subjects were treated in accordance with the requirements of the Declaration of Helsinki.

A pretreatment culture and recultures at 30, 90, and 180 min after the application of final drops were performed on all subjects. All cultures were performed in a double-masked manner. Neither the person who took the specimens nor the microbiologist who evaluated bacterial growth knew which eye belonged to which group. Specimens were taken by rubbing sterile cotton-tipped swabs, which were moistened with brain–heart infusion broth, four times to the inferior palbebral conjunctiva in an identical manner in all groups of eyes. No topical anaesthetics were instilled. The lower eyelid was pulled down in order to avoid the lid margins and lashes. Afterwards, all specimens were inoculated onto

5% blood agar and chocolate agar immediately. Following 48 h of incubation at 37°C, the bacterial growth was evaluated in a quantitative manner as colony-forming units (CFU). The bacterial identification was made according to standard bacteriologic procedures. The mean of CFU counts on the two was recorded. Eyes with pretreatment CFU values less than 25 were excluded from the study.

Comparisons were made using analysis of variance (ANOVA) techniques. Tukey-HSD test was used to determine the significance of differences in CFU values between the groups. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

There was no significant difference among groups in age (*P* = 0.90) (Table 1).

The mean numbers of pretreatment colonies were 190 CFU in Group 1, 204 CFU in Group 2, 193 CFU in Group 3, and 210 CFU in Group 4. There was no significant difference among groups (*P* = 0.85). The differences of colony counts among the groups at 30, 90, and 180 min were statistically significant (*P* < 0.001) (Tables 2 and 3).

During the study, there was no statistically significant change in colony counts in the dexamethasone and the control group (*P* = 0.66 and 0.55, respectively). The CFU numbers decreased significantly at 30 and 90 min in the ciprofloxacin group (*P* < 0.001 and *P* = 0.004) and at 30 min in the combination group (*P* = 0.002) (Figure 1).

In all, 40 eyes were excluded from the study in which pretreatment CFU values were less than 25. The mean age was 56.5 ± 14.9 years (range 24–70). The isolation rate

Table 1 Patient characteristics

	Group 1	Group 2	Group 3	Group 4	<i>P</i> value
Age (years)					
Mean ± SD	54.9 ± 16.9	57.4 ± 14.8	55.8 ± 16.1	57.7 ± 14.7	0.90
Median	55	57	57	58	
Range	22–68	23–74	20–76	23–79	

(Group 1: dexamethasone, Group 2: ciprofloxacin, Group 3: dexamethasone+ciprofloxacin, Group 4: control)

Table 2 Bacterial colony counts and reduction by time and treatment (Mean ± SD)

Subjects	Time (min)			
	0	30	90	180
Dexamethasone group	190 ± 101	196 ± 108	181 ± 124	163 ± 121
Ciprofloxacin group	204 ± 91	107 ± 54	73 ± 46	65 ± 43
Combination group	193 ± 120	113 ± 66	104 ± 71	107 ± 74
Control group	210 ± 56	224 ± 86	205 ± 117	193 ± 113

Table 3 Differences of bacterial colony counts between the groups at 30, 90 and 180 min

Minute	Group comparison	P
30	Dexamethasone–ciprofloxacin	0.001
30	Dexamethasone–combination	0.003
30	Ciprofloxacin–control	<0.001
30	Combination–control	<0.001
90	Dexamethasone–ciprofloxacin	0.001
90	Ciprofloxacin–control	<0.001
90	Dexamethasone–combination	0.027
90	Combination–control	0.002
180	Dexamethasone–ciprofloxacin	0.002
180	Ciprofloxacin–combination	0.021
180	Ciprofloxacin–control	0.002
180	Combination–control	0.007

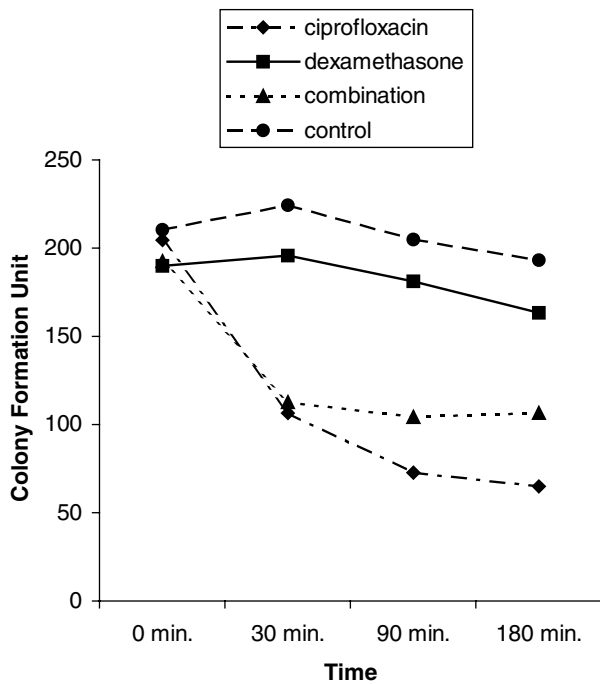


Figure 1 Mean bacterial colony counts and reduction by time and treatment.

of bacteria from the conjunctiva in all eyes was 71.4%. In 19 cases (17%), two or more species were isolated. The cultures of conjunctiva revealed growth of *Staphylococcus aureus* (62%), coagulase-negative staphylococci (43%), *Moroxella* species (6%), *Haemophilus* species (4%), Group B streptococci (2%), and *Proteus* species (2%).

Discussion

The eye features protective mechanisms that are nonimmune in nature such as blink reflex and tears. The

tear film also provides an immunologic barrier to the environment containing lactoferrin, lysozyme, beta-lysin, immunoglobulins, complement, and cytokines, which have antimicrobial effects.³ The conjunctiva contains blood vessels, lymphoid tissue, and immunoreactive cells, including lymphocytes, neutrophils, Langerhans cells, neutrophils, and mast cells. In the normal conjunctival epithelium, small numbers of lymphocytes are interspersed in the epithelial layer, mainly suppressor/cytotoxic T cells.⁴ B cells are present in the substantia propria, but in smaller numbers, arranged in aggregates mostly in the fornices. Langerhans cells are the major antigen-presenting cells of the conjunctiva and are located in the epithelium.⁵ Scattered neutrophils are found in the epithelium and substantia propria. Eosinophils and basophils are not present in normal conjunctiva but are present in disease states.³

The existence of bacterial flora in clinically healthy conjunctiva has been reported.^{6,7} The number of bacteria in the conjunctiva may be important especially in postsurgical eyes. The external ocular tissues of most subjects harbour organisms capable of causing endophthalmitis if introduced into the anterior chamber during surgery. Speaker *et al*⁸ confirmed a previously held belief that most cases of postoperative endophthalmitis are caused by pathogens of the patient’s own flora from the eyelid, conjunctiva, or nose. In our study, topically applied ciprofloxacin significantly reduced colony counts of the healthy conjunctiva during 180 min (Table 2 and Figure 1). Ciprofloxacin can be considered for reducing ocular surface flora before ophthalmic surgery.

Corticosteroids are readily absorbed by the cornea, conjunctiva and sclera after ophthalmic delivery.² Systemic absorption of steroids after topical treatment was reported to be considerable, and if given to a patient with hay fever, it may improve systemic symptoms and decrease the blood eosinophil count.⁹ Steroids may be used in clinical ophthalmic practice for post-traumatic control of inflammation, abnormalities of excessive immunoreactivity, and for diseases that have combined immune and infectious processes. Secondary infections enhanced by corticosteroids can take the form of bacterial conjunctivitis and keratitis. Impaired neutrophil function probably interferes with the control of normal conjunctival flora and predisposes the eye to infections; so it may be important to know the effects of corticosteroid eye drops on ocular bacterial flora. In our study, we found that three drops of topically applied dexamethasone did not cause a significant change in colony counts of the healthy conjunctiva during 180 min (Table 2 and Figure 1).

Corticosteroids exert their effect by binding to receptors in the cytoplasm. The steroid–receptor complex

has a high affinity for nuclear interphase chromosomes and thus binds to chromosomal DNA and leads to new protein synthesis. Cells exposed to corticosteroids synthesize and release phospholipase A₂-inhibitory glycoprotein. The inhibition of phospholipase A₂ leads to a reduction in the release of arachidonic acid, thereby slowing production of arachidonic acid metabolites. Administration of corticosteroids results in a complex series of changes in the actions of cells involved in inflammatory reactions. After a single systemic dose of corticosteroids, there is a net increase in the number of circulating neutrophils, accompanied by a decrease in the margination, migration, and accumulation of neutrophils at sites of inflammation, which reduces the signs of acute inflammation.¹⁰

Corticosteroids directly suppress the action of cells involved in the inflammatory response, inhibiting phagocytosis by neutrophils and monocytes, the production of degenerative enzymes such as collagenase and plasminogen activator by neutrophils, and the production of inflammatory lymphokines and monokines. The inhibition of these factors prevents the vasodilatation and increased vascular permeability components of the inflammatory response.^{1,10,11}

Corticosteroids are commonly used in ophthalmology to decrease postsurgical inflammation and there is evidence linking corticosteroids with postoperative ocular infection.^{2,12,13} Treatment of disorders such as disciform herpes, bacterial corneal ulcers and control of postoperative inflammation is performed with the combined use of topical corticosteroids and antibiotics. In a study, a combination of fluoromethalone and gentamicin eye drops was reported to be effective in the reduction of ocular bacterial flora after cataract surgery.¹⁴ In our study, when dexamethasone and ciprofloxacin were used together, there was a significant reduction in conjunctival flora but less than when ciprofloxacin was used alone. Dexamethasone decreases the reduction of bacterial flora in combination with ciprofloxacin at 180 min, however, the combination reduced colony counts at 30, 90 and 180 min when compared with the control group (Table 3).

The medications used in this study were all preserved with bezalkonium chloride (the concentration is 0.01% for dexamethasone and artificial tear solution, 0.006% for ciprofloxacin). It is possible that bezalkonium chloride may have an effect on conjunctival flora despite a very small amount in three drops.

It is clear that phagocytic killing by leucocytes is an important defence mechanism against bacterial infection, as patients with abnormalities of neutrophil dysfunction are susceptible to recurrent or persistent infections.¹⁵ As

a conclusion, topically applied dexamethasone did not cause a significant change in colony counts of the healthy conjunctiva in the short term. However, it is necessary to investigate whether prolonged use of corticosteroids induces significant changes in the conjunctival flora. Both ciprofloxacin and combination of ciprofloxacin and a dexamethasone were effective in the reduction of ocular bacterial flora.

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