Transscleral Iontophoresis of Gentamicin in Monkeys

Michael Barza,* Cornelia Peckman,* and Jules Baum†

Experiments in rabbits have shown that the novel technique of transscleral iontophoresis is a safe, effective, noninvasive way to produce high concentrations of antibiotics in the vitreous humor. The authors have now studied the effects of repeated transscleral iontophoresis in the eyes of cynomolgus monkeys. Six treatments with gentamicin sulfate, 1.5 mA for 10 min, were applied to both eyes of three monkeys over a 2-week period. The concentrations of gentamicin in the vitreous humor 24 hr after treatment were determined for each eye on three occasions. The mean concentration was 28 μg/ml (range, 11-44 μg/ml) after the first treatment and 12 μg/ml (range, 2-21 μg/ml) after the fifth treatment. Indirect ophthalmoscopy completed after the last treatment showed only small retinal burns up to 2.5 mm in diameter in four eyes in the area of the pars plana over which the electrode had been applied. Electroretinograms were normal after treatment. These experiments confirm the efficacy and safety of transscleral iontophoresis in the primate's eye and suggest that investigations of this potentially useful technique are warranted in humans. Invest Ophthalmol Vis Sci 28:1033-1036, 1987

Physicians are becoming increasingly aware of the need to achieve high vitreal concentrations of antibiotics for the treatment of bacterial endophthalmitis. Accordingly, direct intravitreal injections of cephalosporins, penicillins, and aminoglycosides are often given at the onset of treatment.1-3 However, physicians are frequently reluctant to administer repeated intravitreal injections because of the risk of adverse effects and because of the necessity to take the patient to the operating room for the procedure. We recently reported that the novel method of transscleral iontophoresis affords a highly effective, noninvasive way to product high vitreal concentrations of antibiotics in the rabbit's eye.4 In the present article, we report our results with this technique in the eyes of cynomolgus monkeys. To simulate the potential use of the method in humans more closely, we have measured the drug concentrations and ocular safety of iontophoresis after repeated rather than single applications and have used a better-tolerated modification of our original device on the eye.

Materials and Methods. Three healthy adult male cynomolgus monkeys, each weighing about 4.5 kg, were used in these studies. Both eyes of each animal were subjected to transscleral iontophoresis of gentamicin sulfate on days 1, 3, 5, 8, 10, and 12 of a 12-day experimental period; vitreal aspirations of each eye were done on days 2, 4, and 11. The animals were housed and fed according to the National Institutes of Health Guidelines in an American Association Laboratory Animal Care facility. Two weeks before treatment and 4 weeks after the last iontophoresis treatment, electroretinographic (ERG) testing was done in both eyes of each monkey. Rod (blue light), cone, (30 Hz flicker), and mixed rod and cone (0.5 Hz flashes of white light) ERGs were recorded from each eye of each animal using standard levels of amplification and computer averaging.5 In addition, indirect ophthalmoscopic observations were made on both eyes of each monkey 2 months after the last iontophoresis treatment. The study was performed in accordance with the ARVO Resolution on the Use of Animals in Research.

For each iontophoresis or aspiration procedure, the animals were tranquilized by intramuscular injection of acepromazine (5 mg) and ketamine (50 mg). Topical anesthesia was provided by the application of proparacaine hydrochloride 0.5% drops. After each procedure the animals were treated with neomycin-polyvidone B ophthalmic solution (two drops in each eye). Schiotz tonometry was done just before and after each iontophoresis procedure and each vitreal aspiration.

Transscleral iontophoresis was accomplished using a recent modification of our previously described apparatus.6 The modified device has a smaller central orifice (0.5 mm), ensuring an even greater current density; it also has a shorter iontophoretic column with an expansion chamber to trap any gas bubbles that are formed, and a lower profile, making for a more comfortable fit in the eye. A schematic diagram is shown in Figure 1. After the animals were tranquilized and the eye was anesthetized, a Barraquer wire speculum was inserted into the eye. The eye was rotated nasally with a fine-toothed forceps applied at the limbus, and the electrode was placed over the pars plana in either the superior or the inferior temporal quadrant. The electrode was held in place by suction created by aspirating a tuberculin syringe (Fig. 1). Gentamicin sulfate, obtained as the laboratory diagnostic powder from Schering Corp. (Kenilworth, NJ) and dissolved in distilled water to a concentration of 100 mg/ml, was used to fill the apparatus. Iontophoresis was done for 10 min at 1.5 mA; the current was turned up slowly over 10-15 sec to avoid shocking the animals.

Vitreal aspirations were conducted by inserting a 27-gauge needle about 4 mm posterior to the nasal limbus and into the approximate center of the vitre-
Vitreal aspirates were completed on days 2, 4, and 11 of the experimental period (ie, 24 hr after the first, second, and fifth iontophoresis treatments). Overall, the mean concentration in the six eyes declined from 28 μg/ml after the first aspiration to 20 μg/ml after the second aspiration to 12 μg/ml after the third aspiration (Table 1). There was moderate variability in concentration among eyes and within eyes from treatment to treatment. However, with one exception, all values were between 6 and 44 μg/ml.

**Schiotz tonometry:** Intraocular tensions measured just before each iontophoresis procedure were approximately 12 mm Hg. The tensions measured just after each iontophoresis procedure invariably declined to 0 mm Hg but had returned to normal by the next day. More detailed studies of the time course for the tensions to return to normal were not done because they would have required additional anesthetics.

**Indirect ophthalmoscopy:** Five areas of burn over the pars plana could be seen in four eyes after the 36 iontophoresis treatments. The smallest burn was less than 1 mm and the largest was 2.5 mm in diameter.

**Electroretinography:** The amplitude of the a- and b-waves and the implicit time of the b-waves were within normal limits for each eye before and after the iontophoresis treatments. Thus, there were no ERG

### Table 1. Concentrations of gentamicin in the vitreous humor after iontophoresis treatments

<table>
<thead>
<tr>
<th>Vitreous aspiration</th>
<th>Number of preceding treatments</th>
<th>Monkey 101</th>
<th>Monkey 102</th>
<th>Monkey 103</th>
<th>Mean</th>
<th>SE</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OD†</td>
<td>OS‡</td>
<td>OD</td>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>1</td>
<td>41</td>
<td>44</td>
<td>22</td>
<td>34</td>
<td>11</td>
<td>11–44</td>
</tr>
<tr>
<td>Second</td>
<td>2</td>
<td>28</td>
<td>35</td>
<td>7</td>
<td>14</td>
<td>22</td>
<td>7–35</td>
</tr>
<tr>
<td>Third</td>
<td>5</td>
<td>6</td>
<td>16</td>
<td>2</td>
<td>12</td>
<td>21</td>
<td>2–21</td>
</tr>
</tbody>
</table>

* Each aspiration was completed 24 hr after the preceding iontophoresis treatment.
† OD = right eye.
‡ OS = left eye.
abnormalities attributable to the iontophoresis procedures.

Discussion. Physicians are increasingly prone to use direct intravitreal injections of penicillins, cephalosporins and aminoglycosides in order to produce high vitreal concentrations in the initial treatment of bacterial endophthalmitis. However, because of the risk of trauma and of the necessity to conduct the procedure in an operating room, they are reluctant to carry out repeated intravitreal injections even when to do so might seem clinically appealing. Maurice and we recently reported the use of a novel method of transscleral iontophoresis, which accomplishes the same goals as an intravitreal injection but obviates the need to invade the eye. In contrast to the traditional method of corneal iontophoresis, the new method uses a small area of contact of the ionophoretic column with the eye, which results in a high intensity of current and involves placement of the device posterior to the iris, which bypasses the lens-iris barrier. With this approach we were able to produce vitreal concentrations of cefazolin, ticarcillin, and gentamicin ranging from about 100–200 ìg/ml in the rabbit’s eye. In the present article, we report our experience with repeated applications of transscleral iontophoresis to the eyes of healthy cynomolgus monkeys.

The mean concentration of gentamicin in the vitreous humor following the first iontophoresis was 28 ìg/ml. This is similar to the concentration of gentamicin achieved with a lower current, 0.5 mA, in the rabbit’s eye. However, a simple comparison of vitreal concentrations achieved in the two studies is not very meaningful for several reasons. First, the eye of the cynomolgus monkey is probably somewhat larger than that of the rabbit. Although we could not find published data for the volume of the vitreous humor of the cynomolgus monkey, inquiry of several investigators experienced in working with the eyes of this species suggests that the volume is about 2 ml in the adult cynomolgus monkey (personal communication) as opposed to 1.2–1.5 ml in the rabbit. Therefore, even if the same total amount of drug was transported, the concentration would be lower in the monkey’s eye than in the rabbit’s eye. Second, we used different apparatuses in the two studies, and it is not certain that their efficiency is identical. Third, a much longer interval was allowed to elapse between the iontophoretic treatment and vitreal aspiration in the study with monkeys (24 hr) than in the study with the rabbits (3–3.5 hr). If the half-life of gentamicin in the vitreous humor of the normal cynomolgus monkey’s eye is in the same range as in the normal rabbit’s eye (i.e., 24–32 hr9) or in the normal rhesus monkey’s eye (i.e., 33 hr10), then the concentrations measured in the cynomolgus monkey’s eye were probably about one half of the peak concentration, whereas those in the rabbit’s eye were presumably near the peak concentration. We allowed this long period to elapse in the present study to assure good distribution of the drug through the vitreous humor for purposes of sampling; this precaution was not necessary in the study with rabbits because we took most of the vitreous humor for assay. Of course, it is quite possible that the amount of drug transported into the vitreous humor under identical conditions of iontophoresis may differ between species because of differences in the thickness or vascularity of the sclera, choroid, or retina; the greater the thickness or vascularity of these tissues, the less may be the amount of drug that enters the vitreous humor.

The mean concentration of gentamicin in the vitreous humor declined progressively after repeated treatments and was about 50% as great after the fifth as after the first treatment. One possible explanation for this finding is that there was leakage of vitreous fluid and antibiotic from the needle tracks produced during the aspirations.

There was a moderate degree of variability in the concentration of gentamicin between eyes and within a given eye after repeated treatments; the range was from 2–44 ìg/ml, a variation of about 20-fold. The same degree of variation was noted with ticarcillin and gentamicin in the rabbit’s eye. This variability is greater than the one found with intravitreal injection but less than the one noted with periocular injections of gentamicin1 in the primate eye. Nevertheless, 17 of the 18 values in the monkey’s eye were 6–44 ìg/ml and were presumably higher—perhaps twice as high—shortly after treatment. These concentrations are in the same range as would be produced by the intravitreal injection of 100–200 ìg of gentamicin in the human eye with its vitreous humor volume of about 5 ml and are much higher than those achieved by periocular injection.

The procedure was well tolerated as shown by the results of ophthalmoscopy and electoretinography. The small burns produced by the current were over the pars plana, an area of the eye not used for vision. The animals showed no signs of discomfort during or after iontophoresis, and it seems likely that the procedure could be done in humans with topical anesthesia only. The reason for the transient decrease in intraocular tension following iontophoresis is unclear but may be related to a diminution in function of the ciliary body as a result of electrical injury; a similar decrease in intraocular tension has been reported following corneal iontophoresis.

Taken together, these data indicate that the procedure of transscleral iontophoresis is as effective and
well tolerated in the eye of the primate as in the eye of the rabbit. The results suggest that investigations in humans are warranted.

**Key words:** transscleral, iontophoresis, gentamicin, antibiotic, monkeys

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From the *Department of Medicine, Division of Infectious Diseases, and the †Department of Ophthalmology, New England Medical Center, Boston, Massachusetts. Supported by grant EYO1517 from the National Eye Institute, National Institutes of Health. Submitted for publication: September 9, 1986. Reprint requests: Michael Barza, MD, New England Medical Center, 750 Washington Street, Boston, MA 02111.

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