

Prospective randomized comparison of 1-day versus 3-day application of topical levofloxacin in eliminating conjunctival flora

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PURPOSE. To compare efficacy of a 1-day versus 3-day application of topical levofloxacin in reducing ocular surface bacteria.

METHODS. In this prospective randomized controlled trial, 100 volunteer patients (50 per group) were assigned to receive topical 0.5% levofloxacin four times daily for 1 day or 3 days. Conjunctival cultures were obtained prior to (T0) and after the application of antibiotics (T1). Additionally, all patients received topical levofloxacin at 5-minute intervals for three applications (T2), followed by two drops of topical 5% povidone-iodine (T3). Conjunctival cultures were obtained at timepoints T2 and T3.

RESULTS. A 1-day application of topical levofloxacin significantly reduced ($p = 0.0004$) the number of eyes with positive conjunctival cultures from 41 eyes (82%) to 23 eyes (46%). Similarly, a 3-day application significantly reduced ($p = 0.0001$) the positive culture rate from 37 eyes (74%) to 17 eyes (34%). Two drops of povidone-iodine further reduced the positive culture rate for both groups to 20% (10 eyes for each group). There was no significant difference in positive culture rate between the 1-day and 3-day groups at T0 ($p = 0.4689$), T1 ($p = 0.3074$), T2 ($p = 0.6706$), or T3 ($p = 1.000$).

CONCLUSIONS. The application of topical 0.5% levofloxacin for 1 or 3 days significantly reduced the number of eyes with positive conjunctival cultures. The addition of 5% povidone-iodine further eliminated bacteria from the conjunctiva. The application of levofloxacin for 1 day appears to be as effective as a 3-day application. (*Eur J Ophthalmol* 2007; 17: 689-95)

KEY WORDS. Conjunctiva flora, Endophthalmitis, Levofloxacin

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INTRODUCTION

Endophthalmitis is a rare but serious complication of intraocular surgery. The most common bacteria causing endophthalmitis are normal ocular flora, typically from the conjunctiva or eyelids (1, 2). Topical antibiotics and povidone-iodine are commonly used in the perioperative period to prevent infections following ophthalmic surgery (3). Although the use of antibiotics for endophthalmitis prophylaxis is controversial, and despite the recent publication by the European Society of Cataract and Refractive Surgery (ESCRS) demonstrating little benefit in the periop-

erative application of antibiotics in reducing the risk of endophthalmitis (4), it is a common practice among ophthalmologists (5). The risk of endophthalmitis may be decreased by eliminating bacteria from the ocular surface with the use of topical antibiotics and povidone-iodine. The controversy regarding the use of preoperative antibiotic prophylaxis extends to the choice and dosage of the antibiotics. Previous studies have suggested that a 3-day application of ofloxacin is more effective in eliminating bacteria from the conjunctiva than an application 1 hour before surgery (6, 7). An informal survey of practicing ophthalmologists revealed that a common practice is to

initiate topical antibiotic treatment 1 day prior to surgery. The goal of this research was to compare the efficacy of topical 0.5% levofloxacin in starting 1 day versus 3 days prior to surgery. In order to precisely control the exact timing of antibiotic administration and acquisition of conjunctival cultures, volunteer patients were enrolled in the study rather than patients undergoing surgery. Efficacy of the antibiotic is determined by the elimination of bacteria from the conjunctiva.

METHODS

Upon approval from the Institutional Review Board at Stanford University, and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, volunteers were recruited for the study. The methods of recruitment included placement of informational posters and handouts throughout the Stanford Ophthalmology Clinic. A list of patients who had been examined in the ophthalmology clinic and diagnosed with cataracts were also contacted by phone and asked if they would be interested in the study. All study participants gave their informed consent prior to their inclusion in the study. The inclusive criterion was patient age greater than 50 years to match patients who typically undergo cataract surgery. Exclusion criteria were the use of any topical or systemic antibiotics within 30 days of participation in the study, and allergies to iodine or fluoroquinolone antibiotics.

The Excel software program (Microsoft) was used to generate a block of random numbers that were assigned to each volunteer participant. A total of 100 patients were enrolled in the study, with 50 in each of the two study groups. Baseline conjunctival cultures were obtained from both eyes of each study patient prior to the application of antibiotics (T0). The study subjects were asked to use topical levofloxacin (0.5%) four times daily in one randomly selected eye for either 1 day or 3 days. The patients were asked to return to the clinic the day following completion of the topical antibiotic application. Patients with a follow-up visit scheduled at noon or later were asked to apply one additional drop of levofloxacin upon waking on the day of the follow-up visit. Upon arrival to the clinic, conjunctival cultures were again obtained from both eyes of all subjects (T1). The eye that received levofloxacin was the study eye, whereas the eye that did not receive antibiotic served as the control eye. The study

eyes of both groups received three additional applications of topical levofloxacin, 5 minutes apart, and conjunctival cultures were obtained from the study eye only, 45 minutes following the last antibiotic application (T2). Finally, two drops of povidone-iodine were applied to the conjunctival surface, and cultures were obtained from the study eyes after 5 minutes (T3).

Conjunctival cultures were collected by swabbing the inferior fornix with a moistened cotton swab while pulling down the lower lid, carefully avoiding touching the eyelid and eyelashes. The specimen was immediately inoculated onto plates containing sheep blood agar and chocolate agar, and into blood culture broth media (20 mL BBL Septi-Chek, Becton Dickinson and Company, Cockeysville, MD). The blood culture media plates were incubated with 5% carbon dioxide for 3 days to encourage microaerophilic bacterial growth. The chocolate agar plates were incubated in an anaerobic bag for isolation of anaerobic bacteria for at least 10 days. Cultures in the blood media broth were incubated until positive for growth, or a maximum of 6 days. All cultures were kept at 37 °C. At T0, T1, and T2, cultures were obtained from patients who had not received topical anesthetic since preservatives present in the anesthetic may inhibit bacterial growth. Topical proparacaine with preservative was given just prior to the application of povidone-iodine to minimize any stinging sensation. The individuals who obtained the conjunctival cultures and the microbiologist analyzing the culture results were masked with regard to the study groups, and with regard to control or study eye at time points T0 and T1.

Statistical analysis was performed using Fisher exact test and Mann-Whitney test with Analyse-it software program (Analyse-It Software, Leeds, England). A power calculation determined that 98 patients were required in the study to detect a 50% difference between the two study groups, assuming a positive culture rate of 60% or greater at baseline, power of 80%, and α -error of 5%.

RESULTS

The types of bacteria isolated are displayed in Table I. The most common organisms cultured were Gram-positive bacteria, particularly coagulase-negative *Staphylococcus*. The distribution of bacteria was similar among the 1-day and 3-day groups, as well as among the control and study eyes.

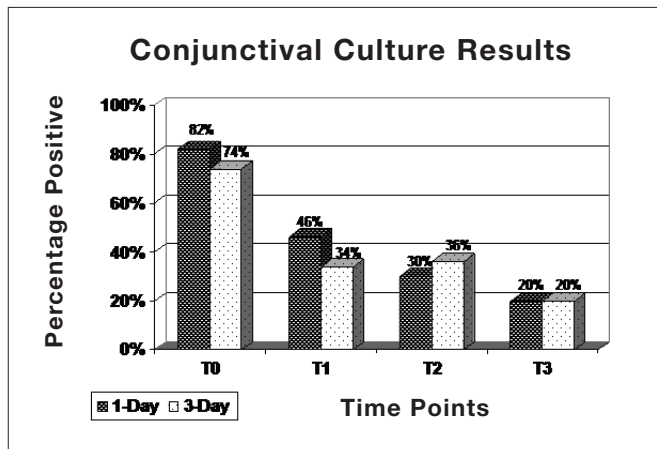


Fig. 1 - Conjunctival culture results of the blood culture broth media. T0 indicates that the samples were obtained at baseline, prior to the application of antibiotics or iodine. T1 was the time point at which the patients received a 1-day or 3-day application of topical levofloxacin. T2 was obtained after an additional three applications of topical levofloxacin, 5 minutes apart. T3 was after the application of topical 5% povidone-iodine.

Table II and Figure 1 summarize the results of the blood culture broth media. The culture results obtained at baseline (T0) were similar between the control and study eyes for the 1-day and 3-day application groups. For the 1-day group, 39 (78%) eyes in the control and 41 (82%) eyes in the study group had positive cultures ($p=0.8031$). In the 3-day group, 38 (76%) and 37 (74%) eyes in the control and study groups had positive cultures, respectively ($p>0.9999$). Because the control eyes did not receive antibiotics, there was no significant reduction in the number of positive conjunctival cultures from T0 to T1. In contrast,

eyes in both the 1-day and 3-day study groups had a significant decrease in the proportion of eyes with positive cultures after the application of topical levofloxacin. The number of positive cultures decreased from a baseline (T0) of 41 eyes (82%) to 23 eyes (46%) after a 1-day application of topical levofloxacin ($p=0.0004$). After 3 days of levofloxacin, the reduction in the number of eyes with positive cultures was from 37 (74%) eyes to 17 (34%) eyes ($p=0.0001$). Similarly, following a 1-day application of antibiotics resulted in only 46% of eyes with positive conjunctival cultures, compared to 76% in the control eyes ($p=0.0038$). After 3 days of topical antibiotics, 34% of eyes in the study group had positive cultures, compared to 66% in the control group ($p=0.0025$).

Additional applications of three drops of topical levofloxacin, 5 minutes apart, further reduced the number of positive cultures from 23 (46%) to 15 (30%) for the 1-day group, but this decrease was not statistically significant ($p=0.1493$). For the group that received 3 days of antibiotics, the three additional doses of topical levofloxacin did not change the positive conjunctival rates. There were 17 (34%) positive cultures at T1 and 18 (36%) at T2 ($p>0.9999$).

The application of two drops of povidone-iodine further reduced the number of positive conjunctival cultures from 15 (30%) positive cultures for the 1-day group and 18 (36%) for the 3-day group to 10 (20%) positive cultures for both groups. However, the decrease was not statistically significant for either group ($p=0.3558$ for the 1-day group and $p=0.1182$ for the 3-day group).

Analysis of the p values for comparison between the 1-day and 3-day group for the study eyes are listed in Table II.

TABLE I - DISTRIBUTION OF BACTERIA ISOLATED AT BASELINE (T0)

	1-day group		3-day group	
	Control, n=50	Study, n=50	Control, n=50	Study, n=50
Coagulase-negative Staphylococcus	34 (72)	39 (78)	34 (74)	31 (72)
Staphylococcus aureus	3 (6)	3 (6)	2 (4)	5 (12)
α -hemolytic Streptococcus	2 (4)	2 (4)	2 (4)	2 (5)
Corynebacterium sp	7 (15)	3 (6)	2 (4)	0 (0)
Micrococcus sp	0 (0)	1 (2)	0 (0)	0 (0)
Propionibacterium acnes	0 (0)	0 (0)	0 (0)	1 (2)
Bacillus sp	0 (0)	1 (2)	0 (0)	1 (2)
Gram-negative rods	1 (2)	1 (2)	6 (13)	3 (7)
Total	47	50	46	43

n indicates the number of eyes in each group. The percentage in parentheses indicates the proportion of bacteria isolated among all bacteria isolated from each group. The total indicates the total number of strains of bacteria isolated

There was no significant difference between the 1-day and 3-day groups for any of the time points collected. The culture results of the blood agar (for microaerophilic and aerobic bacteria) and chocolate agar (for anaerobic bacteria) were stratified into the following categories for data analysis: no growth, 1–10 colony-forming units (CFU), 11–50 CFU, 51–100 CFU, 101–1000 CFU, and >1000 CFU. Table III summarizes the results of the blood and chocolate agar plates.

The overall results of the blood and chocolate agar media demonstrate that the application of topical levofloxacin resulted in a fewer number of positive cultures, and those with positive samples had fewer CFU isolated. Additional applications with povidone-iodine further reduced the number of CFU isolated from the conjunctiva. Statistical analysis using the Mann-Whitney test demonstrated no significant differences between the 1-day and 3-day groups for any of the time points collected.

TABLE II - BLOOD CULTURE MEDIA BROTH RESULTS

	1-day		3-day		p value
	Control eye, n=50	Study eye, n=50	Control eye, n=50	Study eye, n=50	
T0	39 (78)	41 (82)	38 (76)	37 (74)	0.4695
T1	38 (76)	23 (46)	33 (66)	17 (34)	0.3074
T2	N/A	15 (30)	N/A	18 (36)	0.6709
T3	N/A	10 (20)	N/A	10 (20)	1.000

T0 indicates that the samples were obtained at baseline, prior to the application of antibiotics or iodine. T1 was the time point at which the patients already received a 1-day or 3-day application of topical levofloxacin. T2 was obtained after an additional three applications of topical levofloxacin, 5 minutes apart. T3 was after the application of topical 5% povidone-iodine. The number indicates the samples that had positive cultures, and the percentage in parentheses is the proportion of samples that had positive cultures. The p value was calculated using the Fisher exact test comparing the study eyes of the 1-day group versus the 3-day group

TABLE III - RESULTS OF THE BLOOD AND CHOCOLATE AGAR PLATES

CFU	T0		T1		T2		T3	
	1-day	3-day	1-day	3-day	1-day	3-day	1-day	3-day
Blood culture media: study eye								
1–10	17	14	6	9	4	6	2	1
11–50	3	5	2	1	0	0	0	1
51–100	1	2	0	0	0	0	0	0
101–1000	2	0	0	1	0	0	0	0
>1000	1	1	0	0	0	0	0	0
Total	24	22	8	11	4	6	2	2
Chocolate culture media: study eye								
1–10	12	17	12	14	11	14	6	6
11–50	3	5	2	3	3	5	1	0
51–100	2	1	0	1	0	0	0	0
101–1000	0	0	0	0	0	0	0	0
>1000	0	0	0	0	0	0	0	1
Total	17	23	14	18	14	19	7	7

Time point T0 indicates that the samples were obtained at baseline, prior to the application of antibiotics or iodine. T1 was the time point at which the patients received a 1-day or 3-day application of topical levofloxacin. T2 was obtained after an additional three applications of topical levofloxacin, 5 minutes apart. T3 was after the application of topical 5% povidone-iodine. CFU indicates the number of colony-forming units. The number indicates the number of samples (out of 50 samples) that had the corresponding CFU growth on each of the media plates. Total indicates the number of samples with positive cultures

There was a significant decrease in the number of samples with positive growth on the blood agar plates after both the 1-day and 3-day application of antibiotics (T1) compared to baseline (T0). For the 1-day group, positive cultures were reduced from 24 samples to 8 samples ($p=0.0011$). Similarly, the 3-day group exhibited a decrease from 22 to 11 positive samples ($p=0.0327$). Furthermore, topical levofloxacin reduced the number of bacteria present on the conjunctiva. The results of the blood culture solid media showed that seven eyes in the 1-day group and eight eyes in the 3-day group had more than 10 CFU isolated from the conjunctiva at baseline (T0). The application of topical levofloxacin effectively killed bacteria, such that no eyes had more than 10 CFU isolated from the conjunctiva at T2. The application of topical levofloxacin resulted in a significant reduction of bacteria for both the 1-day ($p<0.0001$) and 3-day ($p=0.007$) groups at T2 compared to T0.

The number of samples with positive cultures on the chocolate agar plates decreased from 17 to 14 after a 1-day application of antibiotics ($p=0.6658$). A similar finding was seen with the 3-day group, in which positive cultures were reduced from 23 to 18 samples ($p=0.4162$). The application of iodine significantly reduced the number of positive cultures to seven samples for both the 1-day and 3-day groups ($p=0.0209$ and $p=0.0011$, respectively). The results of the chocolate agar plates demonstrated a reduction in the number of samples with greater than 10 CFU after the application of antibiotics in both the 1-day (from 5 to 3 samples) and 3-day groups (from 6 to 5 samples), although the difference was not significant. The application of topical povidone-iodine further reduced the numbers of samples with greater than 10 CFU to just 1 sample for both the 1-day ($p=0.2044$) and 3-day ($p=0.1117$) groups.

DISCUSSION

Given that postoperative endophthalmitis is most commonly caused by bacteria from the eyelids and conjunctiva, it is conceivable that reduction of bacteria from these sites may decrease the risk of endophthalmitis. Povidone-iodine has been shown to significantly decrease the risk of endophthalmitis (8, 9). Although topical antibiotics have not been demonstrated to lower the risk of endophthalmitis, their use significantly reduces the number of bacteria present on the conjunctiva (10). Furthermore, it has been

shown that a combination of topical antibiotics and povidone-iodine has greater efficacy in eliminating bacteria than either agent alone (10). Despite the recent publication by the ESCRS demonstrating little benefit in perioperative antibiotic prophylaxis (4), topical antibiotics continue to be used in the perioperative period in an attempt to decrease the risk of infection. The recent ESCRS study (4) demonstrated that topical levofloxacin given 1 hour prior to surgery did not significantly reduce the risk of endophthalmitis. However, it has been shown that antibiotics require more than an hour to be effective (7). A study by Ta and colleagues (7) demonstrated that a 3-day application of ofloxacin is significantly more effective in eliminating conjunctival bacterial flora than a 1-hour application, even with concomitant use of povidone-iodine. Therefore, the current study is designed to determine whether a 1-day course of levofloxacin is as effective as a 3-day application.

In an informal survey among local ophthalmologists, we found that a common practice is to prescribe a topical antibiotic just 1 day prior to surgery. Furthermore, surgeons who operate in the afternoon usually advised their patients to use the prescribed antibiotic on the morning of the surgery. In contrast, patients who are scheduled for surgery in the morning are not asked to use their antibiotic on the day of the surgery. Based on this premise, we designed a study of volunteer patients that mimics clinical practice as much as possible.

In the current study, we compared the efficacy of a 3-day versus a 1-day application of levofloxacin in a prospective randomized masked control study. Our results demonstrate that a 1-day application of levofloxacin is as effective in eliminating bacteria from the conjunctiva as is a 3-day application. The positive culture rate was reduced from 82% to 30% for the 1-day group and from 74% to 36% for the 3-day group after the application of antibiotics. The clinical relevance of our study is that for ophthalmologists who normally prescribe antibiotics preoperatively, instructing the patient to use a topical antibiotic just the day prior to surgery is reasonable, and that a 3-day course of antibiotics is probably not necessary to optimize elimination of bacteria from the ocular surface.

Previously, we have reported that a 3-day application of topical ofloxacin is more effective in eliminating bacteria from the conjunctiva compared to a 1-hour application (7). We have also reported that a 3-day application resulted in a significantly greater reduction in the contamination rate of the microsurgical knives used for paracentesis

compared to a 1-day application (6). Although the current study suggests that a 1-day application of levofloxacin is just as effective as a 3-day application, our previous study showed that a 1-hour application of ofloxacin is not effective in eliminating bacteria from the conjunctiva (7). It is noteworthy that the efficacy of a 1-day course of levofloxacin in eliminating bacteria from the conjunctiva in the current study is equivalent to the efficacy of a 3-day course of ofloxacin in a previous study (82% decreased to 46% and 68% decreased to 45%, respectively) (7).

Bacteria are commonly found on the conjunctiva and in the anterior chamber aqueous fluid at the time of the surgery (7, 11-14). However, only a very small percentage of patients develop endophthalmitis. It may be that there are several factors playing key roles in the pathophysiology of endophthalmitis. These may include the virulence of the bacteria, the patient's immune status, and the bacterial load at the time of inoculation. Therefore, the number and types of bacteria on the surgical field, and not just their presence, may be important. We attempted to quantify the number of bacteria present on the conjunctiva by determining the CFU on blood and chocolate agar plates. The results show that topical levofloxacin effectively reduced the number of bacteria present on the conjunctiva. There are several important considerations in interpreting the results of this study. First, the patient population was a group of volunteer participants, rather than actual surgical patients. Although we expect the types of bacteria and the efficacy of treatment of volunteer participants to be similar to that of surgical patients, the compliance of volunteer patients using antibiotics may be less than ideal. Therefore, the efficacy of topical antibiotics may be underestimated. Second, the periorbital skin and eyelids were not scrubbed with povidone-iodine as is typically performed prior to surgery. Third, although we showed that the use of a combination of topical antibiotics and povidone-iodine is very effective in reducing the number of or eliminating bacteria from the conjunctiva, we do not have data on the efficacy of povidone-iodine alone without the use of topical antibiotics. However, previous studies have shown that the combination of topical antibiotics and povidone-iodine is more effective in reducing conjunctival bacterial flora than either agent alone (10). Finally, our data only demonstrated the efficacy of topical antibiotics and povidone-iodine on the conjunctival bacterial flora, and not on the actual rate of endophthalmitis. Therefore, although we showed a reduction in the contamination rate of the conjunctiva, it does not necessarily

imply that the risk of endophthalmitis is decreased with antibiotic application.

In summary, topical 0.5% levofloxacin is highly effective in eliminating or reducing bacteria from the conjunctiva. Topical povidone-iodine further eliminates bacteria from the conjunctiva. The application of topical levofloxacin for 1 day appears to be as effective as a 3-day application. The advantages of a 1-day compared to a 3-day application include simpler instructions for patients and possibly improvement in patient compliance. By reducing or eliminating bacteria from the conjunctiva, the risk of endophthalmitis following ophthalmic surgery may be decreased.

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