J. Kor. Pharm. Sci., Vol. 24, No. 3, S59-S66 (1994)

Studies on Drug Absorption Characteristics for Development of Ocular Dosage Forms: Ocular and Systemic Absorption of Topically Applied β-Blockers in the Pigmented Rabbit

Yong-Hee Lee

Lucky Biotech Institute, P.O. Box 61, Moonji-Dong 104-1, Yuseong-Gu, Daejon 305-380, Korea

(Received September 5, 1994)

The objective of this study was to determine the influence of drug lipophilicity on the extent of ocular and systemic absorption following topical solution instillation in the pigmented rabbit. β-blockers of various lipophilicity were chosen as model drugs, 25 μl of a 15 mM drug solution in isotonic pH 7.4 buffer was instilled, and ocular tissue and plasma drug concentrations were monitored. Ocular absorption was apparently increased in all eye tissues, but non-corneal absorption ratio was decreased by increasing of drug lipophilicity. Systemic bioavailability was ranged from 61% for atenolol to 100% for timolol, and at least 50% of the systemically absorbed drug reached the blood stream from the nasal mucosa. Occluding the nasolacrimal duct for 5 min reduced the extent of systemic absorption of timolol and levobunolol, but did not do so for atenolol and betaxolol. Taken together, the ocular absorption of topically applied ophthalmic drugs would be modest for lipophilic drugs. By contrast, the systemic bioavailability would be modest for drugs at the extremes of lipophilicity, and the nasal contribution to systemically absorbed drug diminished with increasing of drug lipophilicity.

Keywords—Lipophilicity, Ocular absorption, β-Blockers, Bioavailability, Atenolol, Timolol, Levobunolol, Ophthalmic drugs

Introduction

β-Blockers have become the conerstone of glaucoma therapy since the introduction of mixed β_1 and β_2 blocker timolol into glaucoma therapy in 1978. However, the incidence of serious systemic side effect such as cardiovascular and pulmonary systems[1] has been increased[2-3] in topical therapy of β-blockers. Thus, to improve the efficacy of topically applied β-blockers, various approaches have been investigated for maximizing ocular drug absorption with minimizing systemic drug absorption through prolonging the residence time of the applied dose in the conjunctival sac by the formulation selecting a dosing schedule, ie, chronopharmacokinetics[7] coadministration with low doses of vasoconstricting agents, desigining ophthalmic drugs that are preferentially absorbed into the eye and poorly absorbed into the systemic circulation, ie, prodrugand designing ophthalmic drugs that are rapidly inactivated in the systemic circulation, i.e., soft drug studies.

To get the best efficacy in the ocular drug delivery of β-blockers, the basic pharmacokinetic properties of β-blockers in the ocular tissues and blood are necessary. Thus, the objective of this study was to determine the influence of drug lipophilicity on the extent of ocular and systemic absorption of topically applied β-blockers in the pigmented rabbit. Relatively hydrophilic atenolol (log PC 0.16), moderately lipophilic timolol (log PC 1.91) and levobunolol (log PC 2.4), and lipophilic betaxolol (log PC 3.44) were chosen as model drugs.

Materials and Methods

Materials

Male, Dutch-belted pigmented rabbits, weighing 2.0~2.5 kg, were used. All dosing solutions were 15 mM in drug prepared immediately before each experiment in a 10 mM Tris buffer and adjusted to pH 7.4 with an osmolarity of 300 mOsm /kg.

HPLC Assay for β-Blockers

Atenolol was assayed by HPLC under an isocratic condition on a reversed phase Ultrasphere ODS C_{18} column (4.6 ± 250 mm, particle size 5 μm). The mobile phase was a mixture of 6 parts of acetonitrile and 94 parts of 0.0125 M $NH_4H_2PO_4$ solution adjusted to pH 3.0 with H_3PO_4. The other β-blockers were assayed by HPLC under a gradient condition on a reversed phase Navapak ODS C_{18} column (3.9 ± 150 mm, particle size 4 μm). The mobile phase was a mixture of acetonitrile and 0.2% triethylamine HCl solution adjusted to pH 3.0 with HCl. The proportion of acetonitrile was kept at 17% for the first minute and increased to 35% for the next 14 min. the flow rate was 1.0 m*l*/min. Timolol and levobunolol were monitored spectrophotometrically, while atenolol and betaxolol were monitored fluorometrically.

Extraction of Drug From Eye Tissues and Blood

Excised eye tissues were soaked in 200 μ*l* of 0.6% $HClO_4$ at 4℃ for at least 12 hr. Eye tissues and plasma containing atenolol were mixed with a 50 μ*l* internal standard solution and 1 m*l* of 10 mM Tris buffer (pH 7.4 and 260 mOsm/kg), vortexed for 3 min, and centrifuged at 1500 g for 10 min. One m*l* of the supernatant was transfered to a preconditioned solid phase extraction column. After washing the extraction column with water, the drug and internal standard were eluted with methanol. For the other drugs, eye tissues and plasma were mixed with 100 μ*l* of an internal standard solution, 800 μ*l* of 10 mM Tris buffer (pH 7.4 and tonicity 260 mOsm/kg), 500 μ*l* of 1 M ammonium acetate buffer (pH 9.3), and 8 m*l* of diethyl ether in a 15 m*l* screw-capped conical centrifuge tube, vortexed for 3 min, and centrifuged at 1500 g for 10 min. The supernatant was transfered to a 10 m*l* screw-capped conical centrifuge tube containing 200 μ*l* of 0.2 N HCl, vortexed, and centrifuged at 1500 g for 10 min. The supernatant was discarded, while 100 μ*l* of the aqueous phase containing drug and internal standard was injected into the HPLC. The extraction efficacy was greater than 80% for atenolol, 75% for timolol, 80% for levobunolol, and 95% for betaxolol.

Ocular Absorption of Topically Applied β-Blockers

25 μ*l* of a dosing solution was administered into each eye, collecting in the cul-de-sac. At preconditioned times up to 240 min, each rabbit was killed with an overdose of a sodium pentobarbital solution administered via a marginal vein. The ocular surfaces were throughly rinsed with 1.17% KCl solution, and blotted dry. Aqueous humor was collected and anterior segment tissues were excised. The ocular absorption of atenolol was determined up to 240 min in the deepithelized rabbits. The corneal epithelium was removed by the scalpel (No. 11) after drop of local anesthetics proparacaine (25 μ*l* of 0.025%), under general anesthesia with a subcutaneous administration of ketamine (25 mg/kg) and acepromazine (2.5 mg/kg). After complete recovery of general anesthesia, 25 μ*l* of 15 mM atenolol solution was topically applied into the both eyes.

Systemic Absorption of Topically Applied β-Blockers

Fifteen minutes before solution instillation, each rabbit was cannulated in a central ear artery with a polyethylene tubing. Thereafter, 25 μ*l* of a dosing solution was administered into each eye, collecting in the cul-de-sac. At predetermined times up to 120 min (480 min for atenolol), a total of ten 2.5~3.5 m*l* blood samples were collected into heparinized tubes. To evaluate the effect of

nasolacrimal occlusion on systemic drug absorption, a punctum plug fashioned from a 10 mm segment of a polyethylene tubing (PE 20, 1.09 mm O.D.) that had been sealed at one end and be-

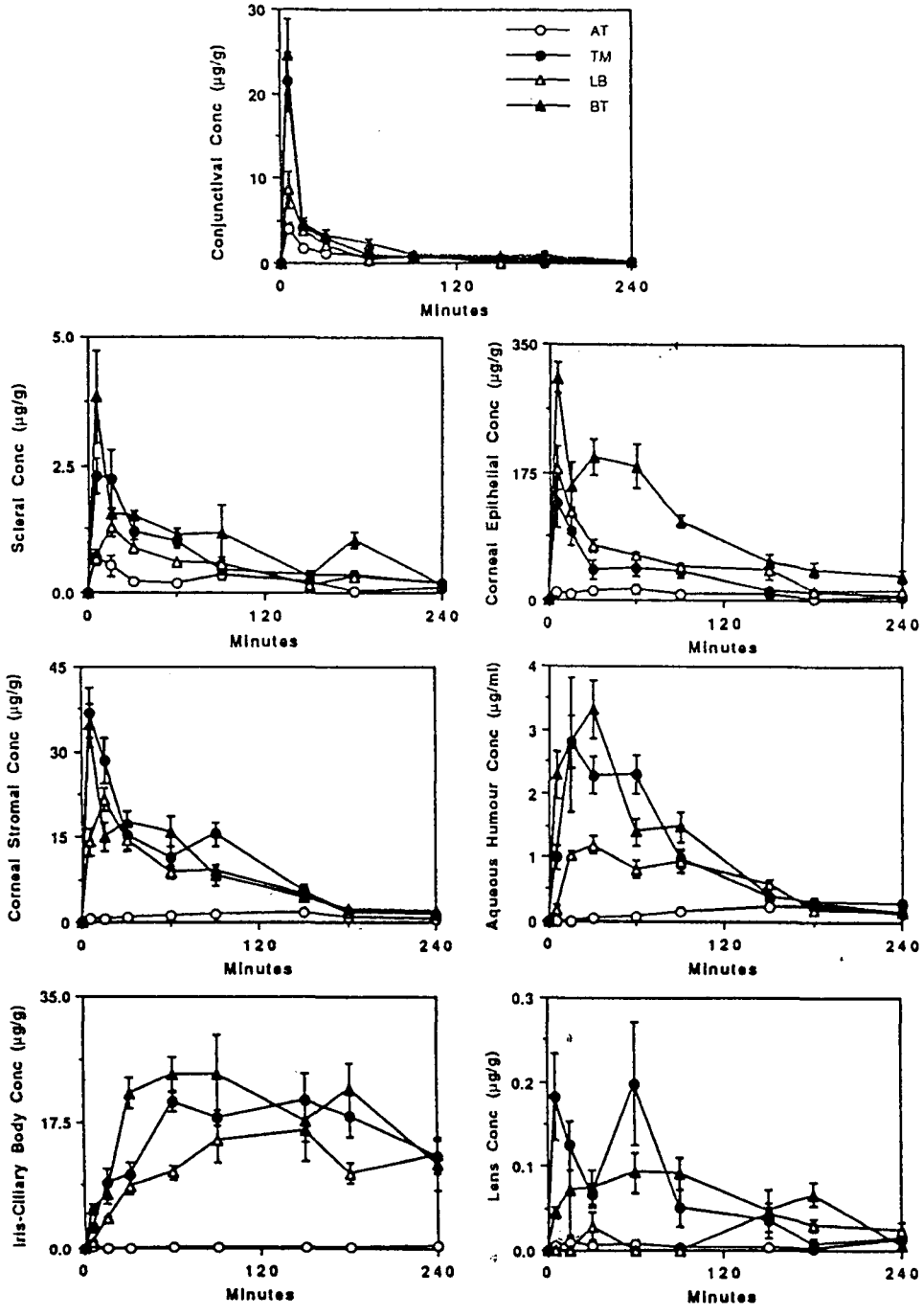

Figure 1—Time course of changes in eye tissue drug concentration following topical instillation of 25 μl of 15 mM atenolol (AT), timolol (TM), levobunolol (LB), and betaxolol (BT) solutions into the pigmented rabbit. Error bars represent s.e.m. for n=4−8.

velled at the other was inserted into the punctum prior to drop instillation and left in place for either 5 or 120 min (480 min for atenolol). The extent of systemic absorption following 120 or 480 min of nasolacrimal occlusion was also taken as the upper limit of conjunctival contribution to systemic absorption. To evaluate nasal contribution to systemic drug absorption following topical solution instillation, a dosing solution was instilled nasally via a polyethylene catheter (PE 50, 1 mm O.D.) preinserted 15~20 mm into the lacrimal sac/nasolacrimal duct. Because no separate oral studies were conducted, the effects of gastrointestinal absorption could not be differentiated and were considered as part of nasal absorption.

Results and Discussion

Ocular Absorption

The drug concentrations in various anterior segment tissues up to 240 min post-instillation of a 15 mM drug solution are shown in Fig. 1. The tissue concentration of b-blockers in the conjunctiva, sclera, corneal epithelium (except atenolol), and corneal stroma (except atenolol and levobunolol) did not show an absorption phase from 5 to 240 min period. The lack of absorption phase in these tissues indicated that the drug input into the tissues was almost complete in 5 min post-dosing, our first sampling time point. Thus, the conjunctiva was not an absorption barrier in the non-corneal absorption of hydrophilic and lipophilic drugs, while the corneal epithelium was not an absorption barrier in the corneal absorption of lipophilic drugs. However, the tissue concentration of β-blockers in the aqueous humor and iris-ciliary body showed an absorption phase. The ocular absorption of drugs were apparently increased by increasing of drug lipophilicity in all eye tissues. These results were agreed to the increase of in vitro conjunctival and corneal Papp by the increase of drug lipophilicity within the log

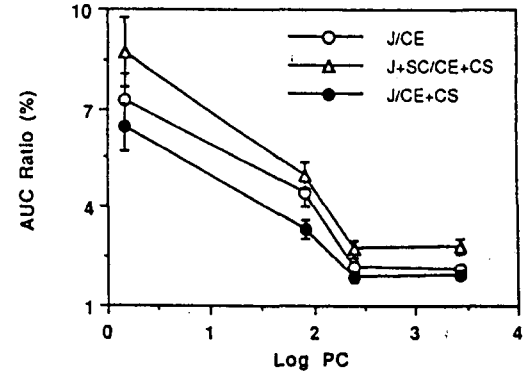

Figure 2—The plot of AUC ratio (%) versus log PC between the conjunctival and corneal tissues following topical instillation of 25 μl of 15 mM β-blocker solutions into the pigmented rabbit eye. Error bars represent s.e.m. for n=4 −8.

PC range of −0.62 (sotalol) and 3.44 (betaxolol)[9]

The non-corneal absorption of b-blockers, expressed by the tissue AUC ratio between the conjunctival and corneal tissues are shown in Fig. 2. The percent AUC ratios were 6.4~8.7% for atenolol, 3.3~5% for timolol, and 1.9~2.8% for levobunolol and betaxolol. Thus, the non-corneal absorption ratio of drugs was decreased by increasing of drug lipophilicity. Actually, the non-corneal absorption ratio of β-blockers into the iris-ciliary body was best for atenolol (65%) and least for levobunolol and betaxolol (5 and 6%) Chien *et al*[10]. reported that, of the three α_2-adrenergic agents evaluated--p-aminoclonidine, AGN 190342, and clonidine, the non-corneal pathway of ocular drug absorption was most prominent for the most hydrophilic p-aminoclonidine. However, the corneal pathway was the major pathway in the ocular absorption of β-blockers independent of drug lipophilicity. The AUC ratio between the conjunctival to corneal tissue was below about 10%. The high concentration of corneal tissues might come from the high tissue binding of drugs and the low corneal clearance of drugs into the aqueous humor, while the low concentration of conjunctival tissues might be due to the low tissue binding of drugs and

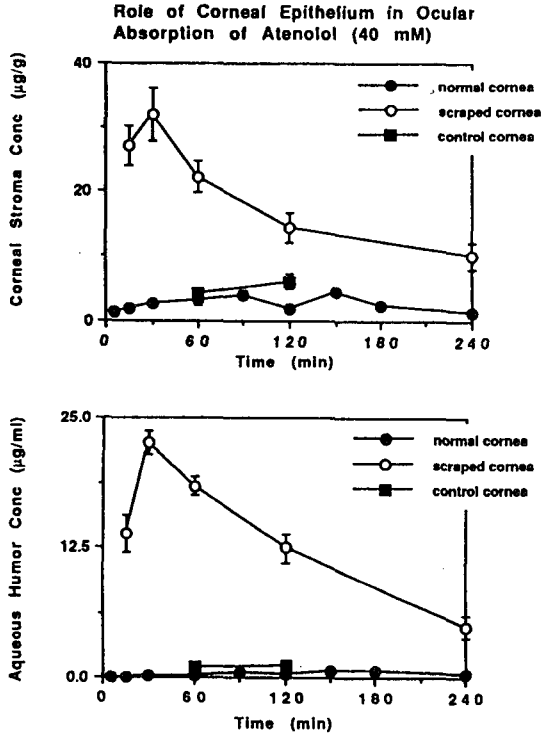

Figure 3—Time course of changes in the corneal stroma and aqueous humor drug concentrations following topical instillation of 25 μl of 40 mM atenolol solution into the deepithelized rabbit eye. Error bars represent s.e.m. for n=4−8.

rapid clearance of drugs from the conjunctiva to the systemic or non-corneal pathway by highly blood circulation.

The ocular tissue concentrations of atenolol in the deepithelized cornea are shown in Fig. 3. By the deepithelization, the peak concentration and AUC of atenolol were 7 and 6 times increased in the corneal stroma, and 31 and 32 times increased in the aqueous humor. Furthermore, the peak times were 3 and 6 times decreased in the corneal stroma and aqueous humor. Thus, the corneal epithelium was the rate limiting barrier in the corneal absorption of atenolol. Therefore, the pa rtition from the tear to corneal epithelium might be the main barrier in the corneal absorption of hydrophilic atenolol.

Systemic Absorption

The time courses of drug concentration in plasma or blood following nasal and ocular administration with 0, 5, and 120 min of nasolacrimal occlusion are shown in Fig. 4. Except for atenolol, peak drug concentration was reached within 10 min following ocular administration. This peak time was comparable in magnitude to that from nasal administration and was not markedly affected by 5 or 120 min nasolacrimal occlusion. For atenolol, the peak time from ocular administration was unusually long (195 min) when compared with nasal administration (34 min), but it was shortened considerably upon occluding the nasolacrimal duct for 5 or 480 min. The shorter peak time was attributed to a larger absorption rate constant associated with insertion of the nasolacrimal plug. The systemic drug bioavailability following ocular administration was found to be lowest for hydrophilic atenolol (41%) and highest for slightly lipophilic timolol (106%), with that of very lipophilic betaxolol (66%) and moderately lipophilic levobunolol (82%) being intermediate (the data in IV and subcutaneous administration are not shown). The areas under the plasma or blood concentration-time curve (AUC) following various modes of administration are shown in Fig. 5, and percent nasal and conjunctival contributions in systemic absorption are listed in Table I. Occluding the nasolacrimal duct for 5 min reduced the AUC for only timolol and levobunolol, but extending the duration of prolongation to 120 or 480 min reduced the AUC for atenolol and timolol but not for levobunolol and betaxolol. The nasal pathway contributed at least 50% towards the absorption of ocularly applied drugs into the systemic circulation, reaching 83% for atenolol and 61% for betaxolol. The implication was that it should be possible to reduce systemic drug absorption by minimizing contact of the applied dose with the nasal mucosa through prolonging its retention in the conjunctival sac. Nevertheless,

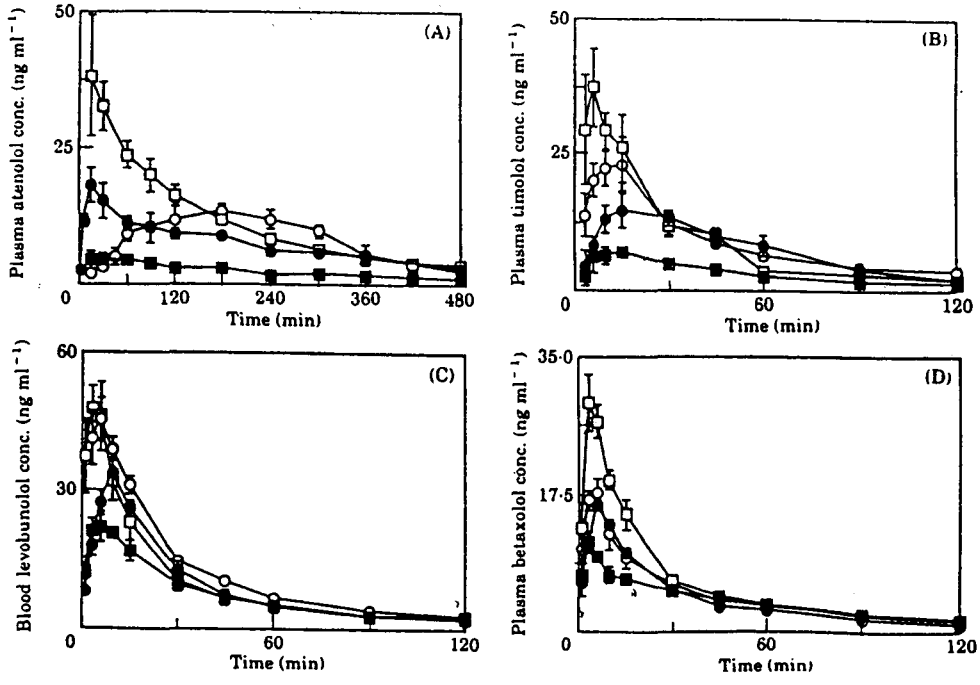

Figure 4—Time course of changes in plasma drug concentration following various modes of administration of 25 μl of 15 mM atenolol (A), timolol (B), levobunolol (C), and betaxolol (D) solutions in the pigmented rabbit eye. Error bars represent s.e.m. for n=4.

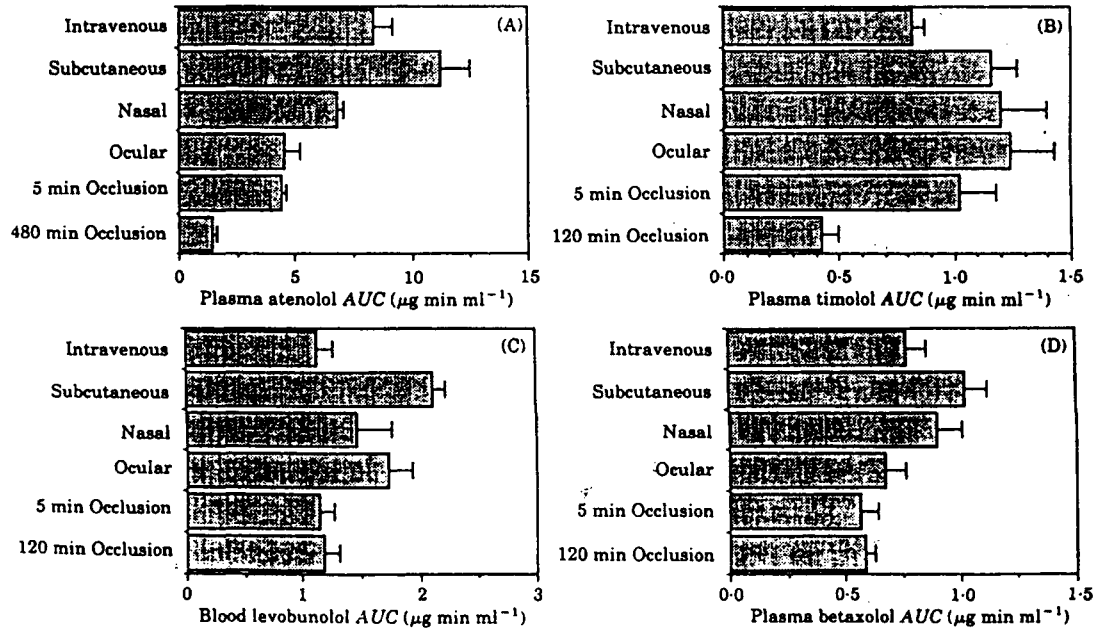

Figure 5—Area under the plasma concentration-time curve (AUC) following various modes of administration of 25 μl of 15 mM atenolol (A), timolol (TM), levobunolol (LB), and betaxolol (BT) solutions into the pigmented rabbit, Error bars represent s.e.m. for n=4.

Table I—*Prcent (%)* Nasal and Conjunctival Contribution in Systemic Absorption*

Drug	Nasal	Oculr	5 min occulusion	Conjunctival
Atenolo	83	56	54	17
Timolol	74	77	63	26
Levobunolol	55	66	43	45
Betzxolol	61/45	38	39	

*AUC of nasal+AUC of conjunctival (120 min occlusion) used as a reference

whether such an approach will bring about a net reduction in the extent of systemic drug absorption will depend on the systemic bioavailability characteristic of the drug. Both hydrophilic atenolol and lipophilic betaxolol, which are less well absorbed into the bloodstream than timolol and levobunolol, were unaffected in their systemic absorption by 5 min of nasolacrimal occlusion. Therefore, formulation changes that afford only modest increase in retention in the conjunctival sac would probably be ineffective in reducing the systemic absorption of extremely hydrophilic drugs such as atenolol, and lipophilic drugs such as betaxolol. By contrast, the systemic absorption of timolol and levobunolol was reduced by the formulations with varying precorneal retention characteristics[4]

Conclusion

The extent of ocular and systemic absorption of topically applied ophthalmic drugs could be possible to control by varying drug lipophilicity. The ocular absorption of drugs will be increased, while the non-corneal absorption ratio of drugs will be decreased by increasing of drug lipophilicity. The ocular absorption of hydrophilic drugs such as atenolol might be low and slow due to the absorption barrier in the corneal epithelium.

Drugs with lipophilicity similar to that of timolol will be well absorbed into the systemic circulation, whereas drugs that are extremely hydrophilic or extremely lipophilic will be absorbed to a much lesser extent. The nasal pathway generally contributes more to systemic absorption than does the conjunctival pathway, although it assumes lesser quantitative importance as drug lipophilicity is increased. The extent of prolongation in retention in the conjunctival sac required to reduce systemic absorption depends on both drug lipophilicity and its intrinsic extent of absorption.

References

1) T.S. Lesar Comparison of ophthalmic β-blocking agents, *Clin. Pharm.* **6**, 451-475(1987).
2) T.J. Zimmerman, J.D. Baumann and Hethe-J. rington (1983) Side effects of timolol, *Sur. Ophthalmol.* **28**, 243-249 (1983).
3) W.P., Munroe J.P. Rindone and R.M. Kershner Systemic side effects associated with the ophthalmic administration of timolol, *Drug Intell. Clin. Pharm.* **19**, 85-89(1985).
4) S.K., Podder K.C. Moy and Lee V.H.L. (1992) Improving the safety of topically applied timolol in the pigmented rabbit through manipulation of formulation composition, *Exp. Eye Res.* **54**, 747-759 (1992).
5) Y.H. Lee and V.H.L., Lee Formulation influence on ocular and systemic absorption of topically applied atenolol in the pigmented rabbit, *J. Ocul. Pharmacol.* **9**, 47-58(1993).
6) Y.H. Lee U.B. Kompella and V.H.L. Lee Systemic absorption pathways of topically applied-adrenergic antagonists in the pigmented rabbit, *Exp. Eye Res.* **57**, 341-349(1993).
7) S., Ohdo G.M. Grass and V.H.L. Lee Improving the ocular to systemic ratio of topical timolol by varying the dosing time, *Invest. Ophthalmol. Vis. Sci.* **32**, 2790-2798(1991).
8) Wang W., Sasaki H., Chien D.S. and Lee V. H.L., Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit : A comparison with corneal penetration. *Curr. Eye Res.* **10**, 571 (1991).
9) Y.H. Lee and V.H.L., Lee Non-corneal absorption of β-blockers in the pigmented rabbit,

in preparation(1994).
10) D.S., Chien J.J., Homsy C.. Gluchowski and D.D..-S., Tang-Liu, Corneal and conjunctival/scleral penetration of p-aminoclonidine, AGN 190342, and clonidine in rabbit eyes, *Curr. Eye Res.* **9**, 1051-1059(1990).