Long-term Ocular Effects of Osmotic Modification of the Blood-Brain Barrier in Monkeys

I. Clinical Examinations; Aqueous Ascorbate and Protein

Douglas E. Gaasterland, John A. Barranger,* Stanley I. Rapoport,† Mary E. Girton,‡ and John L. Doppman§

The blood-aqueous humor barrier in adult rhesus monkeys was opened by intracarotid infusion of a 25% mannitol solution. Each monkey had one to four infusions into the same artery, with at least one week between subsequent procedures. The monkeys were observed clinically for 3 to 212 days. Within an hour pigmented cells and protein accumulated in the aqueous humor of the mannitol-treated eyes. Hypotony developed within a day. Aqueous flare and cells cleared within 2 weeks. Hypotony resolved in 8 to 12 weeks. More than one half of the monkeys had transient anisocoria. In some, the pupil in the treated eye was miotic; in others it was dilated. Direct and consensual pupil responses to light remained intact in untreated eyes and in treated eyes with mydriasis. About one fourth of the monkeys developed edema of the ipsilateral optic disk. This correlated with hypotony. No monkey developed cataract, corneal opacity, or vitreous or retinal change. The aqueous protein concentration was slightly above normal a month or more after the carotid infusions, but was considerably less than plasma protein concentration. Posterior and anterior aqueous ascorbate concentrations in treated eyes were slightly below normal, but far greater than plasma concentration, indicating that ascorbate active transport by the ciliary epithelium was essentially intact despite the widespread, permanent structural alteration that had been caused by the mannitol treatment. Invest Ophthalmol Vis Sci 24:153-158, 1983

The blood-brain barrier (BBB) can be temporarily open by intracarotid injection of a variety of hypertonic agents. This procedure allows delivery of molecules to the brain that normally are not able to cross the BBB. Rapoport and his colleagues have refined this technique and quantitated its effects on permeability of the BBB. Clinical investigators are exploring the possibility of supplying exogenous enzymes to the central nervous system in patients with metabolic storage disorders; other investigators are examining its potential for use in the chemotherapy of brain tumors. These applications may have clinical utility provided there are no unacceptable side effects.

Several studies have indicated that a single intracarotid injection of a hypertonic solution can damage the eye in animals. Such an injection causes profound breakdown of the blood-aqueous barrier in several species. In the rhesus monkey (Macaca mulatta) protein starts leaking from the vascular system into the aqueous humor immediately after intracarotid urea or lactamide. Hypotony lasts 4 to 6 weeks. Soon after the injection, the pigmented epithelium of the pars plana separates from the nonpigmented epithelium. The duration and the long-term functional effects of the morphologic change caused by these injections are not clear. Also, it is not known how much additional damage will be caused by repeated carotid injections.

From the Glaucoma Section, Clinical Branch, National Eye Institute, the Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, the Laboratory of Neurosciences, National Institute on Aging, and the Warren G. Magnuson Clinical Center, Department of Radiology, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland.

Submitted for publication March 2, 1982.

Reprint requests: Douglas E. Gaasterland, MD, Building 10, Room 10N307, National Institutes of Health, Bethesda, MD 20205.

0146-0404/83/0200/153/$1.55 © Association for Research in Vision and Ophthalmology

153
The goal of the current study is to evaluate the extent and duration of changes in monkey eyes after single or repeated intracarotid injections of hyperosmotic mannitol. The latter is important because clinical treatment will no doubt require repeated barrier opening. This report contains the clinical and biochemical observations; the microscopic and ultrastructural alterations will be reported separately.

Materials and Methods

Thirteen young adult rhesus monkeys were studied. They weighed 4.5 to 6.7 kg at the start of the experiments. These monkeys were part of a larger group receiving single or repeated intracarotid hyperosmotic injections to allow study of the central nervous system effects. The 13 monkeys were available for extensive clinical and laboratory ocular examination while still being used for central nervous system observations. Before a monkey was entered into the study, a pretreatment eye examination was done using intramuscular phencyclidine hydrochloride (Sernylan®) anesthesia (0.5 to 1 mg/kg). This agent has little effect on intraocular pressure. Ocular examinations of the monkeys included external examination with a handlight; slit-lamp biomicroscopic examination of the anterior segment; measurement of intraocular pressure with a Perkins’ hand-held applanation tonometer; and indirect ophthalmoscopic assessment of the media and posterior fundus to the midperiphery. Pupil diameter was recorded in a dimly illuminated room by visual comparison with a circle template. Previous studies of similarly anesthetized normal monkeys in the same room have shown the diameter to be 4–5 mm and symmetrical in both eyes, and the direct and consensual light responses are brisk, but limited in amount. During slit-lamp examination the aqueous flare and cellular response were each recorded on a scale from “0” (none) to “4+.”

Hyperosmotic Barrier Opening

The blood-brain barrier was opened by a modification of the method described by Rapoport. A 25% (w/v), commercial solution of mannitol was chosen as the osmotic agent for the study because it can be used in humans. After intravenous pentobarbital anesthesia (12 mg/kg initially, with supplemental doses as required), a cuffed endotracheal tube was inserted. Using sterile technique the femoral artery was exposed and cannulated with a radiopaque catheter. The tip of the catheter was advanced under fluoroscopic control, using minute injections of radiopaque dye (Conray) to identify arterial branches. The tip was placed in the preselected internal carotid artery. Nine of the 13 monkeys were given intravenous Evans blue dye (Chroma-Gesellschaft; 2% in isotonic saline, 2 ml/kg) just before the intracarotid mannitol to serve as a protein marker and qualitative indicator of breakdown of the blood-brain barrier.

Using an automatic pump, 24 ml of mannitol solution, prewarmed to 37 °C, were infused into the carotid artery at a constant rate of 0.8 ml/sec. Infusion was completed in 30 sec; the cardiovascular, respiratory, and neural status were monitored before, during, and after infusion.

After the injection the arterial cannula was withdrawn, the femoral artery ligated, and the wound in the femoral fossa closed. Except for the administration of Evans blue dye, the procedure to modify the barrier was identical for all monkeys each time it was performed. Individual monkeys underwent from one to five carotid infusion procedures (Table 1). Twelve received unilateral mannitol infusions; one was infused once in the left carotid and later, four times in the right (Table 1).

Clinical Observations Following Intracarotid Injections

The examination performed before treatment was repeated afterward at three days, one week, each succeeding week until eight weeks, and then each fourth week. The monkeys were killed on a predetermined schedule by administration of an overdose of pentobarbital to allow morphologic examination of the brain and eyes. There was an exception to this in two monkeys. One monkey died of pneumonia six weeks after the second intracarotid injection; another died of sepsis due to an infected femoral cutdown site three weeks after the third injection. The observations from

Table 1. Observation period for 14 treated eyes of 13 monkeys

<table>
<thead>
<tr>
<th>Days of observation after mannitol infusion</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number eyes</td>
<td>#1</td>
<td>#2</td>
<td>#3</td>
<td>#4</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>24</td>
<td>31</td>
<td>101</td>
</tr>
<tr>
<td>*</td>
<td>198</td>
<td>212</td>
<td>7</td>
<td>1 hr.</td>
</tr>
<tr>
<td>1</td>
<td>119</td>
<td>42</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>28</td>
<td>1.5</td>
<td>75</td>
</tr>
<tr>
<td>*</td>
<td>75</td>
<td>198</td>
<td>94</td>
<td>73</td>
</tr>
<tr>
<td>1*</td>
<td>94</td>
<td>73</td>
<td>66</td>
<td>59</td>
</tr>
</tbody>
</table>

* Eyes that had aqueous ascorbate and protein determinations.
† Paired eyes of one monkey.
Late Effects on Aqueous Ascorbate and Protein

In the observation period when the eyes had become clinically normal, four monkeys were selected for measurement of aqueous ascorbate and protein concentrations. These included the monkey that had received bilateral injections and the monkey with a previously injured eye. (This eye was not examined biochemically.) Aqueous humor was obtained from five treated and two control eyes. Within 1 min the anterior chamber was tapped with a 23-gauge, disposable needle attached to a short section of polyethylene tubing. After 25 to 35 µl of posterior aqueous were obtained, the anterior chamber was tapped with a 25-gauge, disposable needle attached to a 0.5-ml tuberculin syringe. Within 1 min the aqueous samples were analyzed for ascorbate content using a modified spectrophotometric method similar to that used by Pirie.9,10 The method* is based on partial decoloring of an excess of blue, pH 6.6, buffered, 2,6-dichlorophenol indophenol, due to reduction of the dye by ascorbic acid. In our laboratory this method has proven rapid, reliable, and accurate for aqueous humor samples as small as 10 µl. The posterior aqueous sample volumes were determined by the weight difference of the filled polyethylene tube minus the weight after the sample was delivered for analysis. A 25-µl anterior aqueous aliquot was delivered to a dye-filled cuvette using a micropipette. The remainder of the anterior aqueous was used for determination of the total protein concentration using the Bradford method.11

The four monkeys used for aqueous biochemical measurements were observed for an additional two to three weeks before being killed for morphologic examination. The mild inflammation that was present cleared during the first week after the taps.

* Details of the method are available from the author upon request.

Results

The duration of observation of the monkeys after the first intracarotid injection varied from 3 to 212 days. As time passed, monkeys were removed from observation to allow the next injection or to allow morphologic study. Thus, there is a declining number of eyes available for study as the observation periods become longer.

Clinical Observations

In monkeys that received systemic Evans blue dye, the ipsilateral lids, conjunctiva, and anterior aqueous became stained within 10 min; there was no early staining of the other eye. Within days the asymmetry of staining of the lids disappeared as staining of the skin became uniform. Aqueous staining occurred only on the ipsilateral side and persisted for more than 1 week in several monkeys, but cleared by 2 weeks in all monkeys.

All but one of the ipsilateral eyes had a marked (greater than 1 +) anterior aqueous flare and cell reaction at 3 days after each modification. Most of the cells in the anterior chamber were brown. The flare and cells became less pronounced at 7 days. At 4 weeks after the first carotid injection, one half of the eyes showed neither flare nor cells (the aqueous was clear), but one of the six eyes examined at 4 weeks still had a 1+ flare and cell reaction. By 5 weeks the anterior aqueous was essentially clear in all eyes.

Bilateral pupil diameters and responses to light were measured in 11 of the 13 monkeys during the first 3 weeks after the carotid injections. The two exceptions were one monkey with so much aqueous staining by Evans blue dye that the pupil was obscured, and another with previous injury in one eye. Seven of the 11 monkeys had anisocoria during one or more examinations. In three the ipsilateral pupil was smaller than in the control eye. This occurred at 3 days in 2 monkeys, and at 2 weeks in a monkey that previously had symmetric pupil diameters. Miosis did not persist. In one monkey it was noted on only one occasion. In another the smaller, ipsilateral pupil did not react to direct light at 3 days, but at 7 days it had become larger than the control eye pupil, and it reacted sluggishly to direct and consensual light stimulation. Four other monkeys had anisocoria with the pupil on the side of the carotid injection larger than the control eye pupil. This occurred first at 7 to 14 days after treatment in eyes with previously symmetric pupil sizes. Pupils in the control eyes reacted normally to direct and consensual light stimulation throughout the observation period, indicating intact light perception in the treated eyes.
Intraocular pressure was below normal for 8 to 12 weeks after a single carotid injection (Fig. 1). Normal monkey intraocular pressure is 14 to 17 mmHg after phencyclidine anesthesia. Mean pretreatment intraocular pressure for eyes in this study was 16.1 (±0.9 SEM, N = 14) mmHg. After the first injection intraocular pressure was lowest at 3 days. The mean intraocular pressure was 6.0 (±0.8 SEM, N = 14) mmHg at 3 days, 37% of the mean pretreatment pressure. Thereafter, the pressure rose toward normal. The intraocular pressure was less depressed and was affected for a shorter interval after subsequent injections than after first treatments. This occurred even though the subsequent injections were done before the effect of the previous treatment on intraocular pressure had completely cleared. The lowest mean intraocular pressure after the second and third carotid injections was about 50 to 60% of the pretreatment pressure. It was about 70% of the pretreatment pressure after the fourth carotid injection. The intraocular pressure returned to the pretreatment level 4 weeks after the last injection in those eyes that received four treatments (Fig. 1).

In 12 monkeys the fundus of the eye could be examined. The fundus could not be seen in the 13th monkey because of a large amount of Evans blue and protein in the anterior chamber. Edema of the optic disk developed in the treated eye of three of the monkeys 3 to 14 days after carotid injections. Edema of the disk developed in both eyes of the monkey treated bilaterally. Fullness of the disk correlated with depression of intraocular pressure and cleared as intraocular pressure returned toward normal. Disk edema did not occur in control (contralateral) eyes or in the treated eyes of the other nine monkeys even though the pressure was as low as in those with disk edema.

The monkeys did not develop abnormalities of extraocular muscle function, cornea, lens, vitreous, or retina.

Aqueous Humor Ascorbate and Total Protein Concentrations

Aqueous humor ascorbic acid and protein concentrations were determined in five eyes after ipsilateral carotid injections and in two paired, untreated eyes (Table 2). Aqueous samples were obtained at 3 months (one eye) and 6 months (two eyes) after a single injection, and at 4 weeks and 6 weeks after the fourth of four injections (two eyes). Studies in our laboratory of normal rhesus monkeys of the same size, receiving the same diet, have shown that normal anterior aqueous humor ascorbate concentration is about 21 mg/dl and serum ascorbate concentration is less than 1 mg/dl. Normal rhesus monkey serum total protein is about 6.2 g/dl, and aqueous total protein is about 33 mg/dl.

All five eyes tested after carotid injections of mannitol had a considerably higher concentration of ascorbate in the aqueous humor (Table 2) than the normal serum concentration. In three of the five a
recent examination had shown a barely detectable aqueous flare reaction. These three eyes had a lower posterior and anterior aqueous ascorbate concentration and a higher aqueous protein concentration than the other two treated eyes. Ascorbate concentration in the two treated eyes with normal protein concentration and in the two control eyes was more than 20 mg/dl, which is normal.

**Discussion**

This study shows that rapid intracarotid injection of hypertonic mannitol in the monkey causes marked hypotony in the ipsilateral eye. The change is similar to that previously observed after other hyperosmotic agents were injected into the monkey carotid artery. The hypotony and aqueous humor flare and cell reaction clear in two to three months after a single carotid injection of mannitol. This is longer than the recovery period of 4 to 6 weeks in monkeys after carotid infusion of hypertonic urea or lactamide. The recovery process after mannitol was not altered much by subsequent repetition of intracarotid injections. Perhaps the first injection caused sufficient damage that little target tissue remained to be altered by subsequent injections.

Humans may not develop as much ciliary and pars plana epithelial damage as monkeys after comparable per kilogram doses of intracarotid mannitol. In one recent study of five patients, none had a decrease of intraocular pressure after osmotic blood-brain barrier opening with mannitol.

Anisocoria occurred frequently after intracarotid mannitol injections. Ipsilateral miosis occurred in more than 25% of the monkeys within the first week. Whether the miosis was caused by production and release of endogenous mediators, was not explored in the present study. An additional 36% of the monkeys had ipsilateral mydriasis. The dilated pupils responded to direct and consensual light stimulation. Mydriasis tended to appear one to two weeks after barrier opening, following a period in which the pupil size was normal. The anisocoria was transient and not a clinically significant side effect of the injections. Anisocoria appears to occur less frequently in humans who have received similar mannitol injections.

The direct and consensual pupil responses were intact in the control eyes. This indicates that gross visual function was intact in the treated eyes.

Fullness of the optic nerve head was seen in about 25% of the ipsilateral eyes. This correlated with hypotony, which is a possible explanation. The swelling cleared as the intraocular pressure returned toward normal; however, not all hypotonous eyes showed this change. Alternatively, it may have been due to a direct effect of mannitol that occurred in some of the monkeys. Abnormal leakage of intravascular fluorescein into the retina around the optic disk has been observed after intracarotid hypertonic agents. This could explain the fullness of the disk seen clinically in some monkeys. It was not caused by generalized central nervous system changes, because the optic disk of the untreated (control) eyes remained normal.

No lens opacities developed in the treated eyes during the 7 months of follow-up after carotid injections. Extraocular muscle function remained intact to clinical examination. The corneas remained clear. The retina and vitreous appeared normal. The lack of alteration of these structures is reassuring from a toxicologic viewpoint.

Despite extensive structural alteration in eyes of monkeys after hyperosmotic carotid injections, the present study shows that the functional barrier to protein leakage recovers. Anterior aqueous protein concentration in treated eyes 3–6 months after a single carotid injection, or 4–6 weeks after the last of several injections, was three to ten times above the normal value; however, it was far below the concentration in the serum. Anterior aqueous protein in the contralateral eyes was nearly normal. Histologic examination of the eyes has shown almost complete loss of pars plana pigment epithelium and window defects in the pigmented epithelium of the ciliary processes. Despite this loss, the protein concentration in the aqueous was comparatively low, and the eyes were able to create and maintain a large excess of ascorbate in the posterior and anterior aqueous. This lends weight to the impression that active transport of ascorbate occurs in the nonpigmented epithelium. Also, it suggests that the barrier to back-diffusion of ascorbate, after it is transported to the posterior chamber, resides in the nonpigmented epithelium.

**Table 2. Concentration of ascorbate and protein in the aqueous humor of seven eyes of four rhesus monkeys after single or repeated intracarotid hypertonic mannitol infusions**

<table>
<thead>
<tr>
<th>Interval after mannitol infusions</th>
<th>Ascorbate (mg%)</th>
<th>Protein (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>A.C.</td>
<td>P.C.</td>
</tr>
<tr>
<td>§86</td>
<td>21.3</td>
<td>16.3</td>
</tr>
<tr>
<td>177</td>
<td>19.5</td>
<td>13.3</td>
</tr>
<tr>
<td>§191</td>
<td>27.6</td>
<td>26.4</td>
</tr>
<tr>
<td>§58</td>
<td>44.37</td>
<td>30</td>
</tr>
<tr>
<td>§79</td>
<td>58</td>
<td>44</td>
</tr>
<tr>
<td>No treatment</td>
<td>26.4</td>
<td>23.9</td>
</tr>
<tr>
<td>§No treatment</td>
<td>29.2</td>
<td>28.1</td>
</tr>
</tbody>
</table>

†‡§ Symbols identify paired eyes of three monkeys.

---

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933114/ on 09/23/2017
Key words: blood-aqueous barrier, osmotic barrier modification, ocular effects, monkey, hypotony, aqueous humor ascorbate, aqueous humor protein, mannitol.

Acknowledgments

The late V. Everette Kinsey, PhD, of the Institute of Biological Sciences, Oakland University, Rochester, Michigan, encouraged this work and wanted to know what would happen to aqueous ascorbate concentration after the pigment epithelium was injured by hyperosmotic carotid injections. We have appreciated his interest and benefited from it. Venkat N. Reddy, PhD, and Jin H. Kinoshita, PhD, suggested the spectroscopic ascorbate analysis method that proved useful in this study. Larry Merola, MS, provided generous biochemical laboratory guidance and assistance. Richard L. Diggs provided outstanding technical assistance.

References