Antibiotic Resistance of Ocular Surface Flora WithRepeated Use of a Topical AntibioticAfter Intravitreal Injection

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Importance: Treatment with intravitreal (IVT) injections has increased during the last several years as evidence has accumulated demonstrating the efficacy of anti-vascular endothelial growth factor agents in the treatment of neovascular age-related macular degeneration (AMD) and various retinal vascular diseases. Although IVT injections are generally safe, infectious endophthalmitis is a rare but devastating complication, and the risk of morbidity and vision loss from endophthalmitis is high.

Objective: To examine the change in antibiotic resistance of ocular surface flora with repeated prophylactic use of antibiotics after IVT injection for AMD.

Design and Setting: Prospective, nonrandomized cohort study in 2 tertiary academic hospitals.

Participants: Patients 65 years and older with newly diagnosed AMD were recruited by 7 retinal specialists from July 1, 2010, through December 31, 2011.

Intervention: The study group received topical moxifloxacin hydrochloride for 3 days after each monthly IVT injection.

Main Outcome Measure: Resistance to moxifloxacin and ceftazidime in cultured isolates at baseline and monthly for 3 months by change in minimal inhibitory concentration (MIC) of culture isolates was studied.

Results: The study group consisted of 84 patients, and the control group had 94 patients. In the study group, the baseline adjusted MIC increased (from 1.04 to 1.25 \( \mu g/mL; P = .01 \)) as did the MIC for 50% of isolates (MIC\(_{50}\)) (from 0.64 to 1.00 \( \mu g/mL \)) and the MIC for 90% of isolates (MIC\(_{90}\)) (from 0.94 to 4.00 \( \mu g/mL \)). In both groups, the culture-positive rate did not change significantly when adjusted for baseline. No significant change was found in the MIC level, culture-positive rate, MIC\(_{50}\) level, and MIC\(_{90}\) level in the control group. Subgroup analysis found diabetes mellitus to be noncontributory to both the MIC and culture-positive rate. No endophthalmitis or adverse events were reported.

Conclusions and Relevance: Repeated use of topical moxifloxacin after IVT injection significantly increases antibiotic resistance of ocular surface flora. We recommend that routine use of prophylactic antibiotics after IVT injection be discouraged.

Trial Registration: clinicaltrials.gov Identifier: NCT01181713

isolates being resistant. However, these studies were observational and lacked controls or standardization. Kim and Toma conducted a randomized study on change in antibiotic resistance after repeated topical fluoroquinolone use, which revealed an increase in resistance in the treated eye compared with the fellow (control) eye in 24 patients. Antibiotic resistance in these studies was quantified by the Kirby-Bauer diffusion disk method rather than the specific minimal inhibitory concentration (MIC), the standard in quantifying resistance change in microbiology studies.

Because of the low rate of endophthalmitis, studying the effect of prophylactic antibiotics on the rate of endophthalmitis directly would be impractical. In this study, we examine the change in resistance of ocular surface flora with repeated use of an antibiotic after IVT injection. Our study examines the specific MIC in each isolate and consists of the largest sample with controls in the literature so far.

**METHODS**

**OUTCOMES**

Cultures of the ocular surface were obtained with a cotton-tipped applicator of the inferior fornix after instillation of 1 drop of preservative-free minims of tetracaine hydrochloride, 0.5%. An effort was made to minimize contamination from the lashes and skin. The swabs were transported in an amine charcoal media and processed centrally. The swabs were plated on a blood agar and chocolate agar plate and incubated at 35°C in carbon dioxide. The plates were read daily for 2 days, and any growth was documented. Susceptibility testing to moxifloxacin (epi-lometer test, Etest strips; bioMerieux Clinical Diagnostics) was completed for all gram-positive organisms, and ceftazidime susceptibility testing (VITEK2 sensitivity cards, AST-N096; bioMerieux Clinical Diagnostics) was added for Enterobacteriaceae and *Pseudomonas* species. Susceptibility was reported as the MIC, defined as the minimum concentration of moxifloxacin that inhibits a particular isolate. Calculation for the percentage of resistant isolates, MIC for 50% of isolates (MIC<sub>50</sub>), and MIC for 90% of isolates (MIC<sub>90</sub>) were based on the MIC obtained. According to the Clinical and Laboratory Standards Institute guidelines, an isolate of coagulase-negative *Staphylococcus* was considered to be sensitive if the MIC was less than or equal to 0.5, to have intermediate sensitivity if the MIC was equal to 1.0, and to be resistant if the MIC was more than or equal to 2.0.

**INTERVENTION**

A total of 3 loading-dose injections with IVT ranibizumab were given during the first 3 months, after which administration of IVT ranibizumab was determined on an individual basis. Patients were divided into 2 groups based on the treating physicians’ usual practice, with 4 physicians routinely providing postinjection prophylactic antibiotics and 3 not providing prophylactic antibiotics. As such, one group of patients received topical moxifloxacin 4 times per day for 3 days after each IVT injection (intervention group), whereas the other group did not receive any prophylactic antibiotics (control group).

The IVT injections were administered in standard fashion after obtaining informed consent and a culture. Anesthesia was achieved with topical tetracaine hydrochloride, 0.5%; proparacaine hydrochloride, 0.5%; or lidocaine gel, 2%. Povidone-iodine, 5%, drops followed by a 10% povidone iodine swab stick painted over the injection site were applied to the ocular surface, and a sterile eyelid speculum was used. Ranibizumab, 0.5 mg in 0.05 mL, was injected through the pars plana in the inferotemporal or superotemporal quadrant with a sterile, 30-gauge hypodermic needle.

**PARTICIPANTS**

Patients 65 years and older with newly diagnosed neovascular AMD were recruited from July 1, 2010, through December 31, 2011. Exclusion criteria included the presence of an active ocular, periorcular, or systemic infection; previous treatment with IVT medications; or treatment with topical or systemic antibiotics within the preceding 3 months. Patients unable to attend the scheduled follow-up appointments, complete treatment, or give informed consent were also excluded from the study. Recruitment was completed at 2 tertiary academic hospitals. After informed consent was obtained, patients were enrolled into the study by 1 of 7 retina specialists.

Data collected included age, sex, and medical history, including diabetes mellitus and previous intraocular surgery. A thorough ocular examination was completed at baseline. After a diagnosis of neovascular AMD was established, a culture of the conjunctival surface was obtained before IVT injection with ranibizumab. As per the study protocol, patients were seen monthly for 3 months, and at each follow-up visit a history and ocular examination were completed. For the first 2 follow-up visits, a culture of the conjunctival surface was obtained before repeat IVT injection with ranibizumab. On the third follow-up visit, a fourth culture of the conjunctival surface was obtained regardless of whether an IVT injection with ranibizumab was administered. Any incidence of endophthalmitis or serious adverse event was recorded and reviewed on a quarterly basis.

**SAMPLE SIZE CALCULATION**

The current literature reports a mean resistance of ocular surface flora to fourth-generation fluoroquinolones of 4.7% (range, 0%-5.7%). In performing a sample size calculation, we anticipated an increase in resistance from approximately 5% to 20% based on a documented resistance change of second- and third-generation fluoroquinolones of approximately 13%. With a power of 0.8 and an α of .05, we estimated that 142 patients would be required. To account for a coagulase-negative *Staphylococcus* culture-positive rate of 84.9%, the recruitment size was adjusted to 168 patients (84 patients in each study arm).

**STATISTICAL ANALYSIS**

Statistical analysis was performed with SAS Educational Analytic Suite software, version 9.3 (SAS Institute, Inc). A mixed-
Table 1. Baseline Demographic Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Intervention (n = 77)</th>
<th>Control (n = 88)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>80.9</td>
<td>81.2</td>
<td>.71</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>51 (60.7%)</td>
<td>65 (71.1%)</td>
<td>.24</td>
</tr>
<tr>
<td>Male</td>
<td>33 (39.3%)</td>
<td>29 (28.9%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 (11.9%)</td>
<td>8 (8.5%)</td>
<td>.37</td>
</tr>
<tr>
<td>Previous intraocular surgery</td>
<td>46 (54.8%)</td>
<td>51 (54.3%)</td>
<td>.59</td>
</tr>
</tbody>
</table>

Table 2. Number of Organisms Cultured

<table>
<thead>
<tr>
<th>Organism</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococcus</td>
<td>36</td>
<td>46</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Diphtheroids (Corynebacterium spp)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus viridans</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxella</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Mean culture-positive rates with adjustment for baseline. The study group had the higher positive rates at all time points; however, no significant increases were seen from month 1 to 3. Error bars indicate SD.

Figure 2. Culture-positive rate, mean (±SD).

RESULTS

PATIENT CHARACTERISTICS

A total of 178 patients were enrolled in the study, with 84 in the intervention group and 94 in the control group, from June 1, 2010, through December 31, 2011. Seven patients in the intervention group and 6 patients in the control group did not complete the study. The reasons for loss to follow-up included patient hospitalization and death unrelated to ocular treatment or voluntary withdrawal from the study. All patients were analyzed in their original groups. Baseline demographics were similar between the 2 groups (Table 1). The mean (SD) age was 80.9 (6.55) years and 81.2 (6.02) years for the intervention and control groups, respectively (P = .71). Women predominated in both groups, with 60.7% in the intervention group and 69.17% in the control group (P = .24). No adverse events were encountered in the study. No cases of endophthalmitis or other adverse events were identified.

CULTURE-POSITIVE RATE

The overall baseline culture-positive rate was 18.9%. The most common organisms cultured were coagulase-negative Staphylococcus (75.0%), Corynebacterium spp (22.9%), Staphylococcus aureus (6.3%), and Streptococcus viridans (4.9%) (Table 2). Adjusting for the baseline culture-positive rate, the intervention group had a higher culture-positive rate at months 1, 2, and 3 compared with the control group (Figure 1). The respective culture-positive rate for the intervention group and control group was 36.1% compared with 20.8% at month 1 (P = .03), 36.6% compared with 16.8% at month 2 (P = .004), and 39.2% compared with 23.7% at month 3 (P = .06). No significant change was seen in the culture-positive rate from month 1 to 3 in either the intervention group (P = .71) or the control group (P = .68). No significant difference was found in the culture-positive rate in patients with diabetes mellitus and those without (23.9% vs 28.8%) (P = .28).

MINIMUM INHIBITORY CONCENTRATION

The median baseline MIC was 0.64 μg/mL in the intervention group and 0.50 μg/mL in the control group. The intervention group had a significant increase in the mean MIC from 1.04 μg/mL at month 1 to 1.25 μg/mL at month 3 (P = .01) (Figure 2). No statistically significant change was seen in the control group, with a mean MIC of 0.94 μg/mL at month 1 and 0.89 μg/mL at month 3 (P = .68). Although the intervention group had a higher mean MIC compared with the control group at months 1 and 2, these values were not statistically significant (Table 3). However, at month 3 the mean MIC was statistically higher in the intervention group compared with the control group for the ceiling effect of sensitivity testing. A t test was used to detect differences in baseline demographics.
(P = .002). A medical history of diabetes mellitus did not have a significant effect on the mean MIC between the 2 groups (1.01 vs 0.97 µg/mL, P = .76).

**RESISTANT ISOLATES**

In the intervention group, the number of resistant isolates was 0 of 14 (0%) at baseline, 7 of 23 (30.4%) at month 1, 2 of 18 (11.1%) at month 2, and 9 of 18 (50.0%) at month 3. In the control group, the number of resistant isolates was 1 of 9 (11.1%) at baseline, 1 of 13 (7.7%) at month 1, 0 of 9 (0%) at month 2, and 1 of 13 (7.7%) at month 3. The number of resistant isolates in each group was too small to perform a statistical analysis.

**MIC50 AND MIC90**

The MIC50 at baseline for both the intervention and control groups was 0.64 µg/mL. In the intervention group, the MIC50 was 0.75 µg/mL at month 1, 0.64 µg/mL at month 2, and 1.00 µg/mL at month 3. The MIC50 for the control group was 0.64 µg/mL for month 1, 0.47 µg/mL for month 2, and 0.50 µg/mL for month 3 (Figure 2).

The MIC90 for the intervention group was 0.94 µg/mL at baseline, 3.00 µg/mL at month 1, 1.5 µg/mL at month 2, and 4.00 µg/mL at month 3. In the control group, the MIC90 was 0.64 µg/mL at baseline, 1.50 µg/mL at month 1, 0.64 µg/mL at month 2, and 0.94 µg/mL at month 3 (Figure 3).

**COMMENT**

The use of povidone-iodine has been the only proven method in reducing the risk of endophthalmitis after intracocular surgery and conjunctival bacterial growth in patients receiving IVT injections. Furthermore, topical antibiotics have been found to reduce colonization of conjunctival bacterial flora, particularly those most implicated in infectious endophthalmitis. These organisms have been implicated as the source of postoperative and postinjection endophthalmitis. However, when povidone-iodine is used before injection, topical antibiotics have no additional benefit in reducing conjunctival colonization.

More recently, evidence has been emerging to suggest IVT injections can be performed safely without the need for antibiotic prophylaxis. The Diabetic Retinopathy Clinical Research Network reported no case of endophthalmitis in 1276 injections following a standardized injection protocol, including topical povidone-iodine, a sterile eyelid speculum, and topical anesthesia without use of topical antibiotics. Similarly, a study by Bhatt et al found no significant difference in the rate of endophthalmitis after injection with or without antibiotics.

Fluoroquinolones are the most commonly used topical antibiotics in ophthalmology. Antibiotic resistance to the second- and third-generation fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin) has steadily increased. Evidence is mounting regarding the risk of selecting for resistant organisms on the ocular surface with widespread use of prophylactic antibiotics after IVT injection. Kim and Toma randomized 24 patients to receive 1 of 4 topical antibiotics (azithromycin, ofloxacin, gatifloxacin, or moxifloxacin) and compared resistance change to the fellow eye not undergoing treatment. The treated eye in their series demonstrated resistance to fluoroquinolone as early as visit 1 and doubled by visit 3 or 3 months after baseline (66% for gatifloxacin and 75% for moxifloxacin).

In our study, we found that mean MIC levels increase by 20% in the intervention group compared with

**Table 3. Mean MICs in the Intervention and Control Groups**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intervention</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.10 (1.00-1.19)</td>
<td>0.89 (0.78-1.00)</td>
<td>.006</td>
</tr>
<tr>
<td>Month 1</td>
<td>1.04 (0.90-1.18)</td>
<td>0.94 (0.78-1.10)</td>
<td>.34</td>
</tr>
<tr>
<td>Month 2</td>
<td>1.00 (0.84-1.16)</td>
<td>0.83 (0.63-1.04)</td>
<td>.22</td>
</tr>
<tr>
<td>Month 3</td>
<td>1.25 (1.11-1.39)</td>
<td>0.89 (0.73-1.05)</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, mean inhibitory concentration.
a 5% decrease in the control group. The percentage of resistant isolates and MIC\textsubscript{up} were approximately 4 times higher in the intervention group than the control group. To our knowledge, this is the largest series in the literature and the first to examine resistance using MIC rather than the Kirby-Bauer diffusion disk technique. The Kirby-Bauer diffusion disk technique, which reports sensitivity of organisms to antibiotics as sensitive, intermediate, or resistant, is somewhat open to interpretation.\textsuperscript{14-16} The additional advantage of having resistance represented by the MIC level is more sensitive and specific analysis of change in resistance.

Our study had a lower culture-positive rate (overall 18.9% at baseline) compared with the reported rate of approximately 50%\textsuperscript{10} in ocular bacterial flora of patients undergoing IVT injection. The effect of topical tetracaine may have been a contributing factor on this lower rate because topical anesthetic drops have been reported to have antibacterial effects with 24-hour incubation.\textsuperscript{39} However, Kang and Lee\textsuperscript{40} reported that with exposure of less than 2 minutes, there was no inhibition of organisms in in vitro studies. Nevertheless, the lower culture-positive rate may potentially dampen the potential greater increase in resistance because local anesthetic is believed to act through cell membrane lysis and is not affected by degree of resistance; therefore, it would have acted on both sensitive and resistant organisms equally.

Because our culture-positive rate was lower than initially predicted, we performed a post hoc power analysis. With the use of the total mean culture-positive rates of 19.4% and 38.2% in the control and intervention groups, assuming an α of .50, our study power was 0.91. With mean resistances of 6.6% and 22.9% in the control and intervention groups, respectively, our calculated power was 1.00.

Our study was limited by lack of randomization, which introduced a potential selection bias. However, our 2 study groups had similar baseline characteristics and were from 2 tertiary academic hospitals, with each hospital having investigators enrolling patients in the intervention and control groups. The other limitation of our study was the short duration of follow-up of only 4 months. Longer follow-up may be of interest to determine whether the effect of ocular surface flora change is longer lasting even after prolonged cessation of antibiotic use.

Although there is no evidence to prove the efficacy of prophylactic antibiotics after IVT injection in the prevention of endophthalmitis, several studies have reported that IVT injections can be performed safely without prophylactic antibiotics with no increase in the incidence of endophthalmitis. Moreover, evidence suggests that this practice of administering IVT injections may be harmful because of an increase in the growth of resistant organisms on the ocular surface. Because patients with neovascular AMD require an indefinite number of IVT injections, there is theoretically an increased risk of more virulent cases of endophthalmitis caused by more resistant strains of bacteria. As such, we believe that routine use of prophylactic antibiotics after IVT injection should be discouraged.

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References

15. Moss JM, Sanislo SR, Ta ON. A prospective randomized evaluation of topical


