Role of P-Glycoprotein in Ocular Clearance of Rhodamine 123 in Rabbits

Tetsu Kajikawa,[1] Hiromu K. Mishima,[1,3] Teruo Murakami,[2] and Mikihisa Takano[2]

Received December 25, 1999; accepted January 8, 2000

KEY WORDS: P-glycoprotein; ocular clearance; blood-aqueous barrier; rhodamine 123; quinidine; cyclosporin A.

INTRODUCTION

Aqueous humor, secreted into the posterior chamber by the ciliary processes, flows through the pupil into the anterior chamber and leaves the eye mostly through the canalicular system. The rest of aqueous humor is eliminated through the uveoscleral system. Aqueous humor has a role of providing nutrients to the cornea and lens, and of carrying away their metabolic wastes to blood circulation.

Two clearance systems, aqueous humor outflow-mediated clearance and systemic clearance by the highly vascular tissues of the anterior uvea, have been reported in ocular clearance of drugs administered to the eye, although the contribution of each clearance pathway is different for various drugs (1). Recently, several research groups have detected P-glycoprotein (P-gp), an ATP-dependent efflux pump, in blood-aqueous barrier such as capillary endothelial cells of iris and epithelia of ciliary nonpigmented cells, as well as in other normal tissues including the brain, intestine and liver (2–5). This protein transports a variety of structurally and pharmacologically unrelated, hydrophobic compounds out of cells to prevent accumulation in these tissues (6).

Recently, we demonstrated that P-gp in blood-aqueous barrier markedly suppressed the aqueous distribution of rhodamine 123 (Rho-123), a P-gp substrate (7), given intravenously in rabbits (8). In the present study, we further analyzed the role of P-gp as an active efflux system from aqueous humor to blood circulation using Rho-123.

MATERIAL AND METHODS

Chemicals

The following reagents were used: Rho-123 (Kanto Chemical Co., Tokyo, Japan), fluorescein isothiocyanate-dextran 4000 (FD-4, Sigma Chemical Co., St. Louis, Mo), quinidine sulfate dihydrate (Wako Pure Chemicals, Osaka, Japan), and BSS plus® (a solution for eye irrigation, Alcon Japan, Tokyo, Japan). Cyclosporin A (CsA) was kindly supplied by Sandoz Pharmaceuticals (Basel, Switzerland). All other chemicals used were of the highest purity available.

Ocular Clearance of Rho-123 In Vivo

New Zealand male albino rabbits weighing 3.0 to 3.5 kg were used. The investigations utilizing rabbits described in this report conformed to the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985). Rho-123 (2.0 nmol/ml) and FD-4 (2.0 μmol/ml) were dissolved in BSS plus® containing 0.1% dimethyl sulfoxide. The pH and osmolarity of the solution were 7.6 and 278 mOsm, respectively. The mixture (30 μl) was slowly injected into the anterior chamber through a selfsealing tract via the cornea according to published method (9). Aliquot (approximately 100 μl) of aqueous humor was sampled by a 1-ml syringe with a 27-gauge needle once from a limbal paracentesis site on each eye at a designated time after injection. These experiments were repeated to obtain time courses for Rho-123 and FD-4 remaining in the aqueous humor in each rabbit.

To estimate the contribution of P-gp-mediated ocular clearance, 50 μl of quinidine solution dissolved in 50% ethanol (12.5 mM) was applied topically 5 times at 5 min intervals in the same manner as reported previously (8). The corneal surface was washed well with physiological saline 5 min after the last drop of quinidine. Then, the Rho-123 and FD-4 mixture (30 μl) was injected intracamerally. Aqueous humor was sampled at a designated time.

Effect of topical CsA on Rho-123 ocular clearance was also examined. CsA was dissolved in 50% ethanol at a concentration of 1.25 or 12.5 mM. The solution was applied topically in the same manner as the quinidine. For comparison, a solution of quinidine dissolved at a concentration of 1.25 mM was also examined. Aqueous humor was sampled 30 min after intracameral injection of the mixture.

Analysis

Aqueous concentrations of Rho-123, FD-4 and quinidine were determined by HPLC using TSKgel ODS-80 TM column (Tosoh, Tokyo, Japan). Mobile phases used were 40% acetonitrile in 1% acetic acid for Rho-123 and FD-4, and 30% acetonitrile in 1% acetic acid for quinidine. Detection was performed with a fluorometric detector at the following wavelengths: excitation 485 nm and emission 550 nm for Rho-123 and FD-4; excitation 310 nm and emission 380 nm for quinidine.

RESULTS

Elimination of Rho-123 and FD-4 from Aqueous Humor In Vivo

A mixture of Rho-123 and FD-4 was injected intracamerally, and the profiles of elimination from aqueous humor in the absence or presence of quinidine in aqueous humor were tracked (Fig. 1). The aqueous concentrations of quinidine applied topically were in a range from 185.0 ± 17.7 μM to 73.4 ± 30.8 μM (means ± S.D., 4–5 trials) during the 1 h-clearance study of Rho-123 and FD-4. Rho-123 disappeared from aqueous humor faster than FD-4 in the absence of quinidine in aqueous humor.

[1] Department of Ophthalmology, Faculty of Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
[2] Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
[3] To whom correspondence should be addressed. (e-mail: hkmishi@ipc.hiroshima-u.ac.jp)

0724-8741/00/0400-0479$18.00/0

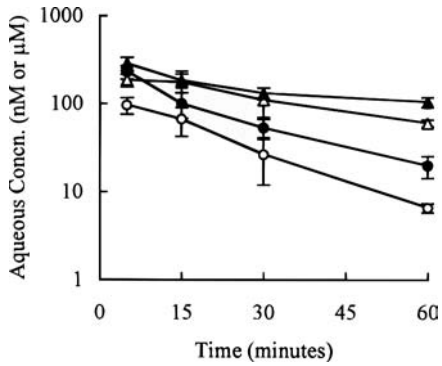

Fig. 1. Elimination of Rho-123 (circle) and FD-4 (triangle) from aqueous humor after intracameral injection in untreated (open symbol) and quinidine-treated (closed symbol) rabbits. The units for Rho-123 and FD-4 in Y-axis are nM and μM, respectively. Quinidine was applied topically as eye drops (12.5 mM solution) at a volume of 50 μl each on the corneal surface 5 times at 5 min intervals. Intracameral doses of Rho-123 and FD-4 were 60 pmol and 60 nmol, respectively. Bars represent the standard deviation of 4 to 5 trials.

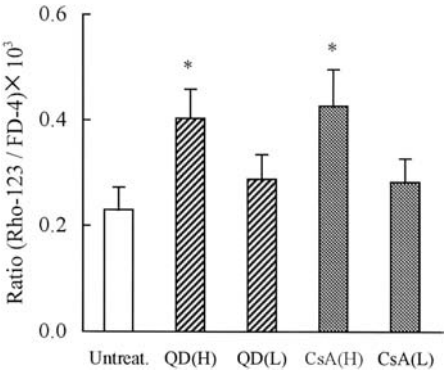

Fig. 2. Effect of topical quinidine (QD) or CsA on the concentration ratio of Rho-123 to FD-4 30 min after intracameral injection in rabbits. Quinidine and CsA were applied topically as eye drops (12.5 mM (high) or 1.25 mM (low)) at a volume of 50 μl each on the corneal surface 5 times at 5 min intervals. Intracameral doses of Rho-123 and FD-4 were 60 pmol and 60 nmol, respectively. Bars represent the standard deviation of 4 to 5 trials. * Significantly different from that of untreated at a level of $p < 0.05$.

Rho-123 elimination was significantly retarded by topical quinidine, though the change in FD-4 elimination was minimal. The concentration ratio of Rho-123 to FD-4 in aqueous humor 30 min after intracameral injection of the mixture was 0.229 ± 0.044 ($\times 10^{-3}$) in the absence of quinidine. This ratio significantly increased to 0.403 ± 0.056 ($\times 10^{-3}$) in the presence of quinidine in aqueous humor ($P < 0.05$).

Pharmacokinetic Analysis of Rho-123 Ocular Clearance

The elimination of Rho-123 from aqueous humor was pharmacokinetically analyzed as a function of aqueous humor outflow-mediated (canalicular and uveoscleral systems), P-gp-mediated, and other remainder clearance. Clearances of Rho-123 and FD-4 in the absence or presence of quinidine were estimated by dividing the dose of each compound by the area under the aqueous concentration-time curve from time zero to infinite (AUC) for that compound.

The P-gp-mediated clearance of Rho-123 was estimated as the decreased ocular clearance of Rho-123 by the presence of quinidine in aqueous humor. The aqueous humor outflow-mediated clearance of Rho-123 was assumed to be equal to FD-4 ocular clearance. Results are summarized in Table I. The P-gp-mediated ocular clearance was approximately 50% of total ocular clearance of Rho-123. The aqueous humor outflow-mediated clearance accounted for approximately one fourth of total ocular clearance of Rho-123.

Inhibitory Effect of CsA on Rho-123 Ocular Clearance

Effect of CsA, another P-gp inhibitor (10), on Rho-123 ocular clearance was also examined. In Fig. 2, the effect of topical CsA on Rho-123 ocular clearance is expressed as the concentration ratio of Rho-123 to FD-4 in aqueous humor, together with the results of quinidine. CsA applied topically at a concentration of 12.5 mM also significantly increased the ratio of Rho-123 to FD-4 in aqueous humor, as did quinidine. In contrast, both CsA and quinidine applied at a low concentration (1.25 mM) did not affect the ratio.

DISCUSSION

The pharmacokinetic analysis of ocular clearances and/or elimination rates of drugs from aqueous humor after administration to the rabbit eye have been reported (11,12). However, up to now, no report has analyzed the participation of P-gp-mediated efflux in the ocular clearance of pharmaceuticals. In the analysis of ocular clearance of Rho-123 in the present study, FD-4 was employed as a marker for estimation of aqueous humor outflow-mediated clearance (aqueous humor clearance). FD-4 ocular clearance was 7.1 ± 1.2 μl/min in the absence of quinidine and 5.0 ± 1.1 μl/min in the presence of quinidine in aqueous humor (Table I). This estimated value is in good agreement with the value of aqueous humor clearance reported by Himmelstein et al. (13), in which they estimated the value (4.7 μl/min) by pharmacokinetically analyzing the pilocarpine ocular clearance in rabbits. On the other hand, some lower values, such as 2–3 μl/min, are also reported (14,15). The reason for

Table I. Pharmacokinetic Analysis of Ocular Clearance of Rho-123 after Intracameral Injection in Rabbits

Ocular clearance (μl/min)	Untreated	Quinidine-treated
Total[a]	27.81 ± 7.32	12.65 ± 3.65[d]
P-Glycoprotein-mediated[b]	(15.16 ± 3.65)	—
Aqueous humor outflow-mediated[c]	7.05 ± 1.20	5.00 ± 1.07
Remainder	(5.60)	(7.65 ± 1.07)

Note: Intracameral doses of Rho-123 and FD-4 were 60 pmol and 60 nmol, respectively. Quinidine was applied topically as eye drops (12.5 mM solution). Each value represents the mean ± S.D. of 4 to 5 trials, and the value in parenthesis was obtained by calculation.

[a] Estimated by dividing the dose by AUC for Rho-123 in aqueous humor.

[b] Difference in total ocular clearance of Rho-123 between the untreated and quinidine-treated eyes.

[c] Estimated by dividing the dose by AUC for FD-4 in aqueous humor.

[d] Significantly different from that of untreated at a level of $p < 0.05$.

the higher value estimated in the present study compared to these values is not apparent, but the intracameral injection of extra solution (30μl) may affect the rate of aqueous flow to some extent. When a mixture of Rho-123 and FD-4 was injected intracamerally, the estimated initial concentration of FD-4 at time zero in aqueous humor according to a one-compartment model was 206 μM on average, indicating that the volume of aqueous humor was approximately 262 μl. This estimated value is also consistent with the previously reported value (200–300 μl) in rabbits (13). There was no damage to ocular tissues by topical quinidine, which was examined by determining the aqueous concentration of protein and LDH activity in treated eyes, as reported previously (8).

The contribution of P-gp-mediated clearance in Rho-123 ocular clearance was estimated by comparing the ocular clearance of Rho-123 in the absence and presence of quinidine in aqueous humor. Quinidine was applied by topical application as eye drops to keep the quinidine concentration in aqueous humor at a concentration high enough to suppress the P-gp function during the study [approximately 50 μM (8)]. In this application mode, the accumulation in the cornea and, therefore, the sustained aqueous distribution of an inhibitor can be expected. In fact, the aqueous concentration of quinidine was maintained at more than 70 μM during the study. The contribution of P-gp-mediated clearance in total ocular clearance of Rho-123 was estimated to be approximately 50% (Table I). The clearance of remaining (remainder) in Rho-123 ocular clearance in Table I, which was almost the same as that of aqueous humor outflow-mediated clearance, may reflect elimination via a paracellular route and/or a transcellular route in the highly vascular tissues of the anterior uvea.

P-gp-related compounds, including immunosuppressing agents, steroid hormones, antibiotics and β-blockers, are frequently used in ophthalmic medical treatments (6). CsA, an immunosuppressing agent, applied at a higher dose (12.5 mM) also suppressed Rho-123 ocular clearance significantly, as did quinidine.

In conclusion, the in vivo P-gp function was clearly shown to be an active efflux system for Rho-123 from aqueous humor to blood circulation. The contribution of P-gp-mediated ocular clearance to total ocular clearance of Rho-123 was estimated at approximately 50%. These results suggest that ophthalmic medical treatments with P-gp-related pharmaceuticals could be greatly improved by modulating the P-gp function in the blood-aqueous barrier.

REFERENCES

1. R. D. Schoenwald. Ocular drug delivery. Pharmacokinetic considerations. *Clin. Pharmacokinet.* **18**:255–269 (1990).
2. J. A. Holash and P. A. Stewart. The relationship of astrocyte-like cells to the vessels that contribute to the blood-ocular barriers. *Brain Res.* **629**:218–224 (1993).
3. J. Wu, J. J. Zhang, H. Koppel, and T. J. Jacob. P-glycoprotein regulates a volume-activated chloride current in bovine non-pigmented ciliary epithelial cells. *J. Physiol. Lond.* **15** :743–755 (1996).
4. R. O. Schlingemann, P. Hofman, J. Klooster, H. G. Blaauwgeers, R. Van-der-Gaag, and G. F. Vrensen. Ciliary muscle capillaries have blood-tissue barrier characteristics. *Exp. Eye Res.* **66**:747–754 (1998).
5. F. Thiebaut, T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan, and M. C. Willingham. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. USA.* **84**:7735–7738 (1987).
6. E. G. Scuetz, W. T. Beck, and J. D. Schuetz. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol. Pharmacol.* **49**:311–318 (1996).
7. J. S. Lee, K. Paull, M. Alvaroz, C. Hose, A. Monks, M. Grever, A. T. Fojo, and S. E. Bates. Rhodamine efflux patterns predict P-glycoprotein substrates in the National Cancer Institute drug screen. *Mol. Pharmacol.* **46**:627–638 (1994).
8. T. Kajikawa, H. K. Mishima, T. Murakami, and M. Takano. Role of P-glycoprotein in distribution of rhodamine 123 into aqueous humor in rabbits. *Curr. Eye Res.* **18**:240–246 (1999).
9. M. P. Langford, M. E. Berg, J. H. Mack, J. P. Ganley, and T. C. Welbourne. Inhibition of glutamate uptake causes an acute increase in aqueous humor protein. *Exp. Eye Res.* **64**:157–165 (1997).
10. Q. Wang, H. Yang, D. W. Miller, and W. F. Elmquist. Effect of the p-glycoprotein inhibitor, cyclosporin A, on the distribution of rhodamine-123 to the brain: an *in vivo* microdialysis study in freely moving rats. *Biochem. Biophys. Res. Commun.* **211**:719–726 (1995).
11. C. H. Chiang and R. D. Schoenwald. Ocular pharmacokinetic models of clonidine-3H hydrochloride. *J. Phamacokinet. Biopharm.* **14**:175–211 (1986).
12. R. D. Schoenwald and D. S. Chien. Ocular absorption and disposition of phenylephrine and phenylephrine oxazolidine. *Biopharm. Drug Dispos.* **9**:527–538 (1988).
13. K. J. Himmelstein, I. Guvenir, and T. F. Patton. Preliminary pharmacokinetic model of pilocarpine uptake and distribution in the eye. *J. Pharm. Sci.* **67**:603–606 (1978).
14. R. F. Jones and D. M. Maurice. New methods of measuring the rate of aqueous flow in man with fluorescein. *Exp. Eye Res.* **5**:208–220 (1966).
15. J. M. Conrad and J. R. Robinson. Aqueous chamber drug distribution volume measurement in rabbits. *J. Pharm. Sci.* **66**: 219–224 (1977).