An Improved Apparatus for Transscleral Iontophoresis of Gentamicin

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The authors previously found that positively charged substances are less well-transported into the vitreous humor by transscleral iontophoresis than are negatively charged substances. There was more bubble formation in the eye cup with positively charged than with negatively charged substances. The authors hypothesized that these bubbles might account for the poorer conductance of the positively charged species by causing interruptions of the current. Therefore, the authors developed a modified eye cup in which the diameter of the fluid column was larger than that in the old device (1.0 rather than 0.5 mm). This modification allowed larger voltage to be applied than with the older device, because bubbles could be more easily cleared from the conjunctiva than with the narrower-bore eye cup. Although the efficiency of the apparatus was the same with the two eye cups (micrograms per milliliter in vitreous humor divided by milliampere minutes of current applied), vitreal concentrations of gentamicin with the modified eye cup were fourfold higher than with the older eye cup (83 versus 19 µg/ml; \( P < 0.001 \)). These studies suggest that modifying the eye cup to permit easier removal of bubbles resulted in improved delivery of gentamicin into the ocular humors. Invest Ophthalmol Vis Sci 33:3543-3545, 1992

We recently showed that negatively charged dyes are better transported by iontophoresis than are positively charged dyes.\(^1\) In that study, in which a newly designed eye cup was used, we got the impression that bubbles, which were generated in the eye cup during iontophoresis, especially with positively charged agents, were interrupting the current. These interruptions led to a reduction in the dosage that could be delivered with positively charged dyes. We modified the iontophoresis apparatus by enlarging the orifice of the contact column of the eye cup to allow bubbles to be more easily dislodged from the eye cup. This study compares the transscleral iontophoresis of gentamicin with the 0.5 mm diameter contact area eye cup to a modified eye cup in which the diameter of the fluid column is 1.0 mm.

Materials and Methods

Healthy, pigmented rabbits weighing 2.2-2.75 kg were anesthetized with intramuscular ketamine hydrochloride 44 mg/kg and acepromazine maleate 0.25 mg/kg. Drops of tropicamide (1.0%) and proparacaine hydrochloride (0.5%) were applied topically. Gentamicin sulfate was obtained as the laboratory diagnostic powder (Schering Corp., Kenilworth, NJ) and was dissolved in distilled, sterilized water to a concentration of 200 mg/ml.

With the eye proptosed, an eye cup was placed on the conjunctiva. The outer edge of the cup was placed at the limbus temporal to the superior rectus muscle. The eye cup was held in place by negative pressure obtained by withdrawing the plunger on a tuberculin syringe approximately 0.45 cc. The eye then was allowed to relax into normal position. The orifice of the contact column of the first eye cup was 0.5 mm in diameter (Fig. 1, device A). When this eye cup was used, the power was turned on and the voltage was slowly brought up to 25 V. After 5 min, the voltage was increased to 50 V and maintained for 5 min. This stepwise procedure was used because there appeared to be a greater tendency for the current to break when the voltage was increased to the higher level in one step. The second eye cup was similar to the first but was modified by enlargement of the aperture of the contact column from 0.5 mm to 1.0 mm in diameter (Fig. 1, device B). After this eye cup was placed on the eye, power was turned on and the voltage was slowly brought up to a constant 100 V. Bubbles, which were observed forming in the contact column of the eye cup, were removed by flushing. The stepwise increment of voltage used with device A was not necessary.
with device B because current could be maintained by flushing bubbles from the eye cup. Current was continuously monitored on an ammeter.

After 10 min of iontophoresis with either eye cup, the power was turned off, the drug was withdrawn from the apparatus, and the eye cup was removed. The eye was rinsed with normal saline to remove any residual drug from the surface. Both eyes of each animal were used, but in random sequence. The care and maintenance of the rabbits used in these experiments conformed to protocols approved by the Tufts-New England Medical Center Animal Research Committee and adhered to the ARVO Resolution on the Use of Animals in Research.

Approximately 3 hr after iontophoresis was completed by either method, the animals were killed by either intracardiac injection of sodium pentobarbital and the eyes were enucleated. The aqueous humor was removed by suction through a 26 G needle. The eyes then were dissected and the entire vitreous humor was separated from the globe. After separation, the vitreous humor was aspirated through a 26 G needle to ensure mixing. The concentration of gentamicin in the ocular humors was determined by a standard agar-diffusion bioassay (well method) using *Bacillus subtilis* ATCC 6633 in Tryptic Soy Agar (Difco Laboratories, Detroit, MI). Samples of 20 μl were double plated in wells. The average zone sizes were calculated and concentrations were determined by comparison with a curve prepared from standards of known concentration.

**Results**

We measured the effectiveness of iontophoresis in two ways: (1) by calculating the estimated total dose (total milliampere minutes of current) that could be achieved under the specified conditions of iontophoresis; and (2) by calculating the concentrations of gentamicin achieved in the aqueous and vitreous humors.

Modifying the apparatus by increasing the diameter of the fluid column in contact with the conjunctiva produced about a fourfold increase in total dose that could be achieved and in the concentrations of gentamicin achieved in the aqueous and vitreous humors.

Even with the modified apparatus, bubble formation continued to be a problem. There was an average of 12 breakages of the current during a 10 min run when the 1.0 mm diameter aperture eye cup was used, although with this modified apparatus the current could be recovered. In contrast, it was impossible to run iontophoresis at 100 V for more than 1 min with the column of 0.5 mm diameter because bubbles were produced that could not be dislodged. Prophylactic flushing, by pushing small amounts through the eye cup at various intervals, did not help keep bubbles from forming in a current-disrupting manner in either device.

The efficiency of the modified and unmodified eye cups was approximately equal when adjusted for dose (ie, micrograms per milliliter divided by milliampere minutes; Table 1). There was no significant difference between the eye cups regarding the vitreal concentrations (*P* = 0.97) and the aqueous concentrations (*P* = 0.19) after adjustment for dose.

The pH of both solutions decreased during iontophoresis. The drop in pH was greater with the modified apparatus than with the unmodified device. This difference probably resulted from the fact that more current was carried with the modified apparatus and, therefore, more electrolysis of solution occurred.

**Discussion**

Our observations led us to believe that a major factor in the poorer iontophoretic conductance of posi-
tively charged agents was the greater tendency for bubbles to form in the eye cup with positively charged than with negatively-charged substances. The reason for this difference in the propensity for bubble formation in the eye cup is not clear. It is possible that positively charged agents diffuse less readily than negatively charged agents into the eye, for example, because of binding of positively charged species by negatively charged scleral polysaccharides. If this were the case, greater current might be required (obtained by higher voltage) to conduct iontophoresis with positively charged agents. This might lead to a greater generation of heat and eventually to "boiling" or outgassing that would produce bubbles. A second possibility may involve a carbonic anhydrase-type reaction, where addition of hydrogen ions may shift the reaction toward the production of CO2. Iontophoresis of positively charged agents into the eye, for example, be-

Table 1. Concentration of gentamicin in the aqueous and vitreous humor after iontophoresis

<table>
<thead>
<tr>
<th>Diameter of contact column aperture</th>
<th>Duration and voltage of iontophoresis</th>
<th>Total mA-min achieved (±SD)</th>
<th>Concentration of gentamicin (µg/ml) in ocular humors (±SD)</th>
<th>Concentration of gentamicin adjusted for dose (µg/ml/mA-min; ±SD)</th>
<th>pH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mm</td>
<td>5 min/25 V ± 5 min/50 V</td>
<td>3.68 ± 0.5</td>
<td>9.2 ± 4.5</td>
<td>19.2 ± 5.4</td>
<td>2.6 ± 1.5</td>
</tr>
<tr>
<td>1.0 mm</td>
<td>10 min/100V</td>
<td>15.16 ± 2.2</td>
<td>51.9 ± 17.4</td>
<td>82.7 ± 19.5</td>
<td>3.4 ± 1.0</td>
</tr>
</tbody>
</table>

* Each pH value is the average of one solution tested twice. All values are ±0.2 pH U. The gentamicin concentration of both solutions was 200 mg/ml. For each eyecup, a single solution was applied sequentially by iontophoresis to eight eyes.

the formation of bubbles. If CO2 were to be detected, the use of a carbonic anhydrase inhibitor, such as acetazolamide, might help limit bubble formation. Our preliminary results (unpublished experiments) have indicated that outgassing the solution by suction, adding detergent (Triton-x 100), and cooling the reservoir by packing it in ice and dribbling cold water over the surface of the eye cup do not help control bubble formation.

The enlargement of the orifice of the contact column with device B produced a striking increase in conductance and allowed us to achieve higher levels in the vitreous humor of the rabbit. However, the efficiency of the two devices (micrograms per milliliter per milliampere minute) was similar. Therefore, the most likely reason for the performance improvement was that more current could be passed with the modified device because bubbles could be cleared more easily from the surface of the conjunctiva as soon as they formed and began to disrupt the current. The vitreal concentrations that resulted with the modified device were reasonably high even though the human eye has a vitreal volume approximately three times greater than that of the rabbit. If iontophoresis had the same efficacy in the two species, vitreal concentrations in the human should be about one-third of those in the rabbit eye. Extrapolating from the concentrations we achieved in the rabbit's eye with the modified device, levels in the human eye should be in a clinically useful range.

Key words: bubble, eye, gentamicin, iontophoresis

References