Effect of Intravitreal Triamcinolone Acetonide on Susceptibility to Experimental Bacterial Endophthalmitis and Subsequent Response to Treatment

Rodney S. Bucher, MD; Edward Hall, MD; David M. Reed, PhD; Julia E. Richards, PhD; Mark W. Johnson, MD; David N. Zacks, MD, PhD

Objectives: Recent reports of high endophthalmitis rates after intravitreal triamcinolone acetonide injection have raised concerns about the safety of this treatment. We sought to evaluate the effect of intravitreal triamcinolone injection on (1) the susceptibility to experimental bacterial endophthalmitis and (2) the subsequent therapeutic response to antibiotic treatment.

Design: For the susceptibility study, the right eye of 40 New Zealand white rabbits received an intravitreal injection of a known quantity of Staphylococcus epidermidis organisms. Half of the eyes received a simultaneous intravitreal injection of triamcinolone acetonide, 4 mg. All eyes were examined daily for signs of endophthalmitis (photophobia, conjunctival injection, and vitritis) using standardized grading protocols (scaled from 0 to 4 with increasing severity). On day 7, vitreous cultures were obtained. For the therapeutic response study, the right eye of 12 rabbits received an intravitreal injection of S epidermidis organisms sensitive to vancomycin. Half of the eyes received a simultaneous intravitreal injection of triamcinolone acetonide, 4 mg. All 12 eyes received an intravitreal injection of vancomycin hydrochloride, 1 mg, on development of the first signs of endophthalmitis. All eyes were examined daily for 7 additional days. On day 7 after treatment, vitreous cultures were obtained.

Results: In the susceptibility study, all 40 eyes developed signs of endophthalmitis. In eyes that received intravitreal bacteria plus triamcinolone, 17 (85%) of the 20 vitreous cultures were positive, whereas only 6 (30%) were positive in the 20 eyes receiving bacteria alone (P = .001). The vitritis was significantly increased in the bacteria plus triamcinolone group compared with the bacteria-only group (17 of 20 vs 7 of 20 with 4+ vitritis, respectively; P = .003). In the therapeutic response study, all 12 eyes developed clinical signs of endophthalmitis within 48 hours. All vitreous samples obtained 7 days after intravitreal vancomycin injection were culture negative. However, the severity of vitritis at the time of vitreous sampling was less in the eyes receiving triamcinolone plus bacteria compared with eyes receiving bacteria alone (0 of 6 vs 5 of 6 with 4+ vitritis, respectively; P = .02).

Conclusions: In eyes with experimentally induced bacterial endophthalmitis, the presence of intravitreal triamcinolone results in a higher culture-positive rate and a higher degree of inflammation, suggesting an impaired ocular immune response and greater susceptibility to infection. However, in eyes with experimentally induced bacterial endophthalmitis receiving early treatment with intravitreal antibiotics, triamcinolone appears to suppress the ocular inflammatory response without impairing the therapeutic effect.

Clinical Relevance: These data suggest that caution must be exercised when combining intravitreal triamcinolone injection with intraocular surgery.

intravitreal injection of a corticosteroid is that it might shift the dose-response curve and thus lower the threshold number of organisms required for the development of culture-positive endophthalmitis.

The main goal of our study was to determine whether intravitreal triamcinolone increased the susceptibility for culture-positive endophthalmitis in an experimental model of bacterial endophthalmitis. In addition, we wished to determine whether triamcinolone affects the therapeutic response to intravitreal antibiotics in eyes with experimental bacterial endophthalmitis.

### METHODS

All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology guidelines for the use of experimental animals, and with the approval of the University Committee on the Use and Care of Animals at the University of Michigan, Ann Arbor. Specific pathogen-free adult male New Zealand white rabbits were used in all experiments. Animals were anesthetized using a mixture of ketamine hydrochloride and xylazine hydrochloride at a dose of 40 mg/kg and 10 mg/kg, respectively. All intraocular injections were given into the right eye of each rabbit and were performed using strict sterile technique. The right eye of each rabbit was prepared, the lids scrubbed with a povidone-iodine solution, and the field covered with a sterile eye drape. Solution containing bacteria or triamcinolone was drawn up in a sterile fashion into the injection site. A sterile lid speculum was used to maintain exposure of the injection site. All injections were performed through the pars plana with the use of a 30-gauge needle on a 1-mL tuberculin syringe. After the injection, a drop of 1% atropine sulfate and lubricating ointment was placed in the eye. All procedures were performed in the animal surgical suite of the Kellogg Eye Center vivarium at the University of Michigan. After the procedure, the rabbits were placed in their cages and allowed to recover using standard animal care protocols.

Rabbits were examined daily for the formation of signs of endophthalmitis. Specific features noted were photophobia, conjunctival injection, and vitritis. Each criterion was graded on a scale of 0 to 4, with increasing values corresponding to increasing severity. Vitritis was assessed with the use of indirect ophthalmoscopy, and the grading scale was formalized as shown in the following tabulation:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Degree of Vitritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No vitritis</td>
</tr>
<tr>
<td>1+</td>
<td>Mild vitreous haze</td>
</tr>
<tr>
<td>2+</td>
<td>Moderate vitreous haze with some retina details visible</td>
</tr>
<tr>
<td>3+</td>
<td>Good red reflex with no retina details visible</td>
</tr>
<tr>
<td>4+</td>
<td>Poor red reflex</td>
</tr>
</tbody>
</table>

Examiners could not be masked to the status of triamcinolone injection because the triamcinolone was easily detectable during indirect ophthalmoscopy. The use of the formal grading scale, however, provided specific criteria for assigning a vitritis score to each eye, thus serving to minimize any examiner bias.

Experimental endophthalmitis was induced by injecting a known dose of vancomycin-sensitive S. epidermidis (American Type Culture Collection [ATCC] No. 12228; ATCC, Manassas, Va) into the vitreous cavity. Organisms were washed and resuspended in a total volume of 0.1 mL of sterile isotonic sodium chloride solution. Bacteria were grown in trypticase soy broth (TSB), 30 g BBL TSB powder per liter (Becton Dickinson and Company, Sparks, Md), and all procedures used standard, sterile microbiological techniques. After incubation overnight with shaking at 37°C, the bacteria were diluted in TSB and grown to an optical density reading of 0.08 to 0.10 absorbance unit as determined using absorbance at 625 nm on a spectrophotometer (Shimadzu UV-2401 PC, Shimadzu Scientific Instruments, Columbia, Md). Serial dilutions were made in sterile isotonic sodium chloride solution to achieve the desired bacterial count per 0.1 mL. Counts were verified by plating aliquots of the serially diluted samples on TSA agar (30 g/L BBL TSB powder, 15 g/L granulated agar [Fisher Chemicals, Fairlaw, NJ]) and growing overnight at 37°C. An aliquot was plated both before and after the injection to ascertain that the bacterial count did not change during the interval required to perform the injection.

For the arm of the study examining the effect of triamcinolone on the dose-response curve for culture-positive endophthalmitis, the right eyes of 40 New Zealand white rabbits were used. Ten rabbits were injected with a dose of 3.5 × 10^1 to 4.0 × 10^3 organisms and 30 were injected with 3.5 × 10^2 to 4.0 × 10^3 organisms. In each group, half of the eyes also received a concomitant intravitreal injection of commercially available 40-mg/mL triamcinolone acetonide, 0.1 mL (Kenalog-40; Bristol-Myers Squibb Company, Princeton, NJ). In addition, in 8 control eyes, 4 received an injection of triamcinolone acetonide, 0.1 mL, alone (to evaluate for signs of sterile endophthalmitis) and 4 received an injection of a higher inoculum (3.5 × 10^2 to 4.0 × 10^3 organisms) of the same bacterial strain to ensure the viability of the organisms. On day 7, the animals were anesthetized as already described, and vitreous samples of approximately 0.1 mL were obtained using a 25-gauge needle passed through the pars plana into the mid vitreous cavity under sterile conditions. The animals were then euthanized with an intracardiac injection of phenobarbital sodium. Each vitreous sample was plated on TSA agar and incubated at 37°C overnight. After 24 hours, the plates were examined for growth.

For the arm of the study testing the effect of triamcinolone on the response to intravitreal injection of vancomycin, 12 New Zealand white rabbits were used and an inoculum of 3.5 × 10^1 to 4.0 × 10^3 organisms was injected. Half of the eyes (n=6) also received a simultaneous intravitreal injection of 40-mg/mL triamcinolone acetonide, 0.1 mL. The eyes were examined daily for signs of endophthalmitis. If endophthalmitis was detected, the eye received an intravitreal injection of 10-mg/mL vancomycin hydrochloride, 0.1 mL, using the protocol already described for sterile intravitreal injection. The eyes were examined daily for 7 additional days, after which the animals were anesthetized and the vitreous cultures obtained. Each vitreous sample was plated on TSA agar and incubated at 37°C overnight. After 24 hours, the plates were examined for growth.

The results for the susceptibility arm of the study are summarized in Table 1. All 40 eyes developed signs of endophthalmitis. Of the eyes that received intravitreal bacteria plus triamcinolone, 17 (85%) of the 20 vitreous cultures were positive. In contrast, only 6 (30%) vitreous cultures were positive in the 20 eyes receiving bacteria alone (P = .001). In the group receiving the 10^2 inoculum size, 14 (93%) of the 15 eyes that also received intravitreal triamcinolone were culture-positive, whereas only 5 (33%) of the 15 bacteria-only eyes were culture-positive (P = .002). Quantitative colony counts on the vitreous samples obtained confirmed that culture-positive eyes from the triamcinolone group had greater bacterial loads than culture-positive eyes that did not receive tri-
amcinolone (data not shown). The group receiving the $10^1$ inoculum size showed a similar trend toward increased culture-positive eyes in the intravitreal triamcinolone group, but the difference did not reach statistical significance, perhaps because of the small sample size.

When assessing the difference in ocular inflammation, there was a significant difference between the triamcinolone with bacteria vs the bacteria-only eyes. Vitritis scores were significantly higher in the 20 eyes with triamcinolone and bacteria, with 17 (85%) having $4+$ vitritis. In the bacteria-only group, only 7 (35%) of 20 eyes had $4+$ vitritis ($P = .003$) (Table 2). Of note, the onset of vitritis in the group receiving triamcinolone appeared to be delayed but surpassed the severity of vitritis in the bacteria-only eyes by day 5 (Figure).

In the control eyes receiving triamcinolone alone, none of the 4 developed signs of sterile endophthalmitis and all vitreous cultures were negative. In the 4 control eyes receiving the $10^3$ dose of organisms, all 4 had signs of endophthalmitis and positive vitreous cultures.

In the therapeutic response arm of the study, all 12 eyes developed clinical signs of endophthalmitis within 48 hours of the injection of organisms. Vitreous samples obtained 7 days after intravitreal vancomycin injection were culture-negative in all eyes. The severity of vitritis at the time of vitreous sampling was markedly less in the eyes receiving triamcinolone plus bacteria (none with $4+$ vitritis) compared with the eyes receiving bacteria alone (5 of 6 with $4+$ vitritis) ($P = .02$) (Table 3).

### Table 1. Susceptibility Study Vitreous Culture Results

<table>
<thead>
<tr>
<th>Inoculum A† (n = 10)</th>
<th>Inoculum B‡ (n = 30)</th>
<th>Combined (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>With triamcinolone acetonide</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Without triamcinolone</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>$P$ Value§</td>
<td>.52</td>
<td>.002</td>
</tr>
</tbody>
</table>

*Data are given as number of eyes with either positive or negative cultures.
†For A, inoculum size was equal to $3.5 \times 10^1$ to $4.0 \times 10^1$ organisms per 0.1 mL.
‡For B, inoculum size was equal to $3.5 \times 10^2$ to $4.0 \times 10^2$ organisms per 0.1 mL.
§Calculated using the Fisher exact test.

### Table 2. Susceptibility Study Vitritis Score Results on Day 7

<table>
<thead>
<tr>
<th>Vitritis Score†</th>
<th>Inoculum B‡ (n = 30)</th>
<th>Combined (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Triamcinolone Acetonide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$4+$</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>$&lt;4+$</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>$P$ Value§</td>
<td>.002</td>
<td>.003</td>
</tr>
<tr>
<td>Without Triamcinolone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$4+$</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

*Data are given as number of eyes with the corresponding vitritis score.
†Vitritis scores are explained in the “Methods” section.
‡For B, inoculum size was equal to $3.5 \times 10^2$ to $4.0 \times 10^2$ organisms per 0.1 mL.
§Calculated using the Fisher exact test.

### Table 3. Susceptibility Study Vitreous Culture Results

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<td></td>
<td>Culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>With triamcinolone acetonide</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Without triamcinolone</td>
<td>1</td>
<td>4</td>
</tr>
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‡For B, inoculum size was equal to $3.5 \times 10^2$ to $4.0 \times 10^2$ organisms per 0.1 mL.
§Calculated using the Fisher exact test.

Any intraocular procedure carries with it the inherent risk of infection. Of particular concern with intravitreal injection of triamcinolone is its immunosuppressive effect, which may make the eye more susceptible to infection. In our model of experimentally induced bacterial endophthalmitis, we found that eyes injected with triamcinolone have a significantly higher rate of culture positivity after 7 days than those injected with the same quantity of bacteria alone. In the absence of triamcinolone...
The immunosuppressive effect of intraocular corticosteroids has also led to concern about their potential masking of early signs of endophthalmitis. In our study, the inflammatory response in the triamcinolone group was delayed despite the fact that the final severity of inflammation was greater in eyes receiving triamcinolone. This delayed immune response might help explain why the bacterial inoculum is not as readily cleared from the eye. That is to say, the organisms might have a longer period to incubate and multiply within the eye in the presence of triamcinolone, before being attacked by ocular immune defense mechanisms.

In our model, the early (within 48 hours) injection of intravitreal vancomycin prevented the formation of culture-positive endophthalmitis, regardless of whether triamcinolone was present in the eye. All eyes receiving intravitreal vancomycin had culture-negative vitreous samples 7 days after treatment. Of note, eyes that received triamcinolone had significantly less inflammation compared with eyes with bacteria only. It appears that the triamcinolone effectively suppressed the ocular inflammatory response associated with infection, resulting in less vitritis in these antibiotic-treated eyes. The suppression of inflammation by intravitreal corticosteroids has been shown to preserve ocular architecture in other rabbit endophthalmitis models.14

Noninfectious sterile endophthalmitis has been described after the use of intravitreal triamcinolone. It has been hypothesized that noninfectious sterile endophthalmitis is caused by an inflammatory reaction to a substance in the formulation.15 We did not observe this reaction in our small number of control eyes receiving triamcinolone alone. Our ability to detect this phenomenon, though, might have been limited by the small number of eyes receiving triamcinolone alone.

The present study demonstrates the potent immunosuppressive qualities of intravitreal triamcinolone in the setting of experimental bacterial endophthalmitis. Although the reported incidence of infectious endophthalmitis after intravitreal triamcinolone injection is low, it appears to be higher than that reported for other intraocular procedures.11,12 Our data suggest that this is at least partially explained by immunosuppression within the ocular microenvironment. Patients should be carefully selected and should be adequately informed regarding the risk of infection. The importance of careful sterile technique cannot be overemphasized, as well as the use of single-dose vials to minimize the risk of inadvertent inoculation of the eye with bacterial contaminants. We advise caution when considering the use of intravitreal triamcinolone in conjunction with intraocular surgery, given the likelihood that bacteria are routinely introduced into the eye intraoperatively. Intravitreal corticosteroid injection will likely continue to be an important therapeutic alternative for refractory macular edema and neovascular maculopathies. However, careful risk-benefit considerations are warranted in light of these findings.

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14. Tervo T, Ljungberg P, Kautiainen T, et al. Prospective evaluation of external ocular microenvironment. Patients should be carefully selected and should be adequately informed regarding the risk of infection. The importance of careful sterile technique cannot be overemphasized, as well as the use of single-dose vials to minimize the risk of inadvertent inoculation of the eye with bacterial contaminants. We advise caution when considering the use of intravitreal triamcinolone in conjunction with intraocular surgery, given the likelihood that bacteria are routinely introduced into the eye intraoperatively. Intravitreal corticosteroid injection will likely continue to be an important therapeutic alternative for refractory macular edema and neovascular maculopathies. However, careful risk-benefit considerations are warranted in light of these findings.