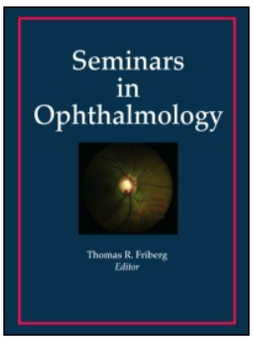

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ORIGINAL ARTICLE

Efficacy of Topical Ofloxacin 0.3 % Administration on Conjunctival Bacterial Flora in Diabetic Patients Undergoing Intravitreal Injections

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ABSTRACT

Purpose: This prospective, randomized case series study aims to evaluate the efficacy of ofloxacin 0.3% eye drops in eradication of conjunctival bacterial flora in diabetic patients undergoing intravitreal injections (IVI). *Methods*: Ninety-two diabetic patients (92 eyes) scheduled to undergo intravitreal injection of ranibizumab due to diabetic macular edema were enrolled in the study. Patients were randomly assigned to three different groups. Group 1 (n=32) received ofloxacin eye drops the day before before IVI (four times); patients in Group 2 (n=29) were administered ofloxacin one hour before IVI (every 15 minutes), while Group 3 (n=31) comprised patients that received combined administration of ofloxacin both one day and one hour before IVI (eight doses). Samples were collected from the injection site before and after antibiotic administration. Culture results from BACTEC broth and positive cultures in blood agar and Sabouraud's dextrose agar plates were measured. *Results*: In Group 1, BACTEC broth positive cultures decreased from 84.4% at baseline to 50% after ofloxacin administration (p=0.007), and blood agar positive cultures reduced from 65.63% to 34.38% (p=0.02). In Group 2, positive cultures significantly decreased in BACTEC broth (from 79.3% at baseline to 48.28%; p=0.027) and in blood agar (from 68.97% to 37.13%; p=0.034). In Group 3, positive cultures decreased from 77.42% at baseline to 32.26% (p=0.0008) and from 58.06% at baseline to 22.58% (p=0.009) in BACTEC broth and blood agar, respectively. No microorganisms were isolated from Sabouraud's dextrose agar plates. *Conclusions*: The combined one day/one hour (eight doses) ofloxacin administration in diabetic patients is extremely effective in reducing conjunctival bacterial flora. The application of topical ofloxacin for one day or one hour before IVI is also significantly effective.

Keywords: Antibiotics, conjunctival bacterial flora, diabetics, intravitreal injections, ofloxacin

INTRODUCTION

During the last few years, intravitreal administration (IVI) of the anti-vascular endothelial growth factor (anti-VEGF) agents has been established as a promising treatment modality of diabetic macular edema (DME). As a result, many diabetics undergo IVIs for a prolonged period of time, even on a monthly basis.[1]

Although the results of anti-VEGF administration are encouraging, the procedure of IVI is not without potential complications, the most serious and feared of which is infectious endophthalmitis. Even though post-injection endophthalmitis is rare, when it occurs it might result in significant vision loss or even loss of the eye. Consequently, with the advent of anti-VEGF factors for the treatment of DME, post-injection endophthalmitis is of rising concern in diabetic patients.[2]

Indeed, diabetic patients constitute a specific group of patients. Firstly, there are implications of being at increased risk of postoperative endophthalmitis.[3] In addition, ocular bacterial flora has been examined and was found to differ from that of non-diabetic patients. Several studies have demonstrated that increased frequency of positive conjunctival cultures, differences in the identified microorganisms, and more

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pathogenic bacteria were prevalent in diabetic patients compared to non-diabetics.[4–8]

It has been postulated that reducing or eliminating conjunctival bacteria flora before IVI prevents post-injection endophthalmitis. Topical administration of antibiotics is considered by many retina specialists to be a preventive measure before IVIs.[9,10] The choice of antibiotic used for endophthalmitis prophylaxis is important. Administration of broad-spectrum bactericidal agents, such as fluoroquinolones, is considered an effective option. The primary objective of our study was to evaluate, for the first time in the literature, the efficacy of ofloxacin in eliminating conjunctival bacterial flora in diabetic patients undergoing IVIs.

MATERIALS AND METHODS

A total of 92 diabetic patients (92 eyes) were enrolled, in a consecutive manner, in this prospective study during a six-month period. The diabetic patients who were examined and scheduled to undergo IVI of ranibizumab due to DME were eligible for the study. Exclusion criteria were age <18 years, any active ocular infection, and any use of systemic or topical antibiotics within 30 days of study enrollment. The study was conducted in compliance with the Declaration of Helsinki. The Institutional Review Board's approval was obtained, and all patients provided a signed informed consent.

Patients were randomized to three different groups. Group 1 (n=32) comprised patients that received ofloxacin 0.3% (Exocin, Allergan Pharmaceuticals Ltd, Ireland) eye drops one day before the scheduled IVI (one drop four times the day before therapy); patients in Group 2 (n=29) were administered ofloxacin one hour before IVI (one drop every 15 minutes), while in Group 3 (n=31) patients received combined administration of ofloxacin for one day and one hour before injection (eight total doses).

Samples were collected at baseline—before any antibiotic instillation and after ofloxacin administration—on the day of the IVI just before the IVI. A single, moistened cotton swab (Transwab® Amies, MW170, MWE, Becton Dickinson, Franklin Lakes, NJ) was used at each time point. Cultures were collected from the IVI site that was the inferotemporal bulbar conjunctiva 3.5–-4 mm posterior to the limbus. No topical anaesthetic was instilled in order to avoid potential alteration of bacterial growth by preservatives. Cultures that were taken the day of IVI were collected before the application of povidone-iodine (PVI). Extreme care was taken to avoid contact with lid margins and eyelashes while collecting the cultures. If a swab was or was suspected to be accidentally contaminated, it was discarded and a new sterile swab was used to re-collect a culture.

The IVIs of ranibizumab were then performed with all of the appropriate precautions. More precisely, we applied topical 10% PVI to scrub the eyelids and eyelashes, placed a sterile adhesive eye drape and used an eyelid speculum, and instilled 5% PVI on the ocular surface.[11]

The collected culture swabs were transported to the lab at ambient temperature and analyzed within two hours from sampling time. Each swab was smeared onto 5% horse blood agar plates, Sabouraud's dextrose agar plates, and then inoculated into a vial of BACTEC culture broth (BACTEC Peds Plus/F, BD). The blood agar and Sabounaud's agar plates and the BACTEC vials were incubated at 37±1°C for two, two, and five days, respectively. A clouding of BACTEC culture vials was recorded as a positive result, which was an evidence of bacterial growth. Cultures on blood agar and Sabounaud's agar plates were deemed positive if one or more colony forming units (CFUs) were observed, while the absolute numbers of CFUs were calculated. The isolated microorganisms were then identified by standard microbiologic methods: gram stain, oxidase test, catalase test, coagulase test and API test (API Staph, Ref 20 500, Biomerieux).

Statistical Analysis

Statistical analysis was performed using the paired samples Student's *t*-test. For the counts of CFUs on the blood agar culture media, the nonparametric Mann–Whitney *U* test was used. A p value less than 0.05 was considered statistically significant. The analysis was performed using SPSS 15.0 software (SPSS, Inc., Chicago, IL).

RESULTS

Among the 92 diabetic patients enrolled, 52 were males and 40 females. The mean age of the patients was 67.3 ± 8.6 years. All of the patients underwent IVI of ranibizumab due to diabetic macular edema. The most commonly isolated bacteria in the baseline samples were coagulate negative *Staphylococcus*, *Staphylococcus aureus*, *Streptococcus* species and *Propionibacterium Acnes*.

The percentages of positive results after inoculation in BACTEC culture vials and of positive cultures on blood agar plates in each group are shown in Tables 1 and 2, respectively. The numbers of CFUs on blood agar cultures of each group are summarized in Table 3. No microorganisms were isolated from the Sabouraud's dextrose agar plates.

One-day application of ofloxacin in Group 1 of the diabetic patients resulted in a statistically significant decrease in the number of eyes with positive bacterial cultures in BACTEC broth media (p=0.007) and blood agar (p=0.02). Similarly, in Group 2, the application of

TABLE 1. Number of positive cultures in BACTEC broth vials at baseline and after ofloxacin 0.3% instillation in each group of diabetic patients.

Positive cultures in BACTEC broth vials						
	Group 1 (n=32)		Group 2 (n=29)		Group 3 (n=31)	
Time of sample collected	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation
Positive	27/32 (84.4%)	16/32 (50%)	23/29 (79.3%)	14/29 (48.28%)	24/31 (77.42%)	10/31 (32.26%)
p*	0.007		0.027		0.0008	

*Student's t-test.

TABLE 2. Number of positive cultures for blood agar at baseline and after ofloxacin 0.3% instillation in each group of diabetic patients.

Positive cultures in blood agar						
	Group 1 (n=32)		Group 2 (n=29)		Group 3 (n=31)	
	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation
Positive cultures	21/32 (65.63%)	11/32 (34.38%)	20/29 (68.97%)	11/29 (37.13%)	18/31 (58.06%)	7/31 (22.58%)
p *	0.02		0.034		0.009	

*Student's t-test.

TABLE 3. Number of colony-forming (CFU) units in each group of diabetic patients measured on blood agar media.

Colony-Forming Units (CFU) on blood agar culture plates						
	Group 1 (n=32)		Group 2 (n=29)		Group 3 (n=31)	
Number of CFU	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation	Baseline	After ofloxacin instillation
Median	1.5	0	1	0	1	0
Range	0–182	0–15	0–122	0–7	0–151	0–3
p *	<0.0001		<0.0001		<0.0001	

*Mann–Whitney U test.

ofloxacin for one hour resulted in a significant decrease in the rate of positive cultures in BACTEC broth and blood agar (p=0.027 and 0.034, respectively). The reduction of bacteria from the conjunctiva that is caused after the combined one-day and one-hour (eight doses) ofloxacin administration in diabetic patients was extremely effective. More precisely, in BACTEC broth media, the number of positive cultures reduced more than half compared to baseline (p=0.0008). Similar results were noted in the number of positive blood agar cultures (p=0.009).

DISCUSSION

During the last few years, treatment with IVI of anti-VEGF drugs has become a new, promising approach against DME. Intravitreal injection of ranibizumab has been approved by the FDA for the treatment of DME.[12] Furthermore, recent, prospective, multicenter clinical trials showed that prolonged treatment with IVI of ranibizumab has excellent visual outcome in patients with DME. However, frequent IVIs may be required on an outpatient basis.[2,13] As a consequence, a dramatic increase has been demonstrated—and further increase is expected—in both the number of diabetics receiving IVIs and the number of injections performed in each patient separately.

Although rare, the most devastating potential complication of IVI is infectious endophthalmitis, which, despite early detection and intervention, can result in substantial morbidity. The rate of endophthalmitis after IVI varies in the literature but is estimated from 0.006% to 0.16% per injection.[14] Nevertheless, the

actual risk per patient of post-injection endophthalmitis is greater than the estimated risk per IVI, since repeated IVIs are frequently administered in each patient.[15]

Diabetes increases susceptibility to various types of infections due to immunologic alterations. Accordingly, diabetic patients might be more predisposed to postoperative endophthalmitis.[3] Based on that premise, there is a postulation that diabetics undergoing IVI of drugs such as anti-VEGFs may be more susceptible to post-injection endophthalmitis. In addition, since IVI of anti-VEGFs tends to become the treatment of choice for DME and this procedure is performed even on a monthly basis in each patient, the number of diabetic patients developing post-injection infectious endophthalmitis is expected to increase.

It is known that the main source of bacteria isolated in cases of post-injection endophthalmitis is the patient's own conjunctival bacterial flora.[16] Interestingly, several studies have demonstrated that the conjunctival flora in diabetic patients differs to a certain extent from that of non-diabetic patients.[4–8] The frequency of positive conjunctival cultures was significantly higher in diabetic compared to non-diabetic patients. Coagulase-negative *Staphylococci* were the most frequent microorganisms isolated, while *Staphylococcus epidermidis* was the most common coagulase-negative *Staphylococcus*.[4] Furthermore, Bilen et al. have shown that *Staphylococcus epidermidis* and *Staphylococcus aureus* were the two most commonly isolated bacteria from the conjunctiva of patients with diabetes.[5] Finally, in two retrospective studies of patients undergoing cataract surgery, preoperative conjunctival bacterial cultures showed that *Staphylococcus aureus, Enterococci*, certain *Streptococci* (except *Streptococcus pneumoniae*), and *Klebsiella* species were more prevalent in diabetic patients.[6,8] Gram-negative bacteria and coagulase-negative *Staphylococci* have been described as the most common pathogens causing endophthalmitis in diabetic patients.[4] Regarding post-injection endophthalmitis, coagulase-negative *Staphylococci* and *Streptococci* cause the most cases of endophthalmitis after IVI of anti-VEGF factors.[17,18]

In our study, we aimed to evaluate the changes in conjunctival flora in diabetic patients after administration of antibiotic before IVI. We used a second-generation fluoroquinolone, ofloxacin, a broad-spectrum bactericidal antibiotic that is active against both Gram-positive and Gram-negative bacteria. Ofloxacin was administered in different dosage regimens in diabetic patients scheduled to undergo IVI of ranibizumab. We tried to evaluate the effectiveness of one day, one hour, and combined one day/one hour topical ofloxacin in eradication of conjunctival bacterial flora. The efficacy of these regimens was estimated counting the number of positive BACTEC broth cultures and the number of positive cultures in blood agar from samples taken from the injection site before IVI. According to our results, ofloxacin is fairly effective in eliminating conjunctival bacteria flora in diabetic patients. More precisely, it resulted in a significant reduction in the percentage of eyes with positive bacterial cultures compared with baseline. However, administration could not completely eliminate all bacteria. Ofloxacin instilled either one hour or one day before IVI resulted in a statistically significant reduction in the percentage of eyes with positive bacterial cultures. However, the instillation of ofloxacin one day before IVI (four doses), combined with four doses within one hour before the injection in group 3, showed the best results. According to the design of the study, no cultures were taken after PVI instillation, since several studies have demonstrated the potent antimicrobial power of this preparation.[19]

In order to reduce the risk of endophthalmitis after IVI, several precautions are taken from retina specialists. However, currently there is no consensus upon IVI technique or pre or post-injection care and best-practice patterns have not been established yet. In a survey on the IVI technique practice patterns of retinal specialists in the United States, it was found that nearly one third of participants administrate prophylactic topical antibiotics pre-injection—either for several days or immediately prior to IVI—while about 81% of doctors in the US and 74% in the UK use prophylactic topical antibiotics post-injection.[9,10] The available evidence regarding endophthalmitis prophylaxis is mainly based on intraocular surgery, particularly cataract surgery.[11] Accordingly, the most accepted prophylactic measure taken before IVI is the preparation of the injection site with topical PVI.[20] On the other hand, there is conflicting evidence about the effect and the necessity of antibiotic prophylaxis in preventing post-injection endophthalmitis.[20] Pretreatment with topical administration of antibiotics is based on the rationale that such application may have a synergistic effect with PVI and enhance the natural defense system in eliminating ocular surface bacteria at the injection site that could enter the intraocular environment during or after the IVI. Elimination of bacterial flora lowers the risk of infection by direct inoculation of pathogens into the sterile intraocular environment at the time of the injection or during the immediate post-injection period where bacteria could enter the vitreous cavity through the needle tract that has been created.[19,21]

Review of the literature demonstrates controversial results. In one study, Moss et al. conclude that pre-injection antibiotic use, combined with PVI, confers no additional benefit in comparison to PVI alone.[19] On the other hand, Stewart et al. conducted a prospective multicenter study and evaluated the needle contamination in patients undergoing IVI. In these patients, except from PVI irrigation, no pre-injection antibiotics were used. The authors reported that bacterial contaminants were present on a substantial proportion of the used needles, indicating that disinfection with PVI might not effectively eliminate the conjunctival bacterial flora.[22] However, since endophthalmitis is a rather rare complication of IVI, controlled prospective clinical trials enrolling a large sample of patients are required to provide definitive conclusions regarding the role of antibiotics

administered pre- or post-injection and endophthalmitis risk.[20] Instead, surrogate markers, such as evaluation of the reduction of conjunctival bacterial flora, are frequently used to study the effectiveness of a prophylaxis regimen, such as administration of antibiotics.[11,15] Reducing the number of bacteria on the ocular surface theoretically results in a decrease in the risk of post-injection endophthalmitis.

To date, no studies have assessed the efficacy of pre-injection administration of ofloxacin in diabetic patients. Among specialists, there is a broad consensus in using PVI, which has become the standard of care in the prevention of post-injection endophthalmitis. However controversy still exists as to the efficacy of prophylactic administration of antibiotics during IVIs. Prophylactic measures for minimizing the risk of endophthalmitis in diabetic patients in the advent of IVI of anti-VEGFs should be used if they are safe and cost-effective. We have chosen to administer ofloxacin pre-injection and our results indicate its effectiveness in eliminating conjunctival bacterial flora, even if it is instilled four times one hour just before IV. This dosage scheme seems to be convenient and sufficient enough to significantly reduce conjunctival bacterial flora in diabetic patients. Nevertheless, further research and investigation are required in order to examine if there is an actual and justified role for antibiotic prophylaxis in diabetic patients undergoing IVIs.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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