Controlled Ocular Timolol Delivery: Systemic Absorption and Intraocular Pressure Effects in Humans

Arto Urtti,* Harr Rouhiainen,[1] Timo Kaila,[3] Veyo Saano[2]

Received January 28, 1994; accepted March 24, 1994

Timolol eyedrops may cause systemic side-effects in glaucoma patients due to absorption of the drug into systemic circulation. In a previous study, timolol concentrations in plasma were reduced if timolol was administered in ocular inserts instead of eyedrops. We compared the intraocular pressure lowering effect and systemic absorption of timolol inserts to those of 0.5 % timolol eyedrops in humans. Inserts of silicone tubing released 90.3±13.9 μg of timolol in 24 hours *in vivo*. Timolol inserts afforded similar decreases in intraocular pressure in open-angle glaucoma patients as did b.i.d. eyedrops, but produced lower peak timolol concentrations in plasma, 0.70±0.10 ng/ml and 0.24±0.05 ng/ml, respectively. After eyedrops, peak concentrations were achieved at 15.0±2.2 min, while application of an insert resulted in a delayed peak (t_{max} = 623±195 min). The insert resulted in a higher systemically absorbed fraction of the timolol dose than the eyedrop, but the peak timolol concentration and daily absorbed amount of timolol were decreased. The release rate of timolol from the inserts *in vivo* was only slightly less than that *in vitro*. Silicone devices are useful for clinical testing of controlled delivery properties of ocular drugs.

KEY WORDS: timolol; intraocular pressure; systemic absorption; controlled release; ocular drug delivery.

Introduction

Timolol eyedrops are commonly used to treat the elevated intraocular pressure (IOP) in glaucoma. Due to its systemic absorption timolol may produce systemic cardiac, respiratory, and central nervous system side-effects especially in patients with predisposing factors (1,2). Systemic side-effects of timolol contributed to the death of more than 30 patients in the United States during 1978-1985 (3). Because of the risk of side-effects, one fourth of patients receive other treatments.

In rabbit studies the systemic absorption of ophthalmic timolol has been reduced by employing prodrugs (4), viscous vehicles (5), controlled release inserts (6), coadministered phenylephrine (5), eyelid closure (8), punctal occlusion (7,8), and different dosing times (9). Punctal occlusion is the only method that has been shown to be effective in humans. Punctal occlusion decreased the amount of systemically absorbed drug by 60 %, but peak concentration in plasma was not affected (7).

Departments of *Pharmaceutical Technology, [1]Ophthalmology, [2]Pharmacology and Toxicology, University of Kuopio, P.O.Box 1627, 70211 Kuopio, Finland.
[3] Department of Clinical Pharmacology, University of Turku, 20520 Turku, Finland.
* Correspondence.

Systemic concentrations of ocularly applied timolol were decreased in rabbits to less than 6 % of that attained after eyedrop instillation by applying the drug in experimental silicone reservoir devices (6). The devices released 7.2 μg/h timolol for 8 hours. The rate of drug release was varied by changing the pH or concentration of the solution in reservoir (10). The devices are potentially useful in experiments where information on optimal drug release rates and patterns are sought.

In the present study, we compared controlled ocular timolol delivery in humans and suitability of silicone devices for clinical testing. The IOP lowering effect and systemic drug absorption from the devices were compared with those of eyedrops.

Materials and Methods

Eyedrops. Commercial timolol maleate (5 mg/ml timolol base equivalents; Blocanol™, Merck & Co) eyedrops were used. The eyedrops were phosphate buffered (pH 6.9), isotonic, and contained benzalkonium chloride (0.1 mg/ml). The commercial droppers delivered eyedrops with mean volume of 33.6 μl (determined from the weights). Mean timolol dose was 33.6 μl × 5 μg/μl = 168 μg.

Preparation of ocular inserts. Medical grade silicone tubing (Silastic™, Dow Corning, Midland, MI with dimensions 1.46 mm i.d., 1.94 mm o.d. and 0.24 mm wall thickness) was cut into 8 mm lengths. The ends were sealed with Silastic™ Adhesive A affording a 6 mm length of empty space inside the device. A 31 G needle was used for pressure relief. After curing, the devices were cut to leave 0.5 mm of adhesive in both ends and hemispheric pieces of cured Silastic™ Adhesive A were glued to the ends. The empty devices were autoclaved and stored in sterile packages.

Timolol maleate (54 mg/ml; eqv. of 40 mg/ml timolol) was dissolved in 0.15 M borax and pH was adjusted to 8.64 with sodium hydroxide. The solution was sterile filtered and 6.3 μl of the solution, containing 251 μg of timolol, was aseptically injected through one end of the silicone devices while pressure relief was provided by 31 G needle at the other end of the device. The devices were filled a few hours before use and stored in sterile packages.

In vitro drug release. Release of timolol from silicone devices in vitro was studied using the rotating bottle method (NF XIV) 3 h after filling of the devices. The speed of rotation was 35 rpm and the dissolution medium was 3.0 ml of isotonic 10 mM phosphate buffer (pH 7.40) at 32 °C. Samples of 200 μl were withdrawn and replaced by fresh buffer. Timolol was analyzed by using RP-HPLC (Beckman System Gold, Beckman Instruments, San Ramon, CA) with a Supelco LC-18-DB column (5 μm, 150 × 4.6 mm). The mobile phase was 30/70 (v/v) acetonitrile/acetic acid pH 4.0. The detection wavelength was 294 nm, flow rate 1.0 ml/min and retention time 2.7 min.

Intraocular pressure study. Clinical studies were approved by the Ethics Committee of the University Hospital of Kuopio and National Agency for Welfare and Health. Tolerability of the devices was demonstrated by evaluation in the eyes of five healthy volunteers.

Twelve recently diagnosed open-angle glaucoma pa-

0724-8741/94/0900-1278$07.00/0

tients participated in the IOP study. They had not been medicated previously. The mean age of the patients (7 women, 5 men) was 70 (61-79 years). Six patients suffered from simple and six from capsular glaucoma. Nine patients had unilateral and three bilateral elevation of IOP. The IOP levels of the patients were followed for one day before timolol devices were applied. The pressure readings were obtained with Schiötz tonometer six or seven times daily.

The timolol-containing device was placed on one eye of all twelve patients and IOP was followed in both eyes for one day. The devices were in the eyes for 5-24 hours. Ordinary glaucoma treatment was started 24 h later by using 0.5 % timolol eyedrops twice daily in nine patients. If the IOP was elevated in both eyes, the patients received timolol eyedrops in both eyes, but unilaterally if only one eye had elevated pressure. Readings were obtained from both eyes.

Systemic absorption. The timolol containing device was placed for 24 h unilaterally in the lower conjunctival sac of six healthy volunteers (four men, two women; ages 28-35). After one week one drop of 0.5 % timolol was carefully instilled to one eye of the volunteers. During application the volunteers were in erect position.

Blood samples were collected at 10, 20, 30 and 40 minutes, 1, 2, 4, 6, 8, 10, 12, and 24 hours after application using cannulas in a forearm vein. After centrifugation plasma timolol was determined as timolol equivalents by using radioreceptor assay of Kaila (11). The assay sensitivities were 40 pg/ml and 50 pg/ml of timolol base for eyedrop and device groups, respectively.

After 24 h the devices were recovered and remaining timolol was determined. The solution in the devices was withdrawn and the device core rinsed with 200 µl of water. Timolol in the silicone walls was extracted in 1 ml of 50 % methanol for 34 days. After this period no more timolol could be extracted from silicone. Timolol in the samples was quantitated with the HPLC-method described above.

Data analysis and simulation. C_{max} and t_{max} values were obtained from the data points. AUC values were determined by using the trapezoidal rule. The STELLA II program (High Performance Systems Inc, Hanover, NH) was used to construct a kinetic model for systemic timolol absorption. Input of timolol was obtained from the 3rd order polynomial fit of in vitro release data. Systemic pharmacokinetic constants, tear flow rate (16 %/min) and conjunctival clearance (10.4 µl/min) were obtained from the literature (6,12,13). Conjunctival clearance was from rabbit data (6). Published (12) values for the elimination rate constant for timolol from plasma (0.169 h^{-1}) and the volume of distribution (147 l) were used. The diagram of the STELLA model is shown in Fig. 1.

Results

Timolol release from the silicone devices. The initial amount of timolol in the devices was 251 µg. During the first eight hours the mean release rate was 6.7 µg/h of timolol base and in 24 h the mean rate was 4.3 µg/h (Fig. 2). In vitro 103.5 ± 6.3 µg (mean ± SD, n = 9) of timolol was released in 24 h. The best polynomial fit for release rate was obtained with equation $9.3959-(0.705t)\pm(0.0206t^2)-(0.000261t^3)$, t is time, (Fig. 2). This was used as drug input in the model (Fig. 1). In vivo, 90.3 ± 13.9 µg (mean ± SD, n = 6) of timolol was released during 24 h. The release rate of timolol in vivo was 13 % slower than in vitro ($P<0.05$, Student's unpaired two-tailed t-test).

Tolerability and retention of the devices. Although the device tolerability was not systematically evaluated, reports from the volunteers indicated no discomfort. In the acceptability test, minor transient local conjunctival reddening was observed after 8 hours in some eyes. No adverse signs were observed in the examination by an ophthalmologist. Mild, short lasting foreign body sensation was reported by the volunteers after ocular application.

All devices were retained well in the eyes of volunteers in the tolerability (8 h) and absorption (24 h) tests. In the IOP study, three devices were expelled from the eyes during pressure measurement.

Intraocular pressures. Compared to the mean pretreatment pressures (29.9±2.8 mmHg), the IOPs during the treatments were significantly lower ($P<0.001$; paired two tailed Student's t-test) in all patients: 18.6±2.9 mmHg (n = 12) and 17.4±3.0 mmHg (n = 9), when timolol insert or b.i.d. eyedrops were applied, respectively (Fig. 3). Ranges of the IOPS before and during timolol device treatment overlapped in two patients. After eyedrop administration overlapping was not observed. The mean IOPs during treatment did not differ between the device and eyedrop groups ($P=0.31$, Fig. 3).

The data for IOP in Fig. 3 includes only those patients where the device was retained for 24 hours (n = 9). Three patients were excluded because the device was expelled from the eye at 5-8 hours during pressure measurement. In two of those patients, their IOP reached the original level nine hours after device displacement, while the IOP of one patient remained low for 24 h.

After unilateral timolol device administration, contralateral pressures decreased only from 21.4 ± 4.5 mmHg to 18.5 ± 3.7 mmHg (mean ± SD). Although the decrease of mean pressure was observed in all twelve patients ($P< 0.001$) the pressure ranges overlapped in six patients, but these patients had normal IOP in the contralateral eye before medications. Significant contralateral pressures effects were not seen after unilateral administration of timolol eyedrops ($P=0.14$, n = 6). Patients with bilaterally elevated IOPs received timolol in both eyes and were not included in the comparison.

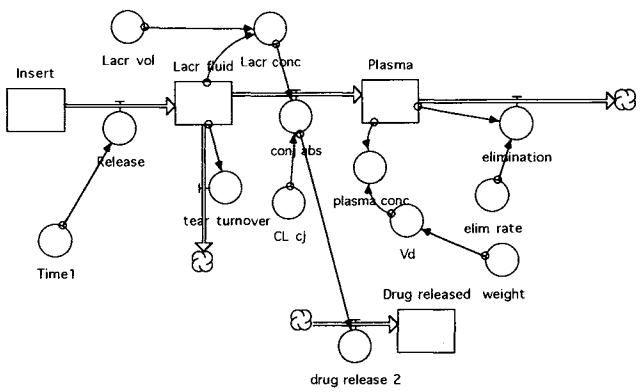

FIGURE 1. Diagram of the kinetic simulation model.

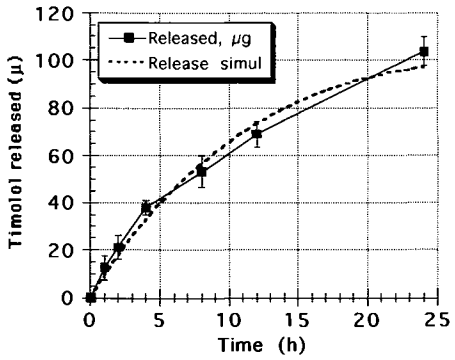

FIGURE 2. Timolol release in vitro from the silicone devices. The dotted line represents the 3rd order polynomial fit of the release data. Means ± SD of nine experiments are shown.

Systemic timolol absorption. After eyedrop administration, timolol concentration in plasma reached its peak (0.70±0.10 ng/ml) at 15.0 ± 2.2 min (Fig. 4, Table). After insert administration, C_{max} was 68 % lower ($P<0.01$) and it occurred later at 623 ± 195 min ($P<0.05$)(Table I). Plasma AUC of timolol after a single dose was equal in both cases (Table I).

Discussion

Acceptability of the Devices. Silicone devices were not designed for clinical drug treatment, but they rather served as experimental tools enabling clinical testing of controlled release concepts for ocular drugs prior to the initiation of development efforts for a controlled release dosage form. The release rates of timolol from the devices are easily modified (10), and their aseptic manufacturing is readily accomplished in a hospital pharmacy. The only clinical problem was occasional expulsion of the device during IOP measurement. This problem might possibly be avoided if a Goldman applanation tonometer, pneumatonometer or non-contact tonometer were used instead of a Schiötz tonometer.

Intraocular Pressures. One controlled release insert releasing 90.3 μg timolol in 24 h decreased the IOP as effectively as twice daily 0.5 % timolol eyedrops (dose 2x168 μg) (Fig. 3). For ethical reasons, timolol eyedrop treatment was initiated the day following the device experiment when some timolol from the insert test probably remained in the ciliary body. Furthermore, controlled release of 40-50 μg timolol decreases IOP and may be a sufficient dose (Figs. 2-3). We conclude that controlled release of timolol decreases the IOP at lower doses than eyedrops in glaucoma patients.

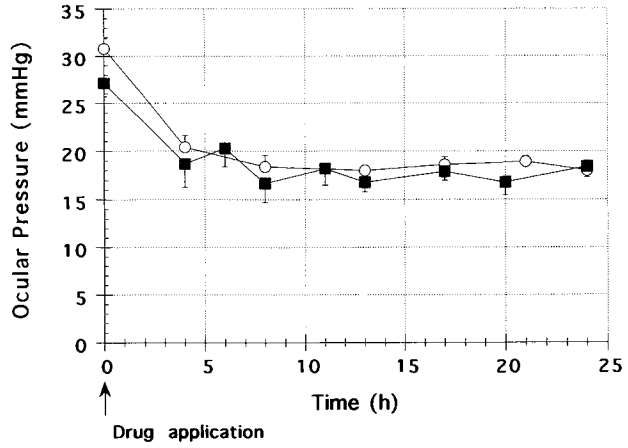

FIGURE 3. Means ± SEM of intraocular pressures in the treated eyes of the open-angle glaucoma patients after application of silicone device (○, n=9) or b.i.d. 0.5 % timolol eyedrops (■, n=4-9).

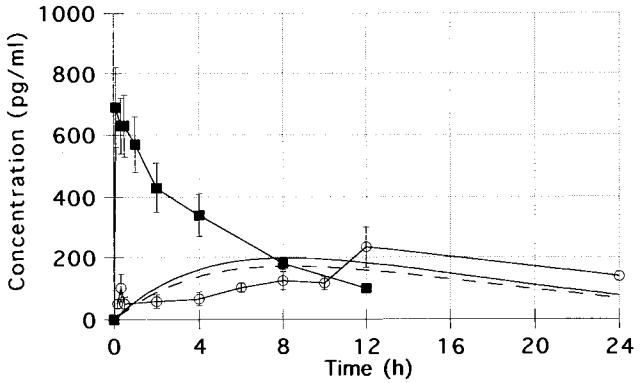

FIGURE 4. Timolol concentrations in plasma after administration of 0.5 % eyedrop (■) or silicone insert (○). Means ± S.E. of 6 volunteers are presented. Smooth line without symbols is the result of simulation assuming i.v. infusion of timolol at the rate of in vitro release. The dotted line represents simulation, where only conjunctival systemic absorption is assumed and the permeability of conjunctiva is that of isolated rabbit conjunctiva.

Systemic Absorption. The systemic absorption of timolol from eyedrops was rapid (Fig. 4). Due to the lack of early data points, we could not reliably determine the apparent rate constant (K_a) for systemic timolol absorption by curve-fitting. Simulation with the model of Fig. 1 was close to the mean data points when the absorption rate constant was set to 0.26 min^{-1}. Accordingly the half-time of systemic timolol absorption ($0.693/K_a$) may be less than three minutes and more than 90 % of the absorption takes place in 10 min after instillation. In humans ocularly instilled solutions enter the nasal mucosa in 1.1±0.7 min (14) with the maximal amount of solution in the nose being observed after more than 10 min following instillation (15). The half-time of solution removal from the nasal cavity to the pharynx is 10-20 min (16). Similarly with rabbits (6,17), the main sites of systemic timolol

Table I. AUC (min * ng/ml) and peak concentration (ng/ml) of timolol in healthy volunteers after eyedrop and silicone device administration.

Volunteer	AUC_{0-24h} device	c_{max} device	AUC_{0-12h} eyedrop	c_{max} eyedrop
A	260	0.26	297	1.10
B	186	0.22	197	0.88
C	277	0.38	269	0.88
D	279	0.39	168	0.77
E	108	0.11	201	0.69
F	84	0.09	104	0.22
MEAN	199	0.24	194	0.70
SEM	36	0.05	27	0.10

absorption from eyedrops in humans are obviously the conjunctiva and nose.

Timolol delivery of 4.3 μg/h for 24 hours resulted in peak levels in plasma that were one third of those seen after a 0.5 % eyedrop (Fig. 4). If the side-effects are related to the peak concentrations, the safety of ophthalmic timolol treatment would be improved by controlled drug release. The decreased C_{max} was due to the slower rate of systemic absorption, not to a smaller fraction being absorbed. Magnitude of this decrease (three fold) was less than in rabbits (> 17 times). This is probably due to the longer half-life of timolol in humans (4.1 h)(12) than in rabbits (20-30 min)(6).

During 24 h the single insert afforded amounts of timolol in plasma equivalent to a single 0.5 % eyedrop as indicated by equivalent AUC values (Table I). Compared to the clinical administration of twice daily eyedrops, the insert delivers only about half the timolol into the systemic blood circulation daily. Since the single dose AUCs were similar and timolol released by the device in vivo (90.3 μg) was less than the timolol contained in one eye drop (168 μg), a greater fraction of the timolol reaches plasma from the device than from eyedrop.

During continuous b.i.d. timolol eyedrop treatment, the previous dose is essentially eliminated before the next dose is administered (Fig. 4). and no accumulation is expected. A long term intravenous infusion of timolol at 6.7 μg/h (the insert release rate during the first eight hours (Fig. 2)), should produce a steady state concentration (C_{ss} = input rate/CL) of 220 pg/ml in plasma. This is not higher than the peak concentration reached after a single ocular insert (Fig. 4). Consequently, the conclusions of this study on systemic timolol absorption would not change upon multiple dosing.

By using the conjunctival clearance of timolol in rabbits (the human value is not known) and tear turnover in humans (0.16 min^{-1}) the systemic absorption from timolol inserts in humans was reasonably well simulated (Fig. 4). However, similar simulation results could be obtained with lower conjunctival clearance together with nasal absorption component. If the human conjunctival permeability is in the same range as in rabbits, very little timolol should enter the nasal mucosa and systemic absorption from insert would primarily take place across the conjunctiva. Although the systemic bioavailability (AUC) and the concentration range in plasma were well simulated, the shape of the curve does not match the real data for unknown reasons (Fig. 4).

In vivo release of timolol from the devices was only 13 % smaller than in vitro release. Timolol release from the devices is based on diffusion across the device walls (12) and increased drug concentration in the lacrimal fluid should lead to decreased concentration gradient and drug release rate. In accordance with the previous model (18), timolol clearance by the ear turnover (1.1 μl/min) and even more so by corneal and conjunctival absorptions (> 10 μl/min) is high enough to maintain sufficient concentration gradient to provide for comparable in vivo and in vitro release rates. Delivery of timolol to systemic circulation is controlled by the device, not by the biomembrane as evidenced by small deviation of the systemic delivery and bioavailability from the simulated intravenous infusion at the rate of in vitro release (Fig. 4). In contrast, in transdermal drug delivery, low skin permeability causes substantial in vivo deviations from the in vitro release and from device controlled delivery (18,19).

In conclusion, controlled delivery of timolol decreases elevated IOP and reduces systemic peak concentrations and drug load. Silicone devices were well-tolerated and may be useful for early clinical testing of ocular controlled release concepts.

Acknowledgments

Excellent technical assistance of Mr. Markku Taskinen and Mrs. Mirja Simonen is acknowledged. We are grateful to the Personnel of the Pharmacy and Eye Department at the University Central Hospital of Kuopio, Finland. This study was supported by $Inter_x$ Research Corp., Merck, Sharp & Dohme Research Laboratories, Lawrence, KS, U.S.A. Dr. Arto Urtti is grateful to Academy of Finland for support and to Drs. James D. Pipkin and Arnold J. Repta for helpful discussions.

References

1. W.P. Munroe, J.P. Rindone, R.M. Kershner. Systemic side effects associated with the ophthalmic administration of timolol. *Drug Intell. Clin. Pharm.* **19**: 85-89 (1985).
2. A. Urtti and L. Salminen. Minimizing systemic absorption of topically administered ophthalmic drugs. *Surv. Ophthalmol.* **37**: 435-456 (1993).
3. W.L. Nelson, F.T. Fraunfelder and J.M. Sills. ADverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978-1985. *Am. J. Ophthalmol.* **102**: 606-611 (1986)
4. S.C. Chang, H. Bundgaard, A. Buur and V.H.L. Lee. Improved corneal penetration of timolol by prodrugs as a means to reduce systemic drug load. *Invest. Ophthalmol. Vis. Sci.* **28**: 487-491 (1987).
5. K. Kyyrönen and A. Urtti. Improved ocular:systemic absorption ratio of timolol by viscous vehicle and phenylephrine. *Invest. Ophthalmol. Vis. Sci.* **31**:1827-1833 (1990).
6. A. Urtti, J.D. Pipkin, G.S. Rork, T. Sendo, U. Finne and A.J. Repta. Controlled drug delivery devices for experimental ocular studies with timolol. 2. Ocular and systemic absorption in rabbits. *Int. J. Pharm.* **61**: 241-249 (1990).
7. M.S. Passo, E.A. Palmer and E.M. Van Buskirk. Plasmal timolol in glaucoma patients. *Ophthalmol.* **91**: 1361-1363 (1984).
8. T. Kaila, R. Huupponen and L. Salminen. Effects of eyelid closure and nasolacrimal duct occlusion on the systemic absorption of ocular timolol in human subjects. *J. Ocul. Pharmacol.* **2**: 365-369 (1986).
9. 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).
10. A. Urtti, J.D. Pipkin, G. Rork and A.J. Repta. Controlled drug delivery devices for experimental ocular studies with timolol. 1. In vitro release studies. *Int. J. Pharm.* **61**: 235-240 (1990).
11. T. Kaila. A sensitive radioligand binding assay for timolol in plasma. *J. Pharm. Sci.* **80**: 296-299 (1991).
12. L.Z. Benet and R.L. Williams. Design and optimization of dosage regimens: pharmacokinetic data. In A. Goodman Gilman, T.W. Rall, A.S. Nies, P. Taylor (eds.), *The Pharmacological Basis of Therapeutics*. Pergamon Press, New York, 1990, pp. 1650-1735.
13. D.M. Maurice and S. Mishima. Ocular pharmacokinetics. In M.L. Sears (ed.), *Handbook of Experimental Pharmacology vol 69, Pharmacology of the Eye*, Springer-Verlag, Berlin-Heidelberg, 1984, pp. 19-116.
14. J.J. Hurwitz, M.N. Maisey and R.A.N. Welham. Quantitative lacrimal scintillography. I. Method and physiological application. *Br. J. Ophthalmol.* **59**: 308-312 (1975).

15. J.J. Hurwitz, M.N. Maisey and R.A.N. Welham. Quantitative lacrimal scintillography. II. Lacrimal pathology. *Br. J. Ophthalmol.* **59**: 313-322 (1975).
16. J.G. Hardy, S.W. Lee and C.G. Wilson. Intranasal drug delivery by spray and drops. *J. Pharm. Pharmacol.* 37: 394-297 (1985).
17. S.C. Chang and V.H.L. Lee. Nasal and conjunctival contributions to the systemic absorption of topical timolol in the pigmented rabbit: implications in the design of strategies to maximize the ratio of ocular to systemic absorption. *J. Ocul. Pharmacol.* 3:159-167 (1987).
18. A. Urtti. Simulation of the absorptive membrane permeability effects on in vivo drug release from controlled release systems. *Proc. Intern. Symp. Control. Rel. Bioact. Mater.* **18**: 431-432 (1991).
19. R. Guy and J. Hadgraft. Rate control in transdermal drug delivery? *Int. J. Pharm.* 82: R1-R4 (1992).