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In-situ Ocular Absorption of Ophthalmic β-Blockers through Ocular Membranes in Albino Rabbits

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Abstract

Ocular membranes have been characterized by in-situ absorption of the ophthalmic β-blockers carteolol (hydrophilic) and timolol and befunolol (lipophilic) using a cylindrical cell.

After introduction of drug solution into the cell on the cornea, sclera (bulbar conjunctival and scleral layer) or palpebral conjunctiva, the disappearance of the drug from the cell was determined as in-situ absorption. The ophthalmic drugs disappeared from the conjunctival and scleral membranes although disappearance from the cornea was hardly observed. The conjunctival membrane showed the highest permeability.

Lipophilic drugs were more permeable than hydrophilic. In-situ apparent permeability coefficients of the ophthalmic drugs through the conjunctiva and sclera correlated with the lipophilicity of drugs. A high drug concentration in the aqueous humor was observed after corneal application. There is a relationship between concentrations of drugs in the aqueous humor and previously reported in-vitro apparent permeability coefficients of the drugs in the cornea.

This in-situ method using a cylindrical cell is a useful method of investigating the ocular absorption of ophthalmic drugs.

Glaucoma, one of the most serious ocular diseases, is characterized by high intraocular pressure, hardening of the eyeball and partial or complete loss of vision. β-Blockers such as hydrophilic carteolol, and lipophilic timolol and befunolol are commonly used as instillation droplets (Novack 1987). They are equipotent at β_1- and β_2-adrenergic receptors, which reduce aqueous humor formation by ciliary processes. Carteolol has some agonist activity, e.g. intrinsic sympathomimetic activity. The usefulness of several β-blockers for glaucoma therapy has been limited by topical and systemic side effects (Van Buskirk 1980; Linkewich & Herling 1981; Görlich 1987). The therapeutic effects are often related to the absorption behaviour of the instilled drugs through ocular membranes such as the cornea, sclera and conjunctiva.

The in-vitro permeability of β-blockers through the ocular membranes has been reported, although the blood circulation and the nervous system were not considered in the in-vitro experiment (Sasaki et al 1995). In-vivo absorption is complicated by the influence of the tear fluid and of drainage. In a previous study we have demonstrated the in-situ absorption behaviour of tilisolol by use of a cylindrical cell (Sasaki et al 1996). The purpose of this study is to investigate the in-situ absorption behaviour of ophthalmic β-blockers of different lipophilicity by measurement of: the depletion in concentration from within a cylindrical cell attached (in-situ) with adhesive to the cornea, palpebral conjunctiva or sclera (bulbar conjunctival and scleral layer) of anaesthetized rabbits; and the concentration of drug in the aqueous humor and vitreous body of previously killed rabbits corresponding to these different locations of the sources of drug.

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Materials and Methods

Materials

Befunolol and carteolol were kindly supplied by the Kaken Pharmaceutical (Tokyo, Japan) and Otsuka Pharmaceuticals (Tokyo, Japan), respectively. Timolol was purchased from Nacalai Tesque (Kyoto, Japan). Pindolol hydrochloride was purchased from Sigma (St Louis, MO, USA). Methyl-*p*-hydroxybenzoate was purchased from Kishida Chemicals (Tokyo, Japan). Salicyl methionine was prepared by standard methods in our laboratory (Nakamura et al 1992). All other chemicals were of reagent grade obtained from Nacalai Tesque. Phosphate-buffered saline (pH 7·4) was prepared by mixing an isotonic phosphate buffer with an equal volume of saline.

Drug disappearance in in-situ experiment

Male Nippon albino rabbits, 2·0–3·0 kg, were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast, Tokyo, Japan). The rabbits were starved for 24 h before use but had free access to water. All experiments in the study conformed to the Guidelines for Animal Experimentation in Nagasaki University.

A plastic cylindrical cell (8 mm o.d., 5 mm i.d., 18 mm length) was designed to fit over the cornea, sclera, and palpebral conjunctiva of the rabbit eye as reported previously (Sasaki et al 1996). The device was secured to the cornea, sclera, or palpebral conjunctiva with a thin film of surgical adhesive (Aron Alpha; Sankyo, Tokyo, Japan) after the rabbit, previously anaesthetized with an adequate dose of a sodium pentobarbitone solution administered via a marginal ear vein, had been placed on its left or right side. Drug solutions (5 mM, 100 μL) in pH 7·4 phosphate-buffered saline were added to the

cell. Samples (0·5 μL) were collected at 10-min intervals for 50 min after slight stirring. Samples were submitted for HPLC assay.

During the development of this procedure, the integrity of the glue-seal and the conjunctival membrane were tested with dye solutions (Sasaki et al 1996). The volumes of drug solutions (measured with a pipette) at the end of experiments were not different from the initial volumes (97–103%).

Drug concentration in the aqueous humor and vitreous body in in-situ experiment

Aqueous humor and vitreous body were collected after the rabbits had been killed by intravenous administration of an overdose of a sodium pentobarbitone solution 30 min after introduction of the drug (100 mM, 100 μL, pH 7·4) into the cell on the cornea. Aqueous humor samples were withdrawn by paracentesis with a 27-gauge, 1·3-cm needle attached to a 1·0-mL disposable syringe inserted through the corneal-scleral junction and slightly upward into the anterior chamber. Vitreous body samples were withdrawn with a 1·0-mL disposable syringe without needle after the middle of the eyeball had been cut with a surgical knife. The samples were also submitted for HPLC assay.

Drug assay

The samples of β-blockers for in-situ experiments (0·5 μL) were mixed with 0·1 M HCl (100 μL) and methanol (100 μL) including internal standard (20 μg mL^{-1} pindolol for carteolol analysis, 500 μg mL^{-1} methyl-*p*-hydroxybenzoate for timolol analysis and 50 μg mL^{-1} salicyl methionine for befunolol analysis). The aqueous humor and vitreous body samples (200 μL) were also mixed with 1 M HCl (20 μL) and methanol (300 μL) containing internal standard. The mixture was centrifuged at 12 000 *g* for 15 min and 50 μL of supernatant was injected into the HPLC system.

The HPLC system (LC-6A, Shimadzu, Kyoto, Japan) was used in the reversed-phase mode for assay of the ophthalmic drugs. Analysis was performed on a Cosmosil 5C18-P packed column (150 mm length × 4·6 mm i.d., Nacalai Tesque). Mixtures of methanol and 50 mM NaH_2PO_4 (25 : 75 for analysis of carteolol; 40 : 60 for analysis of timolol; 45 : 55 for analysis of befunolol, all v/v) were used as the mobile phase with a flow rate of 1·0 mL min^{-1}. Retention of drug was monitored with a UV spectrophotometric detector (SPD-10A, Shimadzu; 250 nm for carteolol; 290 nm for timolol) or, for befunolol, a fluorescence HPLC monitor (RF-535, Shimadzu; excitation and emission wavelengths 300 and 500 nm, respectively).

The apparent absorption rate (K_{obs}, min^{-1}) was estimated from the slope of the profile of the amount of drug remaining in the cell. Apparent clearance (mL min^{-1}, $CL_{app} = K_{obs} \times$ volume) and apparent permeability coefficient (cm s^{-1}, in-situ $P_{app} = K_{obs} \times$ volume/effective area/60) through ocular membranes were calculated from K_{obs}. The effective area and volume were 0·196 cm^2 and 0·1 mL, respectively.

Results

Disappearance of drugs from the cell

Fig. 1 shows the disappearance of carteolol from the cell through the ocular membranes. Carteolol disappeared through the conjunctival and scleral membranes but little disappearance of carteolol was observed through the corneal surface. The conjunctival membrane showed the highest permeability.

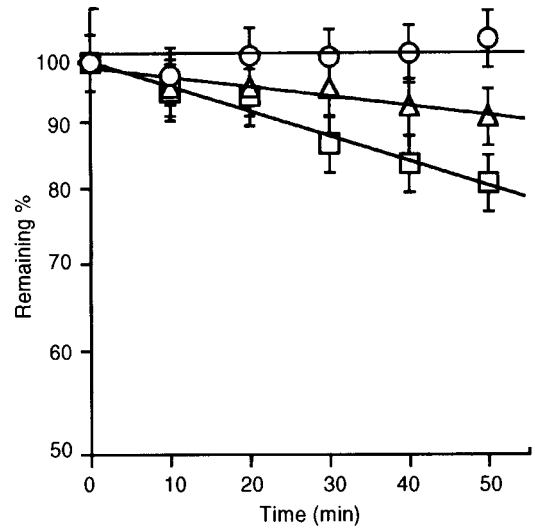

FIG. 1. Disappearance of carteolol after introduction into the cell on ocular membranes: cornea (○), conjunctiva (△), sclera (□). Values are means ± s.e. of at least three experiments.

Figs 2 and 3 show the disappearance of timolol and befunolol, respectively, from the cell through the ocular membranes. Again the drugs disappeared through the conjunctival and scleral membranes, but not through the corneal membrane. The disappearances of timolol and befunolol through the conjunctiva and the sclera were larger than that of carteolol.

Absorption parameters estimated from the disappearance profiles are shown in Table 1. The in-situ apparent permeability coefficients of β-blockers through the conjunctiva were higher than those through the sclera. The in-situ apparent permeability coefficients of drugs through the conjunctiva

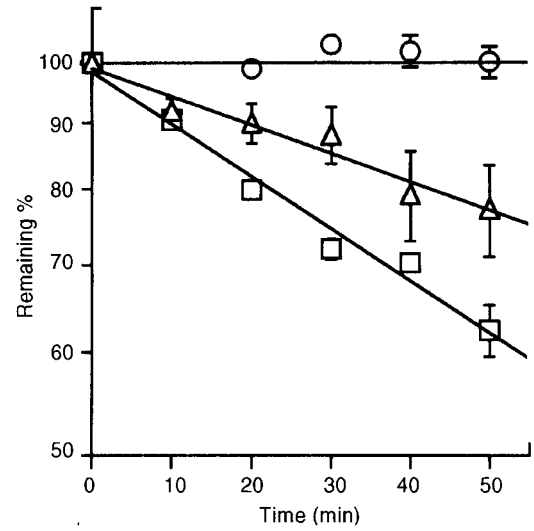

FIG. 2. Disappearance of timolol after introduction into the cell on ocular membranes: cornea (○), conjunctiva (△), sclera (□). Values are means ± s.e. of at least three experiments.

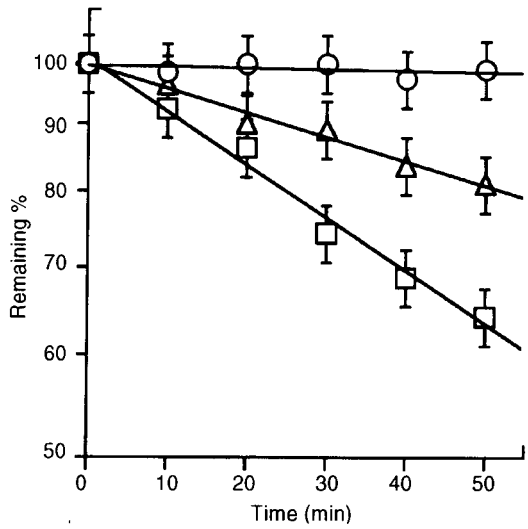

FIG. 3. Disappearance of befunolol after introduction into the cell on ocular membranes: cornea (○), conjunctiva (△), sclera (□). Values are means ± s.e. of at least three experiments.

Table 1. In-situ absorption parameters estimated from disappearance profiles of β-blockers.

Drug	Route	Apparent clearance ($\times 10^{-4}$ mL min^{-1})	In-situ apparent permeability coefficient ($\times 10^{-5}$ cm s^{-1})
Carteolol	Conjunctiva	4·38 ± 1·27	3·72 ± 1·08
	Sclera	1·69 ± 0·20	1·44 ± 0·17
	Cornea	N.D	N.D.
Timolol	Conjunctiva	9·21 ± 0·05	7·82 ± 0·04
	Sclera	5·07 ± 1·61	4·82 ± 1·37
	Cornea	N.D.	N.D.
Befunolol	Conjunctiva	9·37 ± 1·01	7·96 ± 0·86
	Sclera	4·30 ± 0·73	3·65 ± 0·62
	Cornea	N.D.	N.D.

Values are means ± s.e. of at least three experiments. N.D. = not detected.

were 0·9–2·2 times the in-vitro apparent permeability coefficients reported previously (Sasaki et al 1995). In the sclera the in-situ apparent permeability coefficients of the drugs were much higher (2·9–8·1 times) than the in-vitro values.

Fig. 4 shows the relationship between the in-situ apparent permeability coefficients of the drugs and the drug lipophilicities; the table also shows the data for tilisolol (Sasaki et al 1995). A significant relationship was not found for the conjunctiva and the sclera.

Appearance of drugs in the aqueous humor and the vitreous body

The concentrations of the β-blockers in the aqueous humor and vitreous body 30 min after their introduction into the cell on the cornea are listed in Table 2. Corneal application resulted in a high concentration of the drug in the aqueous humor. Drug concentrations in the vitreous body were much lower than those in the aqueous humor. The concentrations of timolol and

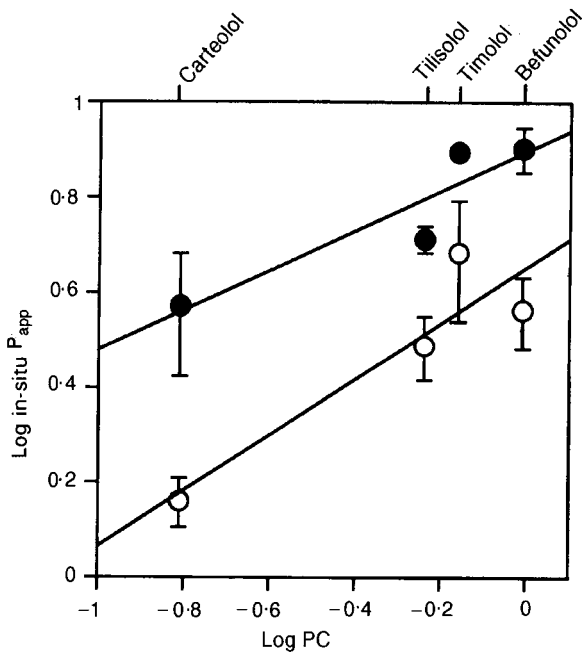

FIG. 4. Relationship between in-situ apparent permeability coefficient (in-situ P_{app}) in conjunctiva and sclera and partition coefficient (PC) of β-blockers: conjunctiva (○); log in-situ P_{app} in conjunctiva = 0·417 × log PC + 0·652, $\gamma = 0{\cdot}919$ (not significant), sclera (●); log in-situ P_{app} in sclera = 0·589 × log PC + 0·896, $\gamma = 0{\cdot}921$ (not significant).

befunolol in the aqueous and vitreous humor were higher than those of carteolol.

Fig. 5 shows a relationship between the aqueous humor concentration and the in-vitro apparent permeability coefficient reported previously, in addition to data for tilisolol (Sasaki et al 1995). A linear relationship was observed in the plot.

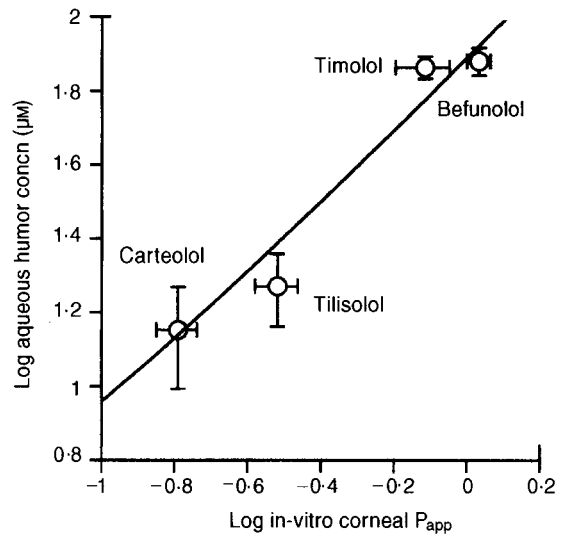

FIG. 5. Relationship between aqueous humor concentration and in-vitro apparent permeability coefficient (in-vitro P_{app}) of β-blockers in cornea. Log aqueous humor concentration = 0·992 × log in-vitro P_{app} in cornea + 1·894, $\gamma = 0{\cdot}974$ (significant correlation $P < 0{\cdot}05$).

Table 2. Concentration (μM) of carteolol, timolol and befunolol in the aqueous humor and vitreous body 30 min after introduction into the cell on the rabbit cornea.

Drug	Aqueous humor	Vitreous body
Carteolol	14·3 ± 4·4	0·07 ± 0·01
Timolol	73·5 ± 5·1	0·18 ± 0·10
Befunolol	76·2 ± 6·4	0·17 ± 0·13

Values are means ± s.e. of at least four experiments.

Discussion

Instilled drug is eliminated from the precorneal area by drainage by the naso-lacrimal duct and dilution by tear turnover (Chrai et al 1973; Himmelstein et al 1978; Shell 1982; Lee & Robinson 1986). Drug in the precorneal area is also eliminated through the cornea, conjunctiva and sclera (Lee & Robinson 1979). The rate and extent of intraocular absorption and therapeutic effectiveness of topically applied drugs are dependent on the transport characteristics of the cornea, sclera and conjunctiva (Ahmed & Patton 1987). Conjunctival and scleral penetration may constitute an important route for some drugs which are poorly absorbed through the cornea and for some drug-delivery systems such as conjunctival inserts (Lee & Robinson 1986; Schoenwald 1990; Sasaki et al 1993). Little information on barrier properties is, however, available for the conjunctiva and sclera compared with that for the cornea.

The corneal composite structure is characterized by three primary layers: epithelium, stroma and endothelium. The stratified epithelial cells with tight junctions are considered to comprise a lipophilic barrier to corneal drug penetration (Pedler 1962; Huang et al 1983; Klyce & Crosson 1985; Grass & Robinson 1988). We have already reported the relationship between the in-vitro apparent permeability coefficients of β-blockers and their lipophilicities in the cornea (Sasaki et al 1995). Aqueous humor concentrations of hydrophilic carteolol are, in fact, lower than those of lipophilic timolol and befunolol after introduction of drug into the cell on the cornea (Table 2) and the aqueous humor concentrations of β-blockers correlated with the in-vitro apparent permeability coefficients after their introduction into the cell on the cornea (Fig. 5). The in-situ apparent permeability coefficients in the cornea could not, however, be calculated because of low disappearance of ophthalmic drugs from the cell on the cornea (Figs 1–3). In a previous report we demonstrated that the corneal route is the dominant route of access to the aqueous humor (40 times more important than the next most important route, the trans-scleral route; Sasaki et al 1996).

The conjunctiva consists of a thin mucous membrane and vascularized tissue lining the inside of the eyelids and the anterior sclera (Stjernschantz & Astin 1993). The conjunctival epithelium offers substantially less resistance than the corneal epithelium because of the preponderance of the paracellular route (Kahn et al 1990). Huang et al (1989) showed that the conjunctiva was much more permeable to hydrophilic macromolecules and to ^{3}H-mannitol than the cornea. The conjunctival membrane showed the highest permeability (Figs 1-3, Table 1). Wang et al (1991) demonstrated the dependency of in-vitro conjunctival permeability coefficients of various timolol prodrugs on drug lipophilicity over a wide lipophilicity range. The in-situ apparent permeability coefficients of β-blockers through the conjunctiva and sclera showed a slight dependence on drug lipophilicity but no significant correlation (Fig. 4). Because the conjunctiva is highly permeable and well vascularized, most of the drug is picked up by the blood and absorbed systemically. Huang et al (1989) demonstrated that conjunctival permeability can be affected by increasing the thickness of the underlying connective tissues.

Sclera is a tough and thick fibrous tissue composed primarily of collagen and mucopolysaccharides (Maurice & Polgar 1977). In-vitro experiments have shown that the thin conjunctival layer lining the anterior sclera has relatively insignificant barrier function against drugs (Sasaki et al 1995). The presence or absence of the conjunctiva apparently had little effect on the amount of pilocarpine entering the eye (Doane et al 1978). We have previously reported that drug was cleared by the blood circulation before it reached the inside of the eye after application to the scleral tissue. This clearance may cause a difference between the apparent permeability coefficients in in-vitro and in-situ experiments.

Thus, ocular membranes are sufficiently different in absorption behaviour to control the extent and pathway for ocular and systemic delivery of drugs. An in-situ experiment using a cylindrical cell is a useful method for investigation of the absorption behaviour of ophthalmic drugs.

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