Iontophoresis of Gentamicin into Aphakic Rabbit Eyes

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The authors evaluated the intraocular penetration of gentamicin (50 mg/ml) into aphakic rabbit eyes following anodal iontophoresis (0.75 mA for 10 min). Gentamicin levels were determined at 0.5, 4, 8, 16, and 24 hrs after iontophoresis (n = 6 eyes for each time) using an agar diffusion bioassay. Peak levels of 72.04 ± 6.1 (X ± SE) μg/ml for the corneas and 77.8 ± 3.0 μg/ml for the aqueous humor were obtained at 30 min after iontophoresis. The peak vitreous level was 10.4 ± 0.4 μg/ml, which was found at 16 hrs after iontophoresis. Therapeutic levels of 6.2 ± 3.0 μg/ml were still present in the vitreous humor 24 hrs after iontophoresis. Iontophoresis appears to be an effective noninvasive method for delivering therapeutic levels of gentamicin into ocular tissues and fluids of the aphakic rabbit eye.

Various methods have been used in an attempt to deliver adequate concentrations of antibiotics into the eye for the treatment and prophylaxis of bacterial endophthalmitis. Systemic, topical, or subconjunctival administration often deliver bactericidal levels into the aqueous humor but not into the vitreous.1,2 Direct intravitreal injection is a currently accepted method of treatment3; however, frequent intravitreal injections may be necessary to maintain bactericidal levels of an antibiotic.4 Baum et al5 suggested that iontophoresis deserves reevaluation in view of the difficulty in attaining high intraocular concentrations of chemotherapeutic agents.

Iontophoresis is a process by which ions in solution are applied to body surfaces and introduced into cells and tissues with the use of electric current. The eye is very suitable for iontophoresis. The cornea is avascular, allowing passage of ions without removal by circulation of the blood, and the aqueous and vitreous humors are good electrical conductors.6 Penetration of antibiotics into the aqueous humor but not into the vitreous humor of phakic rabbit eyes using iontophoresis was reported by Von Sallmann7 and Witzel et al.8

We describe the penetration of iontophoretically applied gentamicin into the cornea, aqueous humor, and vitreous humor of aphakic albino rabbit eyes. Extracapsular lens extractions were performed to remove the lens barrier to the vitreous and to simulate the postoperative condition in which bacterial endophthalmitis often develops.

Materials and Methods

Bilateral extracapsular lens extractions, as described by Mirate et al,9 were performed 48 hrs prior to iontophoresis on New Zealand albino rabbits (2 kg). The rabbits conformed to the ARVO Resolution in the Use of Animals in Research. Immediately before iontophoresis, the rabbits were sedated with xylazine (intramuscularly [im] 12 mg/kg) and anesthetized with ketamine (i.m. 60 mg/kg). The eyes were dilated with two drops of topical 1% atropine and two drops of 10% Neo-Synephrine. Gentamicin sulfate (reagent powder, Schering, Kenilworth, NJ) was dissolved in sterile water to 50 mg/ml (0.094 M). An eyecup was inserted with its periphery at the corneal limbus. The gentamicin solution was placed inside the eyecup and the anode was placed in contact with the solution. The cathode was connected to the shaved abdomen of the rabbit. Constant direct current (0.75 mA) was applied for 10 min with an iontophoretic unit containing a rectifier to convert alternating current to direct current. The eyes were washed with saline at the completion of iontophoresis. The rabbits were sacrificed at 0.5, 4, 8, 16, and 24 hrs (n = 6 eyes for each time). The aqueous humor was removed with a 30-gauge needle attached to a tuberculin syringe, and the volume was measured. The enucleated globe and aqueous humor were placed in sterile containers and quick-frozen in a dry ice–alcohol bath. The vitreous humor was re-
moved intact from the frozen globe and divided midway to obtain anterior and posterior portions. After thawing, each vitreous sample was homogenized by repeatedly forcing it through a 19-gauge needle. Corneas were dissected from the sclera and homogenized with sterile saline (2 ml) using a Polytron PT-10 (Brinkmann, Westbury, NY) at a setting of 4 for 1 min. The corneal homogenate was centrifuged and the supernatant assayed for gentamicin. All samples were assayed by an agar diffusion bioassay using Bacillus subtilis as the test organism. Standard concentrations of gentamicin sulfate (20, 10, 5, 2.5, 1.25, and 0.625 µg/ml) were tested. The sensitivity of the bioassay was 1.25 µg/ml. Sample concentrations were determined by referring to a plot generated from known concentrations of gentamicin. Each sample was assayed in duplicate.

Forty-eight hours after lens extractions, three aphakic control eyes received 50 mg/ml gentamicin solution applied to the eye in an eyecup for 10 min, without current. Control rabbits were sacrificed 30 min after the eye bath, and the eyes were processed as described.

Results
The highest gentamicin concentrations detected were 72.0 ± 6.1 (X ± SE) µg/ml for the cornea and 77.8 ± 3.0 µg/ml for the aqueous humor. These occurred 30 min following termination of iontophoresis (Fig. 1). Corneal concentrations decreased to 10.0 ± 1.6 µg/ml by 4 hrs and continued a gradual decline to 1.6 ± 0.3 µg/ml at 24 hrs. Concentrations in the aqueous decreased more slowly to 62.8 ± 8.2 µg/ml at 4 hrs, to 16.0 ± 1.9 µg/ml at 8 hrs, and to 2.5 ± 0.2 µg/ml at 24 hrs (Fig. 1). The vitreous concentrations increased with a peak at 16 hrs of 10.4 ± 0.4 µg/ml. At 24 hrs, 6.2 ± 0.3 µg/ml gentamicin was present in the vitreous. Gentamicin concentrations detected at 30 min in the anterior vitreous were significantly different than the posterior vitreous (5.9 ± 0.7 µg/ml and 2.4 ± 0.3 µg/ml, respectively; P = 0.0021, two-tailed t). A significant difference between the anterior and posterior vitreous also was observed at 4 hrs (7.6 ± 0.4 µg/ml and 2.7 ± 0.3 µg/ml, respectively; P < 0.0001). Gentamicin concentrations for anterior and posterior vitreous were not significantly different at 8, 16, and 24 hrs postiontophoresis (P > 0.35).

The levels of gentamicin in the cornea, aqueous humor, and vitreous humor were combined to derive the total amount of ocular gentamicin. The total amount as well as the levels in each sample were monitored over a time period of 0.5–24 hrs (Fig. 2). During the assay period, the total levels of ocular gentamicin decreased and a redistribution of the antibiotic was observed. At 30 min, 89.6% of the gentamicin was detected in the cornea and aqueous humor and 10.4% was detected in the vitreous humor. Four hours after iontophoresis, 67.9% of the total was found in the cornea and aqueous humor and 32.1% in the vitreous humor. At 16 and 24 hrs, 90.4% and 88.0%, respectively, of the total gentamicin detected was found in the vitreous humor.

In the control eyes, which received the gentamicin eye bath only, the concentrations of gentamicin in the
cornea, aqueous humor, and vitreous humor were 11.2, 18.5, and <1.25 μg/ml, respectively, at 30 min following the eye bath.

Discussion

Iontophoresis is used in many ways in medicine and dentistry, including the administration of local anesthesia for myringotomy and fluoride for hypersensitive dentin. The amount of drug delivered by iontophoresis can vary depending upon the mA employed, the duration of application of the current, and the concentration of the drug. Entry of many ionized substances into the eye can be facilitated by iontophoresis. Hill et al reported antiviral levels of vidarabine monophosphate in the cornea and aqueous humor using iontophoresis. Leopold and Nichols demonstrated that streptomycin did not penetrate the normal cornea with topical corneal baths, but rapid entry into the aqueous could be obtained with iontophoresis of 5,000 units of streptomycin (2 mA for 3 min). We obtained bactericidal levels of gentamicin in the cornea and aqueous humor 30 min after iontophoresis. These concentrations were seven- and fourfold those obtained in the cornea and aqueous humor, respectively, compared with the corneal baths used in this study, and six- to sevenfold those reported in the aqueous humor (12.0 μg/ml) and in the cornea (11.5 μg/ml) 1 hr following a subTenon’s injection of 20 mg gentamicin. Iontophoresis of gentamicin resulted in vitreal concentrations 30 min after iontophoresis that exceeded the MIC concentration (3.13 μg/ml) for pseudomonas. Even at 24 hrs the levels were greater than the MIC levels for pseudomonas.

Albino rabbits were used in this study. Kane et al reported that levels of gentamicin obtained in the cornea, aqueous humor, and vitreous humor of pigmented and albino eyes are not significantly different after intravitreal injections. Based upon this data, we predict that no significant differences in tissue distribution would be found following iontophoresis of gentamicin into pigmented and nonpigmented rabbit eyes.

Iontophoresis is a relatively simple, noninvasive procedure that can be performed without apparent damage to corneal epithelium. While iontophoresis may not be a substitute for direct intravitreal injections in the therapy of bacterial endophthalmitis, its role, especially as an adjunct in treatment, should be investigated.

Key words: gentamicin, iontophoresis, endophthalmitis, cornea, vitreous

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