Indomethacin and the Epinephrine-Induced Breakdown of the Blood-Ocular Barrier in Rabbits

Kensaku Miyake, Fumio Kayazawa, Ryoko Manabe, and Yoshiko Miyake

Using aqueous and vitreous fluorophotometry, the authors examined the blood-aqueous and blood-retinal barrier functions in three groups of pigmented rabbits. Epinephrine (1.25%) was applied topically five times daily and indomethacin (0.5% sesame oil suspension) was applied topically three times daily to one eye of each of the animals in Group 1; under the same regimen, epinephrine and indomethacin placebo were administered to one eye of each of the animals in Group 2 and epinephrine placebo and indomethacin placebo were administered to one eye of each of the animals in Group 3. Fluorophotometry was done 1, 2, and 3 months after drug administration. The results showed that epinephrine induced disruption of the blood-aqueous barrier 2 and 3 months after drug administration, and that the magnitude of this disruption increased with time. Epinephrine also induced disruption of the blood-retinal barrier 3 months after drug administration. Indomethacin significantly prevented disruption of the blood-aqueous barrier at 2 and 3 months and significantly prevented disruption of the blood-retinal barrier at 3 months. The magnitudes of the barrier disruptions in eyes treated with both epinephrine and indomethacin were slightly higher than, or the same as, those of the control eyes. The results strongly indicated that the epinephrine-induced disruption of the blood-ocular barrier was partially caused by prostaglandins and other cyclo-oxygenase products whose biosynthesis was initiated by epinephrine. Invest Ophthalmol Vis Sci 28:482-486, 1987

The effects of epinephrine on the blood-ocular barrier are unknown, but are of interest when studying the pathogenesis of epinephrine-induced macular edema, which is believed to be an established clinical entity.

Adrenergic agents, such as epinephrine, stimulate endogenous prostaglandin synthesis in several tissues, including rabbit or bovine irides. Prostaglandins have a wide range of ocular biological reactions, including inflammatory reactions, breakdown of the blood-ocular barrier, miosis, and regulation of intraocular pressure. Prostaglandins were initially believed to cause an acute increase in intraocular pressure. However, animal studies have shown that prostaglandins produce a prolonged reduction of intraocular pressure. The ocular hypotensive effects of epinephrine are not fully understood. Bhattacherjee and Hammond and Camras et al found that indomethacin, an inhibitor of prostaglandin biosynthesis, inhibits epinephrine-related ocular hypotensive effects both in rabbits and in humans. Camras et al stressed that the ocular hypotensive effect of topically applied epinephrine can be partially ascribed to prostaglandins. These findings stimulated a consideration of epinephrine maculopathy pathogenesis. Clinically, aphakic cystoid macular edema is very similar to epinephrine maculopathy. The two entities are often confused, and one may be misdiagnosed as the other. The pathogenesis of aphakic cystoid macular edema is not fully understood; postulated etiologic factors include hypotony, inflammation, and vitreous traction. Indomethacin applied topically has been used to prevent aphakic or pseudophakic cystoid macular edema. This suggests that prostaglandins and other cyclo-oxygenase products biosynthesized intra- and postoperatively play some role, especially in transient postoperative cystoid macular edema. Thus, prostaglandins and related compounds may be common factors in both aphakic cystoid macular edema and epinephrine maculopathy.

We have, to date, no animal model of epinephrine maculopathy. This study examined the effects of epinephrine on the blood-aqueous and blood-retinal barrier function, and also the effects of topically applied indomethacin on the epinephrine-induced disruption of the blood-ocular barrier. These studies were done by fluorophotometry, the most sensitive method for examining the function of the ocular barrier. This study was done in rabbits; the findings may not be transferable to cystoid macular edema in humans.
Materials and Methods

Pigmented Dutch strain rabbits weighing 1.5 to 2.5 kg were used. They were treated according to the ARVO Resolution on the Use of Animals in Research. All rabbits were examined by slit-lamp and indirect ophthalmoscopy, and only those that showed no signs of inflammation or other diseases that might produce abnormal fluorophotometric readings were included in the study. The selected animals were divided into three groups in a prospective, randomized, and double-masked fashion by one of the authors, (RM, a pharmacist), who was not involved in ocular examinations and measurements. Animals in Group 1 received 1.25% epinephrine eye drops (Epista Senju Co., Osaka, Japan) five times daily and 0.5% indomethacin drops (Senju Co., Osaka, Japan) three times daily for 3 months. Animals in Group 2 were given 1.25% epinephrine eye drops and indomethacin placebo eye drops in the same regimen as animals in Group 1. Animals in Group 3 received epinephrine placebo eye drops and indomethacin placebo eye drops, also in the same regimen as animals in Group 1. The medications were given to one eye of each animal. In our preliminary experiment, we confirmed that indomethacin applied topically for 3 consecutive months had no significant effect on the blood-ocular barrier. The functions of the blood-aqueous barrier and blood-retinal barrier were evaluated by a slit-lamp fluorophotometer (Gamma-Inami, Tokyo, Japan) similar to that described by Waltman and Kaufman27 and by Fluorotron Master (Coherent Radiation, Palo Alto, CA). Slit-lamp fluorophotometry was done after dilating the pupil with 0.5% tropicamide, and the background fluorescence in the retina-choroid, the crystalline lens, and the cornea was measured. During fluorophotometry, there was no difference in pupil size between the two eyes of each animal. Aqueous and posterior vitreous fluorescein concentrations were measured at the center of the anterior chamber and 3 mm from the retina and 2 mm from the myelinated nerve bundle 1 h after intravenous administration of 10 mg/kg fluorescein sodium.

Fluorotron Master is especially useful in the middle or posterior vitreous fluorescein measurement of rabbit eyes. We used the method described by Zeimer et al28 for minimizing the influence of lens and postinjection chorioretinal fluorescence. After pre- and bolus scan, we did a vitreous fluorophotometric scan at the same time aqueous fluorophotometry was done (1 hour after dye administration). The mean posterior vitreous concentration was calculated by averaging the corrected values between 3 mm and 5 mm from the chorioretinal peak. Particular attention was given to each scan to be initiated at the same point of the posterior retina, and areas of the optic disc and the myelinated nerve bundle that would be associated with high auto-fluorescence were avoided. Fluorophotometry was performed in both eyes of each animal 1, 2 and 3 months after drug administration. As a parameter indicating the function of the blood-aqueous barrier, we used the treated eye/fellow eye ratio of the aqueous fluorescein concentration. This parameter is basically the same as the one described by Sander et al.29,30 As a parameter for the blood-retinal barrier, we used the treated eye/fellow eye ratio of the (mean) posterior vitreous fluorescein concentration.

Results

Figures 1 and 2 summarize the results. We used the following statistical tests: analysis of variance to evaluate the differences among the three groups; Bartlett's
test to evaluate the uniformity of variance; and the Duncan test as a multiple range test. Three animals in Group 3 were withdrawn from the study because of abnormal ocular signs and death. The treated eye/fellow eye ratios of aqueous fluorescein concentration 1 month after drug administration in Groups 1, 2, and 3 were 0.98 ± 0.14 (mean ± SD), 1.06 ± 0.15, and 0.96 ± 0.18, respectively. There were no statistically significant differences among the groups. At 2 months, the ratios were 1.00 ± 0.14, 1.29 ± 0.19, and 1.05 ± 0.16, respectively. There were statistically significant differences between Groups 1 and 2 (P < 0.01) and Groups 2 and 3 (P < 0.01), but not between Groups 1 and 3. At 3 months, the ratios were 1.16 ± 0.17, 1.58 ± 0.21, and 1.01 ± 0.19, respectively. There were statistically significant differences between Groups 1 and 2 (P < 0.01), and Groups 2 and 3 (P < 0.01), but not between Groups 1 and 3. In Group 2 the ratio at 3 months was significantly larger than that at 2 months (P < 0.01), which was also significantly larger than that at 1 month (P < 0.01).

The treated eye/fellow eye ratios of posterior vitreous fluorescein concentration of Groups 1, 2, and 3 were 0.99 ± 0.23, 1.04 ± 0.23, and 1.07 ± 0.24, respectively, 1 month after drug administration. There were no statistically significant differences among groups. At 2 months, the ratios for the three groups were 1.05 ± 0.28, 1.00 ± 0.16, and 0.93 ± 0.15, respectively. There were no statistically significant differences between groups. At 3 months, the ratios for the three groups were 1.06 ± 0.26, 1.35 ± 0.11, and 0.97 ± 0.14, respectively. There were statistically significant differences between Groups 1 and 2 (P < 0.01) and Groups 2 and 3 (P < 0.01), but not between Groups 1 and 3.

**Discussion**

We report that topical application in rabbits of 1.25% epinephrine five times daily induced substantial disruption of the blood-aqueous barrier 2 and 3 months after drug administration. Also, we found that these disruptions were prevented by simultaneous topical application of 0.5% indomethacin three times daily. The epinephrine-induced disruption was recognized earlier in the blood-aqueous barrier than in the blood-retinal barrier. We also found that in the eyes treated with epinephrine, the disruptions increased in magnitude up to 3 months, especially in the blood-aqueous barrier. In eyes treated with epinephrine and indomethacin, however, the disruption of the blood-aqueous barrier was only slight, and disruption of the blood-retinal barrier was not recognized until 3 months. These findings are important for considering the nature of the epinephrine-induced blood-ocular barrier disruption, and also for a discussion of the pathogenesis of epinephrine maculopathy.

Epinephrine maculopathy was first reported by Kolker and Becker in 1968; subsequent reports have documented this disorder by fluorescein angiography. This maculopathy shows cystoid macular edema, which clinically resembles those in aphakias and several other ocular pathologies. Thomas et al statistically supported the concept of epinephrine macular edema. They also speculated that in aphakia, epinephrine and its toxic photo-oxidation products are more accessible to posterior ocular tissues. They state that this accessibility is related to an epinephrine maculopathy based on several studies, mainly those conducted by Kramer and Zigman. Based on this historical review, one could conclude that epinephrine induces macular edema when topically instilled to aphakic eyes. There is a question as to whether epinephrine induces hyperpermeability in retinal capillaries. Several recent studies demonstrated that adrenergic agents can induce endogenous prostaglandin synthesis in rabbit or bovine irides. Furthermore, indomethacin, an inhibitor of prostaglandin synthesis, can inhibit epinephrine-induced ocular hypotension. These reports led to our current studies, especially because endogenous prostaglandins were recently postulated as a factor causing cystoid macular edema after intraocular surgeries.

In these studies, we demonstrated that indomethacin could prevent the epinephrine-induced disruption of the blood-ocular barrier. This suggests that the barrier disruption in rabbit eyes is at least partially mediated by endogenous prostaglandins or other cyclo-oxygenase products. Although there may be a difference between rabbits and humans in metabolism and synthesis of prostaglandins, and also in the synthesis of prostaglandins relating to epinephrine application, the current results may have some relevance to epinephrine maculopathy.

Our studies demonstrated that epinephrine applied topically can induce the disruption of the blood-retinal barrier, even in phakic rabbits. This finding may be partially due to the specificity of the experimental animals used and also to the high sensitivity of fluorophotometry in demonstrating alterations of the blood-retinal barrier. The frequent dosing of epinephrine (five times daily) may also account for the disruption of the blood-retinal barrier. In eyes treated with epinephrine alone, the disruption of the blood-aqueous barrier was recognized earlier than the disruption of the blood-retinal barrier. There are reports that epinephrine applied topically accumulates more in the anterior uvea than in the retina, especially in phakic subjects. However, these reports cannot fully explain our observation in eyes with combined treatments of epinephrine and indomethacin of only a slight disruption of the blood-aqueous barrier and essentially no disruption of the blood-retinal barrier at 3 months. From
these findings, we postulate that the main factor in the epinephrine-induced breakdown of the blood-ocular barrier might be prostaglandins initiated by topical application of epinephrine.

That epinephrine applied topically induces synthesis of prostaglandins in the iris that may lead directly to the disruption of the blood-aqueous barrier or, more specifically, the blood-iridial barrier, may explain the time difference we found in the disruption of the blood-aqueous and blood-retinal barriers from the "prostaglandin theory." In the retina, the amount of prostaglandins may be reduced by the active transport of the ciliary epithelium in normal phakic eyes. For this reason, some time is needed before substantial disruption of the blood-retinal barrier occurs.

Further investigations are necessary before application of the pathogenesis of epinephrine-induced maculopathy to humans based on our findings in rabbits, since the disruption of the blood-aqueous and blood-retinal barriers in rabbits are not the same as those in human cystoid macular edema. In fact, Camras et al performed a similar study in which multiple dosing of 2% epinephrine twice daily was given to cynomolgus monkeys, animals more closely related to humans than are rabbits. The researchers did not produce a significant change in any of the following: aqueous humor flare by slit-lamp biomicroscopy, protein concentration in the aqueous humor, fluorescein concentration in the aqueous humor as determined by fluorophotometry after intravenous fluorescein administration, and evidence of cystoid macular edema by fluorescein angiography.

We have begun to study the effect of indomethacin on humans. Additional studies on the effect of these combined drugs in aphakic eyes should be done, and differences between aphakic and phakic subjects should be evaluated. In vivo assay of prostaglandins in the uvea and the vitreous in these experimental conditions will also be important. Other parameters not considered in the current study include the influence of epinephrine on the aqueous flow, on the relationship between the aqueous flow and the function of blood-aqueous barrier, and also on the general blood flow.

Key words: epinephrine, prostaglandins, blood-ocular barrier, indomethacin, cystoid macular edema

Acknowledgment
Mr. Yoshihatsu Kawase, a biostatistician, did the statistical analysis.

References
25. Kraff MC, Sanders DR, Jampol LM, Peyman GA, and Lieber-