Effect of Plasma Osmolality and Intraocular Pressure on Fluid Movement Across the Blood-Retinal Barrier

Shunji Tsuboi and Jonathan E. Pederson

The inward permeability of the blood-retinal barrier to carboxyfluorescein was determined in monkey eyes with and without rhegmatogenous retinal detachment (RD). In the absence of changes in the diffusional permeability of the retinal pigment epithelium (RPE), inward permeability changes reflect changes in fluid flow across the RPE. Intravenous injection of mannitol resulted in a 15 mosmol/kg increase in plasma osmolality which decreased inward permeability 37% in eyes with RD and 21% in eyes with vitrectomy alone. When the intraocular pressure was raised 20 mm Hg above normal, inward permeability decreased 29% in eyes with RD and 32% in normal eyes. It is concluded that fluid flow across the blood-retinal barrier is influenced by both plasma osmolality and intraocular pressure. Invest Ophthalmol Vis Sci 29:1747-1749, 1988

Fluid transport across the retinal pigment epithelium may occur by three possible mechanisms: (1) hydrostatic pressure differential between the subretinal space and suprachoroid; (2) osmotic pressure differences across the retinal pigment epithelium; or (3) active solute-linked water transport.1 The relative proportion of these components is unknown, although it has been suggested that 70% of the fluid transport across the RPE is driven by solute-linked water transport.2 Intravenous injections of mannitol accelerate resorption of Hanks'-filled subretinal blebs in rabbits, revealing the importance of osmotic factors.3 Changing the intraocular pressure from 0 mm Hg to 38 mm Hg accelerates bleb resorption time in Hanks'-filled blebs in rabbit eyes by 39%.4 Thus, the importance of each of these three factors has been identified in the rabbit. However, no comparable information regarding the monkey is available.

In the present study, the effect of intraocular pressure and plasma osmolality was evaluated in normal monkey eyes as well as monkey eyes following vitrectomy or with chronic rhegmatogenous retinal detachment. Changes in the inward permeability of the blood-retinal barrier to carboxyfluorescein was used as a measure of the alteration in fluid movement across the RPE induced by changes in the intraocular pressure or plasma osmolality. The suitability of this method has been previously discussed.5

Materials and Methods. Thirteen cynomolgus monkeys of either sex weighing 2-6 kg were used. Six animals had total vitrectomy in one eye and retinal detachment in the other eye. Three animals had retinal detachment in one eye with the other eye untouched. Eight normal eyes of four additional animals were used. Rhegmatogenous RDs with a large retinal hole were created by the procedure described in detail elsewhere.6 At least 5 months later, when a total funnel-shaped RD was observed, vitreous fluorophotometry was performed on all eyes. Animal care conformed to the ARVO Resolution on the Use of Animals in Research.

Determination of the inward permeability (Pin) of the blood-retinal barrier in the cynomolgus monkey eye is described in detail elsewhere.5 Briefly, under intraperitoneal pentobarbital sodium anesthesia, 20 mg/kg of carboxyfluorescein (CF, Eastman Kodak, Rochester, NY) was injected intravenously. One hour later, vitreous fluorophotometry was performed through a dilated pupil with a flash fluorophotometer described by Brubaker and Coakes.7 Both eyes were measured on each occasion. In normal eyes, readings were taken from seven points, 1.5 mm apart, along the ocular axis, ie, 1.5-10.5 mm from the retina. Three readings from posterior, mid, and anterior vitreous were taken in vitrectomized eyes and eyes with RDs. Blood samples were taken at 15 and 75 min after injection and total concentration of CF in plasma was measured. The exponential decay was then calculated assuming first-order kinetics.8 Probenecid, an inhibitor of CF transport in the RPE,9 was used both intraperitoneally (150 mg/kg) and intravenously (25 mg/kg) in each experiment. The ratio of unbound to total concentration of plasma CF was previously determined as 0.54 in the presence of probenecid.8

Pin was determined 2 to 4 times, at least 2 weeks apart, for each eye. A control experiment was followed by an experiment where either IOP or plasma...
osmolality was raised, and vice versa. The IOP, determined by an applanation tonometer, was regulated by cannulation. The anterior chamber was cannulated through a self-sealing incision with a 27 gauge needle connected to a reservoir under an operating microscope. The needle was fixed by sutures at the limbus. The reservoir was raised so that the IOP was higher by about 20 mm Hg than control, where IOP was kept at 10 mm Hg. To raise blood osmolality, 35 ml of 20% mannitol were injected IV 5-20 min prior to CF injection. Blood was collected before injection, and 15 and 45 min after injection and blood plasma osmolality was determined by a freezing point osmometer (Precision Systems, Natick, MA). The cannulation was also employed in experiments with mannitol and corresponding "control" experiments in the normal eyes.

Pin was calculated from the following equations, assuming that the amount of CF present in the posterior hemisphere of the vitreous cavity 1 hr after injection, (CF)t=60, moved across the blood-retinal barrier:

$$P_{in} = \frac{(CF)^{t=60}}{S} \int_{0}^{60} C_p d t$$  \hspace{1cm} (1)

and

$$(CF)^{t=60} = (271F^{1.5} + 177F^{3} + 102F^{4.5} + 48F^{6} + 15F^{7.5} + F^{9})/614 \times V(C/F)$$  \hspace{1cm} (2)

where $S$ = surface area of the posterior hemisphere, 4.5 cm$^2$, $C_p$ = unbound plasma CF concentration, $F^{1.5-9}$ = measured vitreous fluorescence 1.5-9 mm from the retina, $V$ = volume of the hemisphere, 1.3 cm$^2$, and $C/F$ = resolution coefficient for vitreous fluorophotometry, 1.6. In vitrectomized eyes and eyes with RDs, CF distributed evenly in the vitreous cavity, so that $(CF)^{t=60}$ was simply expressed as $FV(C/F)$.

**Results.** The effect of plasma osmolality on Pin was studied in normal eyes, vitrectomized eyes, and eyes with RDs and is summarized in Table 1. Plasma osmolality, $308 \pm 2$ (SE) mosmol/kg, was increased by IV mannitol to $324 \pm 3$ at 15 min and $321 \pm 3$ at 45 min after injection. IV mannitol significantly decreased Pin in eyes with RD and vitrectomized eyes. The decrease was 37% in eyes with RD and 21% in vitrectomized eyes. However, the decrease in Pin was not statistically significant in normal eyes.

The effect of IOP was studied in normal eyes and eyes with RD (Table 2). By application of an additional 20 mm Hg on the vitreous side of the blood-retinal barrier, Pin was significantly decreased in normal eyes and eyes with RD. The decrease was 32% and 29%, respectively.

**Discussion.** The present study in the monkey confirms the fact that intraocular pressure and plasma osmolality affect fluid movement across the retinal pigment epithelium. This conclusion is based on the validity of the assumption that the change in inward permeability of carboxyfluorescein is due to a change in fluid movement. If mannitol were to cause an increase in the permeability of the RPE, then one would expect an increase in inward permeability. This is opposite to what was observed in the study. Thus, it can be assumed that the mannitol-induced decrease in inward permeability is a result of increased outward fluid movement across the blood-retinal barrier. Alteration in intraocular pressure would not be expected to alter the diffusional permeability of the RPE and thus the same assumption would apply.

Mannitol affects both plasma osmolality and IOP. Since an IOP rise causes a decrease in Pin, a mannitol-induced decrease in the IOP would underestimate the effect of osmolality. Thus, IOP was kept constant by cannulation in the normal eyes throughout the injection of mannitol. Why the mannitol effect is significant only in eyes with RDs and vitrectomized eyes is unknown. Mannitol-induced fluid flow may be minimized by the sensory retina and solid vitreous gel in the normal eye. Furthermore, CF entering from the ciliary body would be included in the vitreous cavity of eyes with RDs and vitrectomized eyes where vitreous CF concentration is uniform. Although integrity of the RPE barrier function is maintained in eyes with chronic RDs, an abnormal CF leak from blood vessels of the detached sensory retina could be involved.
It is difficult to assign a value for hydraulic or osmotic fluid permeability of the retinal pigment epithelium, since the precise hydrostatic pressure or osmotic pressure difference across the RPE is not known. Elevation of intraocular pressure also changes the suprachoroidal hydrostatic pressure, such that the pressure drop across the RPE may not change appreciably. Similarly, unstirred layer effects in the extravascular choroid following systemic mannitol injection would cause an underestimation of the true osmotic fluid permeability of the RPE.

It would appear that under normal circumstances, osmotic and hydrostatic forces play a definite but small role in fluid transport across the RPE. However, following damage to the RPE, such as with sodium iodate, the importance of these factors may be enhanced.4 One might also speculate that a similar situation could occur following retinal cryopexy where the hydraulic permeability of the RPE would be increased and the protein content of the extravascular choroid would be increased.

Key words: blood-retinal barrier, retinal pigment epithelium, permeability, carboxyfluorescein, mannitol, intraocular pressure, cynomolgus monkey

From the Department of Ophthalmology, University of Minnesota, Minneapolis, Minnesota. Dr. Tsuboi's current address is: Department of Ophthalmology, Osaka University Medical School, Osaka 553, Japan. Supported by the James S. Adams Scholars Award from Research to Prevent Blindness, Inc., and NIH grant EY-03277 (JEP). Submitted for publication: August 10, 1987; accepted June 13, 1988. Reprint requests: Jonathan E. Pederson, MD, Box 493 UMHC, University of Minnesota, Minneapolis, MN 55455.

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