Subconjunctival Gentamicin Prophylaxis Against Postoperative Endophthalmitis in the Rabbit

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Postoperative endophthalmitis is one of the most dreaded complications of cataract surgery and presently occurs in 3 to 4 cases out of a thousand. Although several published studies indicate some decrease in the incidence of postoperative bacterial endophthalmitis after topical and subconjunctival antibiotic administration prior to or at the time of surgery, the use and route of administration of prophylactic antibiotics remains controversial.

This study was undertaken to utilize an experimental surgical model in the rabbit to simulate the postoperative human condition in order to evaluate the efficacy of antimicrobial prophylaxis. The numbers of Staphylococcus aureus organisms which would cause reproducible postoperative infections were determined, and the effect of subconjunctival gentamicin administration on the incidence of subsequent intraocular infections was evaluated.

MATERIALS AND METHODS

Female New Zealand albino rabbits, weighing 2.5 to 3 kg were examined for evidence of ocular disease. Each animal was given 30 mg of chlorpromazine hydrochloride intramuscularly in the flank, followed 20 to 30 minutes later by 25 to 40 mg of sodium pentobarbital administered intravenously through a 23 gauge butterfly cannula in a marginal ear vein. Local anesthesia was obtained using 2% lidocaine hydrochloride. The pupils were dilated with cyclopentolate 1% and phenylephrine hydrochloride 10%. A wire lid speculum or traction sutures was used to separate the eyelids, and a 4-0 silk bridle suture was placed under the superior rectus muscle. When an extracapsular cataract extraction was to be performed, a 160-degree limbal incision was initiated with a razor blade knife and completed with corneal section scissors. A No. 11 Bard-Parker knife blade was used to impale the lens as far superiorly as possible and gently lifted in order to split the anterior capsule and then rotated to eviscerate the lens substance (Figure 1). A watertight wound closure was obtained using 7 or 8 interrupted 8-0 silk sutures.

In the case of the eyes undergoing peripheral iridectomy, a 4 mm superior limbus incision was made with a razor...
GENTAMICIN

FIGURE 1: Technique of extracapsular lens extraction employing a No. 11 Bard-Parker knife blade to impale and manipulate the lens substance.

blade knife and then peripheral iris was grasped with a forceps and excised. 1 to 2 interrupted 8-0 silk sutures were placed to obtain adequate closure.

Following closure of the corneal wound, the eye was grasped inferior to the cornea and 0.1 ml of sterile normal saline containing varying numbers of either Staphylococcus epidermidis or Staphylococcus aureus were administered in the anterior chamber. Rarely did any of the solution containing the microorganisms return through the paracentesis site as the anterior chamber volume had been appropriately decreased by surgery. In selected animals, 20 mg of gentamicin sulfate was administered subconjunctivally into the inferior quadrants at the termination of the procedure.

Postoperatively, all eyes received Atropine sulfate 1% and cyclopentolate 1% at least once daily and were evaluated by two examiners on each day for seven days. An anterior chamber paracentesis was performed on the first postoperative day and subsequently on eyes which appeared clinically infected. All paracenteses were performed employing proparacaine hydrochloride drops and pledgets at the site of the insertion of the 25 gauge needle on a 1 cc tuberculin syringe. The eye was grasped with a forceps. the needle was inserted into the anterior chamber and 0.1 ml of aqueous was rapidly aspirated and the needle withdrawn. The aqueous humor thus obtained was inoculated into glucose broth and incubated at 37°C for 18 hours.

Group I: Establishing the virulence of Staphylococcus epidermidis. Nineteen rabbits (32 eyes) underwent extracapsular cataract extraction and received 0.1 ml of varying numbers of Staphylococcus epidermidis at the completion of surgery. Twenty eyes received 10,000 organisms, 10 eyes received 40,000 organisms, and 2 eyes received 19,000,000 organisms.

Group II: Establishing the virulence of Staphylococcus aureus. Ten rabbits (20 eyes) underwent extracapsular cataract extraction and received varying doses of Staphylococcus aureus organisms. Four eyes received 1,000, 8 eyes received 5,000, 2 eyes received 50,000 and 6 eyes received 5,000,000 organisms.

Group III: Determining the rates of postcataract Staphylococcus aureus infection in gentamicin-treated eyes. Eight rabbits underwent bilateral extracapsular cataract extraction, received 5,000 Staphylococcus aureus in each eye, and were given 20 mg of subconjunctival gentamicin in the right eye (8 eyes).

Group IV: Comparing the rates of postiridectomy Staphylococcus aureus infection in gentamicin-treated and untreated eyes. Sixteen rabbits (16 eyes) underwent peripheral iridectomy in one eye and received 5,000 Staphylococcus aureus organisms. Eight of the eyes were left untreated while 8 received 20 mg of subconjunctival gentamicin.

Group V: Gentamicin penetration into the aqueous humor. Four animals (8 eyes) underwent an extracapsular cataract extraction and the right eyes were given 20 mg of subconjunctival gentamicin. A paracentesis was performed on the right eye of 2 animals at 15 minutes, 1 hour, and again at 4 hours at the time of sacrifice. Two of the animals underwent paracentesis on the right eye at 30 minutes and again at 2 hours when sacrificed. At the 2 and 4 hour period, a paracentesis was performed on the untreated left eye as well and 10 ml of intracardiac blood was aspirated.

60

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Staphylococcus aureus and epidermidis test organisms had been isolated from recent cases of human bacterial conjunctivitis. The organisms were quantitated by using a viable colony count method\(^4\) and then diluted with sterile saline in order to provide the approximate numbers of organisms desired. Prior to the experiment, *Staphylococcus aureus* was found to have a minimal inhibitory concentration (MIC) of 0.3 mcg/ml of gentamicin sulfate.

**RESULTS**

Postoperatively, a mild clinical infection was characterized by a turbid anterior chamber and slight corneal haze associated with ciliary injection in the animal. The presence of a discharge, if minimal, was not considered significant because of the exposed sutures used to perform the closure of the wound. A moderate infection was characterized by a greater anterior chamber haze, the presence of an hypopyon, as well as definite evidence of corneal edema. A severe case of postoperative endophthalmitis was characterized by a profuse anterior chamber exudate which obscured the outline of the pupil, massive corneal edema and a markedly injected eye.

Table 1 indicates that only 5 of 32 eyes in Group I that received varying doses of intraocular *Staphylococcus epidermidis* at the completion of the cataract surgery demonstrated clinical evidence of postoperative endophthalmitis. Organisms could be recovered by paracentesis of 3 of 32 eyes only on the first postoperative day (all of which showed evidence of infection). In all cases, the signs of intraocular infection were relatively mild (Figure 2) and persisted for 4 days with the exception of the one postoperative endophthalmitis occurring after 19,000,000 organisms which was severe. In this case, many leukocytes and a few Gram-positive cocci were observed in the paracentesis smear; however, the organism could not be isolated in culture.

The animals in Group II infected with different doses of *Staphylococcus aureus* showed a much greater incidence of positive clinical signs and positive recovery of organisms from the anterior chamber by culture on the first postoperative day. Evidence of clinical infection was detected in 2 of 4 eyes.

**FIGURE 2:** Post-cataract extraction eye which received *Staphylococcus epidermidis* (40,000 organisms) demonstrating signs of mild intraocular infection on the first postoperative day; the organism could not be recovered.

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose (No. ORGS/0.1 ml)</th>
<th>Treatment</th>
<th>No. of Eyes</th>
<th>Positive Clinical Signs</th>
<th>Positive A.C. Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>—</td>
<td>20</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>40,000</td>
<td>—</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>19M</td>
<td>—</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>40,000</td>
<td>Genta.</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

OPHTHALMIC SURGERY
### TABLE 2

INCIDENCE OF POST-CATARACT ENDOPTHALMITIS FOLLOWING STAPHYLOCOCCUS AUREUS INOCULATION

<table>
<thead>
<tr>
<th>Dose (No. ORGS/0.1 ml)</th>
<th>No. of Eyes</th>
<th>Positive Clinical Signs</th>
<th>Positive A.C. Culture</th>
<th>Incidence of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>50-75%</td>
</tr>
<tr>
<td>5,000</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>88%</td>
</tr>
<tr>
<td>50,000</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>1 M</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
</tbody>
</table>

receiving 1,000 organisms whereas this increased to 7 of 8 eyes receiving 5,000 organisms. With greater dosages of *Staphylococcus aureus*, the incidence of infection by both clinical course and aqueous humor recovery of the organisms was 100% (Table 2). The two infections with 1,000 organisms were considered mild; however, infections associated with a greater number of *Staphylococcus aureus* were moderate to severe (Figure 3) and were characterized by a progressive worsening clinical course. Anterior chamber paracenteses obtained from animals receiving 5,000 organisms were positive in 7 of the 8 eyes on day 1, 4 eyes on day 2 and only 1 eye on day 3, despite the persistence of the inflammatory reaction.

The animals in Group III who received subconjunctival gentamicin sulfate in the right eye appeared to be protected from infection (Table 3). Neither the gentamicin-injected or contralateral untreated eyes of these animals showed any evidence of intraocular infection (Figure 4). In contrast, 88% of the control eyes which were infected after cataract surgery (Group II) showed evidence of postoperative suppurative endophthalmitis. Histopathological examination of several infected eyes indicated the severe nature of the inflammatory reaction.

None of the 8 rabbit eyes in Group IV that received subconjunctival gentamicin after peripheral iridectomy demonstrated clinical or microbiological signs of infection; whereas 6 of the 8 eyes which did not receive antimicrobial administration demonstrated mild anterior chamber inflammation with the recovery of organisms on the first postoperative day (Table 4). In the cases of post-iridectomy infection, organisms could not be recovered after the first day and the inflammatory signs completely resolved by the third postoperative day.

The results of gentamicin penetration into the serum and aqueous humor of the 4 rabbits in Group V are illustrated in Table 5. In all of the eyes receiving gentamicin after cataract surgery, the aqueous must be considered as secondary or post-inflammatory. Adequate anti-staphyllococcal aqueous levels were achieved in 15 minutes and were 75-100 μg/ml at 1 hour. After 4 hours, 18.8-23 μg/ml could still be recovered from the injected

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**FIGURE 3:** An example of a severe postoperative *Staphylococcus aureus* infection (5,000 organisms) seen on the first day after surgery in which the organism was isolated.
TABLE 3
INCIDENCE OF POST-CATARACT STAPHYLOCOCCUS AUREUS ENODPHTHALMITIS IN GENTAMICIN-TREATED VS. UNTREATED RABBITS

<table>
<thead>
<tr>
<th>Dose (No. ORGS/0.1 ml)</th>
<th>No. of Eyes</th>
<th>Treatment</th>
<th>Positive Clinical Signs</th>
<th>Positive A.C. Culture</th>
<th>Incidence of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,000</td>
<td>8</td>
<td>—</td>
<td>7</td>
<td>7</td>
<td>88%</td>
</tr>
<tr>
<td>5,000</td>
<td>8 O.D.</td>
<td>Genta.</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>5,000</td>
<td>8 O.S.</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

TABLE 4
INCIDENCE OF POST-IRIDECTOMY STAPHYLOCOCCUS AUREUS INFECTIONS IN GENTAMICIN-TREATED VS. UNTREATED RABBITS

<table>
<thead>
<tr>
<th>Dose (No. ORGS/0.1 ml)</th>
<th>No. of Eyes</th>
<th>Treatment</th>
<th>Positive Clinical Signs</th>
<th>Positive A.C. Culture</th>
<th>Incidence of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,000</td>
<td>8</td>
<td>—</td>
<td>6</td>
<td>6</td>
<td>75%</td>
</tr>
<tr>
<td>5,000</td>
<td>8</td>
<td>Genta.</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

TABLE 5
GENTAMICIN PENETRATION FOLLOWING SUBCONJUNCTIVAL ADMINISTRATION (20 mg.) IN THE POST-CATARACT RABBIT EYE

<table>
<thead>
<tr>
<th>Time</th>
<th>Gentamicin Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous Humor</td>
</tr>
<tr>
<td>15 min.</td>
<td>6.2</td>
</tr>
<tr>
<td>30 min.</td>
<td>4.7 - 7.8</td>
</tr>
<tr>
<td>1 hr.</td>
<td>75 - 100</td>
</tr>
<tr>
<td>2 hr.</td>
<td>24.3 - 3.31</td>
</tr>
<tr>
<td>4 hr.</td>
<td>18.8 - 23</td>
</tr>
</tbody>
</table>

*None of these eyes were infected with bacteria.

FIGURE 4: Appearance of a gentamicin-treated (20 mg) eye which had received 5,000 Staphylococcus aureus following extracapsular cataract extraction.

eye and there was significant gentamicin penetration into the contralateral non-injected eye. This could be explained by the reasonable serum levels obtained at the same time of sacrifice.

DISCUSSION

The controversy regarding antimicrobial prophylaxis prior to or at the time of cataract surgery has not been resolved and has been adequately discussed elsewhere. A number of authors have reported that the use of preoperative topical antibiotics contribute to a reduction in the rate of postoperative infections. However, it is very difficult to sterilize the
GENTAMICIN
eye or even reduce conjunctival bacterial counts with many of the commonly used topical regimens. Several investigators have indicated that subconjunctival antibiotics at the time of surgery lessen the incidence of infection; on the other hand, Aronstam and Chalkley et al. have demonstrated post-cataract infections despite antimicrobial prophylaxis. Because human studies on postoperative infections are retrospective and fraught with so many variables, this study was undertaken to evaluate whether subconjunctival antibiotics at the time of surgery would effect the incidence of experimental endophthalmitis under reasonably controlled circumstances.

A reduction in the 3.4/1000 incidence of postoperative infections in the modern age would be of great value and antimicrobial prophylaxis might offer such a hope. Gentamicin sulfate is a broad spectrum aminoglycoside which provides excellent intraocular drug levels after subconjunctival administration. It was decided to administer the drug subconjunctivally immediately after the surgical procedure in order to achieve significant antimicrobial concentrations inside the eye at the time one might suspect that microorganisms would have been inoculated and would be multiplying in the case of cataract surgery in man. Preoperative inoculation might allow leakage of the drug into the eye, causing endothelial toxicity; therefore, this was not employed.

Staphylococcus epidermidis was the first organism selected and demonstrated only minimal virulence in doses of 10,000, 40,000 and 19,000,000 orgs/0.1 ml. Even with the administration of 19,000,000 organisms, only 1 of 2 eyes showed evidence of infection. Similarly, Maylat and Leopold reported that doses of 5 to 15,000,000 coagulase negative staphylococci were needed to cause a significant intraocular reaction.

Staphylococcus epidermidis caused mild post-cataract infections and the organism could only be recovered for 1 day in 3 of 32 eyes. This lack of virulence is noted clinically by virtue of the fact that this organism is so abundant in the postoperative flora but has only rarely been documented as a cause of endophthalmitis.

Since Staphylococcus aureus is felt to be the most common cause of documented postoperative bacterial endophthalmitis this was selected as the test organism in the remainder of the study. The animals in Group II which received varying intraocular doses of Staphylococcus aureus showed a much greater incidence of clinical and microbiologically proven infections. When 5,000 orgs/0.1 ml were injected, an 88% incidence of infection was obtained; therefore, resorting to higher doses appeared to be unnecessary. The minimum number of organisms which could cause reproducible infection was selected, again to simulate the probable ocular conditions which prevail at the time of human cataract surgery. This dose of 5,000 orgs/0.1 ml was quite similar to those employed by other authors who studied experimental infections of the rabbit anterior chamber.

In striking comparison, the 8 animals that underwent bilateral extracapsular cataract extraction in Group III and received 20 mg of subconjunctival gentamicin in the right eye showed no incidence of infection in either the treated or the untreated eye as can be seen by Table 3. The fact that gentamicin aqueous levels in the contralateral eye in these animals 4 hours after subconjunctival injection (Table 5) exceeded the mean inhibitory concentration for the Staphylococcus aureus organism seems to explain the absence of infection in these non-treated eyes. Therefore, the use of subconjunctival antimicrobial administration appears to have such excellent penetration so as to prevent post-cataract endophthalmitis in both eyes.

The animals undergoing peripheral iridectomy also demonstrated significant difference in the incidence of infection when subconjunctival gentamicin was administered (Table 4). Only 6 of the 8 non-treated eyes developed postoperative
infection and in all of these cases it was relatively mild and the eyes were not lost. The presence of a lens separating the aqueous humor from the vitreous body would appear to play an important role in preventing the development of destructive postoperative infection.

SUMMARY

1. An experimental aphakic rabbit model was employed in order to evaluate the correlation of the clinical course of postoperative endophthalmitis and the ability to recover microorganisms in the postoperative time period, the ocular antibiotic penetration of gentamicin, and the value of antibiotic prophylaxis.

2. *Staphylococcus aureus* is a far more pathogenic organism than *Staphylococcus epidermidis*. A concentration of 5,000 organisms resulted in an 88% infection rate whereas 50,000 organisms and greater resulted in 100% infection rate after extracapsular cataract extraction.

3. *Staphylococcus aureus* could be isolated from the eyes demonstrating infection following cataract extraction for 2 days (3 days in one case) and then could no longer be recovered despite the persistence of intraocular inflammation. The infection which appeared in animals receiving 5,000 organisms of *Staphylococcus aureus* after peripheral iridectomy was significantly milder than that which occurred following cataract extraction. In addition, the microorganisms could only be isolated on the first postoperative day.

5. When 20 mg of gentamicin sulfate was administered subconjunctivally following the post-cataract extraction or post-iridectomy administration of the 5,000 organisms of *Staphylococcus aureus*, none of the eyes developed clinical signs of endophthalmitis nor could bacteria be recovered.

6. Subconjunctivally administered gentamicin sulfate rapidly penetrated into the aphakic rabbit eye; 75-100 μg/ml appears in the aqueous humor at 1 hour and persists in bactericidal concentrations for at least 4 hours in both the treated and contralateral eyes.

7. Antimicrobial (subconjunctival gentamicin sulfate 20 mg) prophylaxis at the time of surgery in the experimental animal significantly reduced the incidence of experimental postoperative bacterial endophthalmitis.

REFERENCES


13. Chalkley THF, Shoch D: An evaluation of prophylactic subconjunctival antibiotic

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