Importance of the Noncorneal Absorption Route in Topical Ophthalmic Drug Delivery

Imran Ahmed and Thomas F. Parson

Transcorneal permeation has traditionally been the mechanism by which topically applied ophthalmic drugs are believed to gain access to the internal ocular structures. Relatively little attention has been given to alternate routes by which drugs may enter the eye. A system has been developed which allowed the investigation in vivo of the contribution of noncorneal absorption to intraocular drug levels after topical dosing. Using timolol and inulin as probe drugs, it was shown that the noncorneal absorption route may contribute significantly to drug penetration into intraocular tissues. Furthermore, results demonstrated that drugs absorbed by the noncorneal route appear to enter certain intraocular tissues by a mechanism which bypasses the anterior chamber. These studies suggested that intraocular penetration via noncorneal routes involves penetration of drug across the conjunctiva/sclera. Neither reentry from the general circulation after drug absorption into the blood or drug delivery by the local vasculature accounted for the observed results. In terms of topical ophthalmic drug delivery, the noncorneal absorption route may be important for drugs that are poorly absorbed across the cornea due to their physical-chemical properties. We have demonstrated this using inulin as a model for a poorly absorbed, high molecular weight substance. Invest Ophthalmol Vis Sci 26: 584–587, 1985

Drug absorption across the conjunctiva and the sclera is generally regarded as nonproductive, based on the assumption that drug entering these membranes is picked up by the general circulation and does not contribute to intraocular drug levels.1-3 Hence, most efforts at improving ocular drug delivery are aimed at enhancing corneal permeability. When such studies are conducted in vivo, the anterior chamber generally serves as the sampling compartment for measuring intraocular drug bioavailability.

Previous studies in our laboratory have shown that drug concentration in the aqueous humor does not always reflect that in the surrounding intraocular tissues.6 Furthermore, it has been suggested that drug entry into the eye via a noncorneal route may be one of the causes for such an observation.7 There is other evidence that conjunctival and scleral absorption may be important for drug entry into the eye in some cases.8,9

The relative contribution of the noncorneal absorption route to drug levels observed in intraocular tissues after topical instillation of timolol and inulin was measured in this study. Drug absorption into the eye was determined both when the instilled solution was allowed access to the entire precorneal area and also when corneal access to the drug was prevented.

The results clearly demonstrate that noncorneal absorption may contribute significantly to intraocular drug levels following topical dosing. The relative importance of this route may depend upon the particular drug being investigated. In addition, the anterior chamber is not an appropriate sampling compartment for drugs which are absorbed by noncorneal routes. Such findings may be significant in the design and evaluation of ocular drug delivery systems.

Materials and Methods. The levoisomer of timolol (carbon-14 labelled and unlabelled) was provided by Interex Research Corporation (Lawrence, KS) as its maleate salt. Proposol, Eonofluor, and Aquasol II were purchased from New England Nuclear (Boston, MA). Unlabelled inulin was obtained from Sigma Chemical Company and tritium labelled inulin was obtained from Amersham Corporation (Arlington Hts, IL). All other chemicals were analytical reagent grade.

Male, New Zealand albino rabbits, 2.0–2.4 kg, were anesthetized with a 3-ml intramuscular injection of a 3:2, ketamine:xylazine combination. A 5-mm plug made out of polyethylene tubing (PE 50) was inserted into the puncta in each eye, and the globe of the eye was proposed. A hollow, glass cylinder (o.d. 16 mm, i.d., 14 mm and 0.5 cm in length) was glued onto the surface of the globe along the corneoscleral junction of one eye with a cyanoacrylate adhesive (Superglue®). The diameter of the glass cylinder was sufficient, in all cases, to cover the entire corneal surface and overlapped slightly onto the conjunctiva (≤1 mm). The eye was then returned to the socket. During the development of this procedure, the integrity of the glue-seal was tested repeatedly with dye solutions (fluorescein and coomassie brilliant blue) placed both inside and outside the cylinder. It was determined that over the time course that these studies were conducted, that no leakage through the seal occurred. The other eye was left as is.

The rabbits were placed in plastic restraining boxes (Plas Labs; Lansing, MI) in their normal, upright position. A 0.65% drug solution (inulin or timolol maleate) containing both unlabelled and radiolabelled (300 μCi per ml) molecules was prepared in isotonic phosphate buffer. The solution pH was adjusted to 7.0. Twenty-five microliters of the drug solution were then instilled under the upper lid in each eye with a
Hamilton® microsyringe. At selected times postinstillation, the rabbits were sacrificed with an overdose of pentobarbital. Immediately following the death of the animals, the precorneal area was thoroughly rinsed with normal saline and gently blotted to remove excess fluid.

Approximately 150 μl of aqueous humor were aspirated following limbal puncture. The iris-ciliary body, lens, cornea, the entire vitreous humor, bulbar conjunctiva and the sclera in toto were subsequently removed from each eye. The entire procedure took less than 3 min. The iris-ciliary body, cornea, lens, conjunctiva and sclera were rinsed with 1 ml of saline and gently blotted with Kimwipes® to remove residual fluid. Tissue standards were prepared by spiking two of each tissue type with 25 μl of the drug solution. The tissues were digested in Protosol®. After adding scintillation fluors (Aquasol II® for the aqueous humor and Econofluor® for the tissues), samples were stored overnight and subsequently counted with a liquid scintillation spectrometer (Beckman LS 7000). Values were converted to mcg of drug based on total radioactive activity and were normalized for the tissue weight. Chemical identification of the 3H-tracer was carried out using a TLC procedure (Avicel Cellulose, 250 μ, Analtech Inc.; Newark, NJ). The plates were developed in a preequilibrated chamber using nBuOH:EtOH:H2O (33:20:47) mobile phase and a 5% phenol-sulfuric acid in ethanol for visualization. Separate experiments were performed using the same protocol where the rabbits were either dosed in one eye (cornea open or blocked) and the tissue levels were measured in both the dosed and the undosed eye postinstillation, or where the animals were administered the entire dose as an iv bolus and the intraocular tissue concentrations were measured. Studies were also conducted where drug levels in ocular tissues were measured in rabbits which were first sacrificed and then dosed orally or where the animals were administered the entire dose as a constant infusion (except for the conjunctiva) are lower than that observed in these intraocular tissues. Sclera and conjunctiva levels remain essentially unchanged. The comparative concentrations at 20 min postinstillation shown in Table 1 are reflective of the results at each time point in the concentration versus time profile carried out for a period of 4 hr.

Table 1 shows the results of the study in which timolol was instilled into the eye both when corneal access was permitted and when it was blocked. In the case where corneal access was permitted, the highest timolol levels were observed in the conjunctiva, followed by the cornea, sclera, iris-ciliary body, the aqueous humor, lens and vitreous humor, respectively. When corneal access was blocked, however, aqueous humor and corneal drug levels dropped drastically, to <5% of its value compared to that observed when corneal access was permitted. Although iris-ciliary body, lens and vitreous levels are lower in the absence of corneal access, significant timolol levels are still observed in these intraocular tissues. Sclera and conjunctiva levels remain essentially unchanged. The comparative concentrations at 20 min postinstillation shown in Table 1 are reflective of the results at each time point in the concentration versus time profile carried out for a period of 4 hr.

Table 2 shows the results of similar studies conducted using inulin as the test substance. Although the absolute concentrations of inulin within the eye (except for the conjunctiva) are lower than that observed for timolol, the pattern observed in the presence and absence of corneal access is similar. Furthermore, the noncorneal contribution to drug levels in the iris-ciliary body and the vitreous humor are between 70 and 80% of that observed when corneal access is permitted. In fact, statistically, the values for the iris and vitreous with and without corneal access do not differ significantly at the 95% confidence level. Lens levels of inulin were found to

### Table 1. The concentration of timolol maleate in various ocular tissues 2 min following the topical instillation of 25 μl of a 0.65% timolol maleate solution, in the presence and absence of corneal access

<table>
<thead>
<tr>
<th>Concentration (mcg/g)</th>
<th>With corneal access</th>
<th>Without corneal access</th>
<th>Without access × 100 with access</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td>7.87*</td>
<td>0.03</td>
<td>0.4</td>
</tr>
<tr>
<td>Cornea</td>
<td>84.5</td>
<td>2.61</td>
<td>3</td>
</tr>
<tr>
<td>Lens</td>
<td>0.37</td>
<td>0.16</td>
<td>43</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>0.08</td>
<td>0.03</td>
<td>37</td>
</tr>
<tr>
<td>Iris-ciliary body</td>
<td>8.13</td>
<td>1.03</td>
<td>13</td>
</tr>
<tr>
<td>Sclera</td>
<td>9.51</td>
<td>10.7</td>
<td>112</td>
</tr>
<tr>
<td>Bulbar conjunctiva</td>
<td>115</td>
<td>118</td>
<td>103</td>
</tr>
</tbody>
</table>

* The mean standard error of the mean and the number of determinations in parentheses.
be not detectable either in the presence or absence of corneal access. This is not surprising, and further in vitro studies have shown that inulin, possibly due to its large molecular size, has a very low affinity for lens tissue.

As described in the Materials and Methods section, studies were done to determine if the observed results could be accounted for either by the drug reaching intraocular tissues via systemic return, or if the local vasculature could be carrying drug to the intraocular tissues. When rabbits were dosed in one eye, and tissue levels were measured in the contralateral eye, these levels were less than 1% of those observed in the dosed eye. When the entire drug dose was administered intravenously, tissue levels were less than 5% of those observed after topical dosing. In the case where rabbits were sacrificed prior to topical drug administration, tissue levels were not statistically different from those observed in live animals. These experiments indicate that neither systemic return or local vasculature contribute significantly to the intraocular drug levels measured in these studies.

Discussion. To date, almost all efforts in ophthalmic drug delivery have been aimed at studying transcorneal permeation and optimizing corneal absorption of drugs. While the possibility of noncorneal routes by which drugs may enter the eye has been pointed out,1,11 relatively little attention has been given to its potential applications and implications in ophthalmic drug delivery.

The results presented in this study clearly establish that the noncorneal route may contribute significantly to overall drug penetration into intraocular tissues. An important point to note is that drugs absorbed via the noncorneal route appear to enter certain intraocular tissues bypassing the anterior chamber. Therefore, aqueous humor levels may not be an adequate measure of the effectiveness of some ocular drug delivery systems.

Furthermore, it may be possible to exploit the noncorneal absorption route to promote site-specific delivery of some drugs to intraocular tissues, without exposing the anterior chamber to unnecessarily high drug levels. The results suggest that intraocular penetration via the noncorneal route involves penetration across the conjunctiva and sclera, and that neither reentry from the general circulation after drug absorption into the blood, or drug delivery by local vasculature can account for the observed results. This is consistent with previous findings.8,9

Finally, it has been shown with inulin, a large molecular weight compound that is generally considered to have poor transcorneal permeability, that much of its intraocular access is via the noncorneal route. Further efforts at maximizing noncorneal absorption for similarly poorly penetrating compounds of therapeutic interest may prove productive.

In summary, the noncorneal route is established as being of potential significance in contributing to the intracocular access of certain compounds. The compounds studied here gained intraocular access via the noncorneal route, and essentially bypassed the anterior chamber. It appears important that further studies be aimed at identifying physical-chemical drug properties that may make this route significant; at ways of optimizing the noncorneal route by understanding the mechanism by which this occurs; and at potentially taking advantage of this route in the design and evaluation of ocular drug delivery systems. All of the above aspects of noncorneal drug absorption are currently being investigated.

Key words: noncorneal, ophthalmic, topical, timolol, inulin, conjunctiva, sclera, cornea

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References

Spontaneous Ocular Shedding of HSV-1 in Latently Infected Rabbits

Earl J. Berman and James M. Hill

The unscarified corneas of rabbits were inoculated with 50 µl of 2–4 × 10^6 PFU/ml of herpes simplex virus, type 1 (HSV-1), McKrae strain in 10 separate experiments over a 12-month period. Sixty of 104 (57.7%) rabbits survived to postinoculation (PI) day 20. These sixty rabbits were swabbed with dacron-tipped swabs for twenty consecutive days (PI days 20–39). The tear film collected on the swabs was immediately placed in tissue culture tubes with confluent primary rabbit kidney (RK) cell monolayers. The RK monolayers were monitored for cytopathic effects indicative of HSV-1. Fifty-eight of the sixty rabbits (96.7%) inoculated had at least one positive episode. Ninety-three of the 120 (77.5%) eyes of the latently infected rabbits had at least one positive episode. Virus was detected in 72 of the 93 positive eyes (77.4%) between PI days 20 and 29 and in 21 of the 93 positive eyes (22.5%) between PI days 31–39. A total of 2400 swabs were taken and 324 were positive (13.5%). All of the 58 positive rabbits were used later for ocular induction of HSV-1 and all 116 eyes of the latently infected rabbits shed virus for at least four consecutive days during induction. Invest Ophthalmol Vis Sci 26:587–590, 1985

The history of spontaneous HSV-1 shedding in the tear film of latently infected rabbits is a very important aspect of ocular studies of reactivation. Spontaneous HSV-1 ocular shedding must be documented before reliable data concerning induction, reactivation, and inhibition can be accumulated. Kwon et al. have shown that the longer the postinoculation (PI) time the fewer the detectable spontaneous sheddings that will occur. Kwon et al. used a broad time frame (PI days 40–220) and 10 rabbits. In this report, a very narrow PI range (PI days 20–39) and 60 rabbits are used. Shimomura et al. reported the importance of spontaneous HSV-1 ocular shedding in relation to induction of HSV-1 ocular shedding.

A complete, detailed history of spontaneous shedding is important for planning experiments and evaluating results from induced reactivations. If the history is not available, one possibility is that spontaneous shedding may be confused with induced shedding. Another possibility is that if there is no induction following some specific procedure, then without a spontaneous history, there is no proof that the rabbit has the ability to shed virus in the tear film. Spontaneous shedding proves that both HSV-1 latency and reactivation are operable. The following details the spontaneous ocular shedding of herpes simplex virus McKrae strain in rabbit eyes between PI days 20–39 in rabbits used over a 12-mo period in 10 separate experiments.

Materials and Methods. Virus inoculation: Rabbit (2–3 kg) eyes were inoculated with HSV-1, McKrae strain (2–4 × 10^6 PFU/ml). These investigations using rabbits conformed to the ARVO Resolution on the Use of Animals in Research. The virus was grown on rabbit kidney (RK) cells and titrated on African green monkey kidney cells (CV-1). The harvested virus was frozen at −80°C in 3-ml aliquots. A 50 µl suspension of HSV-1 was placed in the lower cul-de-sac of each rabbit eye and the closed eyelid was gently massaged against the unscarified cornea for 1 min. This inoculation resulted in an acute HSV-1 infection which was verified by slit-lamp biomicroscopy. A single 3-ml aliquot was used for each experiment and each of the 104 rabbits were inoculated from the same pooled stock of virus.

Determination of viral shedding: Starting on PI day 20, eye swabs were taken from rabbits every day for twenty consecutive days with a sterile dacron-tipped swab gently rotated in the upper cul-de-sac, across the cornea, and then into the lower cul-de-sac where the swab was allowed to absorb tear film in the fornix for 5 sec. The swabs were then immediately placed in tissue culture tubes with confluent RK monolayers.