Blood-ocular barrier permeability in monkeys

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Abstract

The permeability of the blood-ocular barrier was investigated in five monkeys using vitreous fluorophotometry (VFP). Inward permeability (P_{in}) of the blood-retinal barrier was calculated by a computer simulation method. Kinetic VFP was performed after intravitreal injection of fluorescein (F) or fluorescein monoglucuronide (FG). The estimated mean value of P_{in} ($\times 10^{-6}$ cm/min) was 4.8 (SD 1.2). The mean rates of loss (per hour) of F from the anterior chamber (K_a) and the vitreous (K_v) were 0.11 (SD 0.01) and 0.13 (SD 0.03), respectively, which were approximately three and four times greater than those of FG (0.04 (SD 0.01) and 0.03 (SD 0.01), respectively). Probenecid administered intraperitoneally decreased both the K_a and the K_v of F significantly but had no effect on the K_a or the K_v of FG, suggesting that F was excreted from the eye with the aid of the active transport mechanism. The results of comparative studies of the rates of loss of F from the anterior chamber (K_a) and from the vitreous (K_v) suggested that active transport was more predominant in the blood-retinal barrier than in the blood-aqueous barrier.

Studies performed using vitreous fluorophotometry (VFP) have demonstrated the presence of certain mechanisms in the blood-ocular barrier (BOB), such as the inhibition of intraocular penetration of some substances from the blood and the excretion of some substances from the eye via active transport.¹ However, little has been learned about the physiological mechanism of active transport in the BOB because the metabolism of fluorescein-Na (F) used in VFP interferes with the determination of this mechanism. Systemically administered F has been shown to be metabolised rapidly to fluorescein monoglucuronide (FG)² and that it is more difficult for FG than F to undergo active transport.^{3,4} Thus, when the intraocular active transport function is studied using VFP, the intraocular kinetics of both F and FG should be followed.^{5,6}

Rabbit eyes have been used widely for the study of the intraocular active transport function,^{1,3,4,7} but the structure of the retinal vasculature in rabbits differs markedly from that in humans. Therefore, it is difficult to extrapolate the results obtained in rabbits to humans. Moreover, the direct injection of F into the human vitreous is restricted. In this study we used the monkey eye to analyse the permeability function of the BOB since it resembles the human eye in anatomy and retinal vasculature. This function was classified into inward and outward permeabilities by monitoring the fluorescence kinetics.

To analyse the inward permeability (P_{in}) of the BOB, particularly of the blood-retinal barrier

(BRB), F was injected intravenously, and a P_{in} coefficient of the BRB was calculated using VFP and a computer simulation method.^{5,8} In this study, the outward permeability (P_{out}) of the BOB was estimated by injecting F or FG into the vitreous and determining changes in the concentration periodically using VFP. Our study demonstrated that the P_{in} of the BRB in the monkey eye is nearly equivalent to that in the human eye, which allowed us to speculate on the homeostasis of the human vitreous in relation to BOB function.

Materials and methods

MATERIALS

Ten eyes of five adult cynomolgus monkeys (all male) weighing 3.0-5.0 kg were used. The following experiments were performed under a light anaesthesia induced by an intramuscular injection of ketamine hydrochloride and pentobarbitone sodium.

REFRACTION MEASUREMENTS

Both pupils of each monkey were dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide. The dioptric power (D) of each eye was measured by refractometer (Ophthalmometer, Zeiss Jena, Germany), and the optical elements such as anterior chamber depth, lens width, and vitreous cavity length were determined using A-mode ultrasonography (Ophthalmoscan 200, DBR Sonometrics, Lake Success, NY, USA).

VITREOUS FLUOROPHOTOMETRY

After dilatation, the eyes were evaluated further by indirect ophthalmoscopy. No fundus abnormalities were found. The vitreous body of each eye was examined using a slit-lamp with an aspheric +58.6 D lens (El Bayadi-Kajiura).⁹ No vitreous abnormalities, such as liquefaction, lacuna formation, or any type of posterior vitreous detachment were apparent.

VFP then was performed on all 10 eyes. The fluorophotometer used in this study has been described in detail elsewhere.^{10,11} In short, a modified Haag-Streit model 360 slit-lamp was used to record equivalent F concentration profiles in the ocular media. The angle between illumination and measuring light paths was 14 degrees, and the beam dimensions at the focal plane in air were 3 mm $\times$ 150 μ m and 2.8 mm $\times$ 150 μ m, respectively. The meter readings of this system were linearly related to the F concentrations in a range from 7.0×10^{-11} to 8.0×10^{-7} g/ml.

After topical anaesthesia with 0.4% benoxinate hydrochloride, a low vacuum contact lens

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Figure 1 Schematic illustration of the simulation model.

was placed on the cornea using methylcellulose solution. Measurements were taken before (baseline) and 60 minutes after antecubital vein injection of 10% F (Fluorescein, Alcon, Fort Worth, TX, USA) at a dose of 7 mg/kg body weight.

To determine the concentration of protein-unbound free F (PUF) in plasma, blood was sampled from the antecubital vein on the side opposite to the dye injection at 10 and 65 minutes following fluorescein injection by methods previously described.¹²

ESTIMATION OF INWARD PERMEABILITY OF THE BLOOD-RETINAL BARRIER

To estimate the P_{in} of the BRB with our computer simulation technique,^{5,8} we adopted the values at 14 dispersed sampling points, utilising serial VFP measurements. The value for the anterior vitreous was derived from seven points 0.4 mm apart starting from a point 2.0 mm posterior to the centre of the crystalline lens; the value for the posterior vitreous was derived from seven points 0.4 mm apart between the 2.4 and 4.8 mm anterior points from the retinal surface. All these VFP readings were corrected for the baseline value.

The model we used for computer simulation of intraocular F kinetics has been described in detail.^{5,8} Briefly, the eyeball is divided into anterior and posterior segments (Fig 1). The posterior part is assumed to be ellipsoid. The length of the ellipsoid axis can be altered according to the actual length of the vitreous cavity obtained from A-mode ultrasound measurement. The anterior and posterior parts were divided into eight compartments, each consisting of a thin shell. The thickness of each shell is 0.8 mm for the monkey eye. In the posterior half, P_{in} and P_{out} are the two parameters of the BRB permeability coefficient, and $D-p$ represents the

dye diffusion coefficient in the posterior vitreous cavity. Similar parameters in nature were used for the anterior part of the eye. Based on this model, dye kinetics in the vitreous cavity were simulated, and P_{in} and $D-p$ values were estimated for each eye.

CHANGES OF INTRAOCULAR FLUORESCENCE FOLLOWING INTRAVITREAL INJECTION OF FLUORESCIN OR FLUORESCIN MONOGLUCURONIDE

One month after the previous experiment, indirect ophthalmoscopy revealed no fundus abnormalities. Intraocular autofluorescence was then measured by fluorophotometry. Ten μ l of a 1 mmol solution of F, prepared in isotonic phosphate buffered saline (pH 7.4), was injected directly into the centre of the vitreous cavity through a 30 gauge needle placed on the pars plana in the right eye of the monkeys. The left eye was injected in the same manner with the same amount and concentration of FG (synthesised by Chugai Pharmaceutical Co, Ltd, Tokyo), prepared in isotonic phosphate buffered saline. Its purity was specified by the manufacturer to be 99.17% FG and 0.21% F. Intraocular fluorescence was measured 6, 8, 10, 12, and 24 hours after injection.

EFFECTS OF INTRAPERITONEAL INJECTION OF PROBENECID

Two months following the intravitreal injection, the subjects were examined to ensure that no fundus or vitreous abnormality had developed. Probenecid (Sigma Chemical Co, St Louis, MO, USA), a known inhibitor of active transport of F from the eye,¹³ then was given intraperitoneally (150 mg/kg) to the five monkeys, twice, just before and 6 hours following intravitreal injection of F or FG. Intraocular fluorescence again was measured by VFP.

DATA ANALYSIS

The data were analysed using standard statistical methods. Student's unpaired t tests were used to compare groups. Paired t tests were performed for paired parameters. Differences were considered significant when the probability value indicated a chance of random occurrence of less than 5%.

Results

REFRACTION AND OPTICAL ELEMENTS

The values of the refraction and optical elements are shown in Table 1. The refraction ranged from -0.5 to 0 D and was emmetropic.

Mean axial length was 18.57 mm (SD 1.81), corresponding to ~78% of that of the human eye.¹⁴ The mean ratio of each optical element to axial length in monkeys was 0.16 for anterior chamber depth, 0.18 for lens width, and 0.66 for vitreous cavity length. These ratios of each element to the axial length in monkeys' eye was similar to that obtained from the human eye (0.15, 0.18, and 0.68, respectively).¹⁴

Table 1 Refraction and optical element measurements (n=10)

Parameter	Range	Mean (SD)
Dioptric power (D)	-0.5-0	-0.3 (0.3)
Anterior chamber depth (mm)	2.50-3.36	2.93 (0.33)
Lens width (mm)	3.15-3.65	3.40 (0.15)
Vitreous cavity length (mm)	11.20-14.75	12.24 (1.47)
Axial length (mm)	16.80-21.50	18.57 (1.81)

Figure 2 Time courses of concentration changes in the anterior chamber and vitreous following intravitreal injection of fluorescein and fluorescein monoglucuronide.

INWARD PERMEABILITY OF THE BLOOD-RETINAL BARRIER

The mean estimated P_{in} value by the computer simulation method was 4.8×10^{-6} cm/min (SD 1.2).

CHANGES OF INTRAOCULAR FLUORESCENCE

FOLLOWING INTRAVITREAL INJECTION OF F OR FG
The time course of intraocular fluorescence measured following intravitreal injection of F or FG was studied with emphasis on two values: the anterior chamber value represented by the central value of the anterior chamber, and the vitreous value represented by the value in the vitreous 6 mm anterior to the retina (Fig 2). Values were displayed by converting them to actual concentrations of F or FG, which were based on each fluorescence intensity. The concentration of F in the anterior chamber and vitreous decreased rapidly with time compared with the FG concentration in those sites. Furthermore, the F concentration in the mid-vitreous showed consistently higher levels than in the anterior chamber in contrast to the FG concentration, which showed only a slightly higher level in the mid-vitreous.

The rates of loss (per hour) of F and FG from the anterior chamber (K_a) and vitreous (K_v) were calculated using fluorescence values obtained 12 and 24 hours following intraocular injection (Table 2). To compare the rates for F and FG, we determined $K_a(F)/K_a(FG)$ and $K_v(F)/K_v(FG)$. The mean ratios were 2.9 (SD 0.5) and 4.7 (SD

1.4), respectively; the difference was statistically significant ($p < 0.05$).

EFFECTS OF INTRAPERITONEAL INJECTION OF PROBENECID

Intraperitoneal administration of probenecid, an inhibitor of active transport of F from the eye, significantly decreased the mean values (per hour) of K_a and K_v of F to 0.09 (SD 0.04) ($p < 0.05$) and 0.05 (SD 0.03) ($p < 0.05$), respectively (Table 2), compared with the values without probenecid. The values for K_a and K_v of FG (0.04 (SD 0.01) and 0.03 (SD 0.01), respectively), however, remained unchanged with the administration of intraperitoneal probenecid (Table 2).

Discussion

Our present study revealed that refraction of the monkey eyes was emmetropic and that the structure of monkey eyes, based on the ratio of each optical element to the axial length, was similar to human eyes. P_{in} ($\times 10^{-6}$ cm/min) of the monkey eyes was 4.8 (SD 1.2), which is ~77% of the value in humans (6.2 (SD 1.8)).⁸ These findings allow us to state that passive transport functions similar to those in human eyes exist in the BRB of monkeys.

Cunha-Vaz and Maurice¹ reported that F infused into the rabbit vitreous cavity was excreted via the BRB by active transport into the bloodstream. Active transport function in the BOB has also been analysed.^{3-6, 15-21} We found that the K_v (per hour) of FG was nearly the same as that of sucrose ($K_v = 0.04$)²² or gentamicin ($K_v = 0.035$), which was found to be excreted from the eye only by passive transport.²³ This, together with the fact that the K_v of F is about four times greater than the K_v of FG in the vitreous, allows us to assume that F is excreted from the eye mainly by active transport in the monkey eye. Further, intraperitoneal administration of probenecid, an inhibitor of active transport, caused a decrease in K_a (per hour) and K_v of F but had no measurable effect on K_a and K_v of FG. These results strongly suggest that F and FG are excreted from the eye mainly by active and passive transport, respectively.

The value of $K_v(F)/K_v(FG)$ was found to be greater than that of $K_a(F)/K_a(FG)$, and the difference was statistically significant. This finding, together with the K_v values obtained from the kinetics of fluorescence in the mid-vitreous (which is surrounded by the retina and thus reflects the retinal dye excretion), contributes to the assumption that intraocular active transport is greater in the BRB than in the blood-aqueous barrier. This result agrees with Miyake's²⁴ who used enucleated human eyes to study the active transport of F from the vitreous cavity to the bloodstream *in vitro*. He found that the retina represented the main region of blood-ocular active transport (76% of the active transport rate).

The value of P_{in} of the BRB of the monkey was determined to be nearly equivalent to that of the human eye. This monkey model was extremely useful because it allowed us to evaluate direct

Table 2 Rate of loss (per hour) from anterior chamber (K_a) and vitreous (K_v) following intravitreal injection of F or FG (n=5)

	Without probenecid		With probenecid	
	F	FG	F	FG
K_a	0.11 (0.01) ^a	0.04 (0.01) ^a	0.09 (0.04) ^a	0.04 (0.01) ^a
K_v	0.13 (0.03)	0.03 (0.01)	0.05 (0.03)	0.03 (0.01)

F=fluorescein; FG=fluorescein monoglucuronide; K_a =rate of loss from anterior chamber; K_v =rate of loss from vitreous.

^aStandard deviation (SD).

infusion of F or FG into the eye, which could not be performed in humans. Using direct infusion, we observed the presence of an active transport system and some of its characteristics in the BOB. We speculated that the active transport system plays an important role in maintaining vitreous homeostasis not only in the monkey eye but also in the human eye. Our findings may provide important clues for understanding the function of the BRB in the human eye.

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