Relationship between Steroid Permeability across Excised Rabbit Cornea and Octanol–Water Partition Coefficients

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Abstract □ Permeability rates were determined across excised rabbit corneas for 11 steroids. Permeability coefficients for each steroid were calculated, and their logarithms were plotted against their respective log octanol–water partition coefficients. A parabolic relationship resulted, with an optimum log permeability and coefficient observed at a log P_0 of 2.9. From these experimental results, an improvement in ophthalmic bioavailability of dexamethasone acetate as compared to dexamethasone is predicted and correlates with literature results.

Keyphrases □ Permeability—of various steroids across excised rabbit corneas, related to octanol–water partition coefficients □ Partition coefficients, octanol–water—related to permeability of various steroids across excised rabbit corneas □ Steroids, various—permeability across excised rabbit corneas related to octanol–water partition coefficients

The amount of drug that ultimately penetrates the cornea is often largely determined by physiological factors such as the extent of drainage, blinking, and/or tearing during the first 4–6 min after topical dosing (1). The time-honored approach to resisting drainage has been through the use of viscous solutions. Water-soluble polymers such as methylcellulose, hydroxypropyl methylcellulose, and polyvinyl alcohol impart a slight lowering of surface tension and an increase in viscosity to ophthalmic solutions. The increase in viscosity prolongs contact of the drug in the eye, thereby resisting drainage (2, 3); a lowering of the surface tension improves mixing with the precorneal tear film (4). In rabbits, a two- to threefold improvement in pilocarpine bioavailability has been found for viscous aqueous solutions (2, 3).

Another approach to improving ophthalmic bioavailability has been to increase drug absorption into the eye. For example, it was reasoned (5) that the enhancement of the lipophilicity of epinephrine through the use of a dipivalyl ester would facilitate penetration through the lipoidal layers of the cornea. Prodrug concentrations of 0.1%, administered twice daily for 1 month, significantly reduced intraocular pressure in hypotensive patients (6). The prodrug is reported to be about 100 times more effective than epinephrine in the management of glaucoma and about 100–400 times weaker than epinephrine in affecting the cardiovascular system of dogs and cats (7).

In terms of improved efficacy, the final evaluation of an analog or prodrug must come from *in vivo* studies. However, optimization of permeability based on molecular modification can be assessed more reliably from a model devoid of extraneous physiological factors (8, 9). Such a model should focus primarily on enhanced permeability as a function of molecular modification.

The purpose of this study was to determine the relationship between the permeability of various structurally related steroids across an excised rabbit cornea and their octanol–water partition coefficients.

EXPERIMENTAL

Materials—Ten tritium-labeled steroids and one ^{14}C-labeled steroid were purchased[1]. Identification of each steroid was confirmed by TLC using two solvent systems[2]. This step was accomplished by dissolving unlabeled steroids[3] in methanol along with the labeled steroid and determining R_f values for each by scintillation[4] and UV[5] spectroscopic methods. Purification consisted of removing volatile, labile tritium, using low temperature vacuum evaporation of alcoholic and then aqueous solvents (11). Monitoring the aqueous distillate for radioactivity indicated that removal of labile tritium by solvent evaporation was complete after three distillations[6]. All other chemicals were reagent grade.

Excised Corneal Preparation Procedure—A 1–1.5-kg young albino rabbit was sacrificed by injecting 20–30 ml of air into the marginal ear vein. The intact eye along with the lids and conjunctival sac was then enucleated. According to a published procedure (12, 13), the exposed cornea of the enucleated eye was placed carefully on a specially designed corneal holder, which maintained the cornea curvature and held the eye in place. Various tissues of the eye were dissected away, leaving the cornea, a small ring of scleral tissue, and palpebral conjunctiva, which was tied to the corneal holder.

The conjunctiva and scleral tissue served as a gasket and permitted the cornea to be suspended within the block system without inducing significant trauma or curvature distortion to the corneal endothelium or epithelium (12, 14). The block system[7] consisted of two cylindrical compartments separated by the cornea. The compartment adjacent to the endothelial surface of the cornea was designated the internal side, whereas the compartment adjacent to the epithelial side was referred to as the external side.

Study Procedure—Within 20 min of death, the cornea was mounted within the preheated block system and clamped into place; 6.0 ml of preheated (37°) glutathione Ringers solution[8] was added to the internal side. Then 6.0 ml of preheated glutathione Ringers solution, containing labeled and unlabeled steroids, was added to the external compartment. These solutions were prepared about 1 hr prior to the start of the experiment.

[1] From New England Nuclear, Boston, Mass.: 6,7-^3H-prednisolone, lot 747-276; 1,2-^3H-hydrocortisone, lot 853-184; 7-^3H-testosterone, lot 772-277; 1,2-^3H-desoxycorticosterone, lot 853-169; 1,2-^3H-cortexolone, lot 951-024; 6,7-^3H-triamcinolone acetonide, lot 635-258; 6,7-^3H-dexamethasone, lot 998-003; and 6,7-^3H-dexamethasone acetate, lot 690-1348. From Amersham/Searle, Arlington Heights, Ill.: 4-^{14}C-progesterone, lot CFA.148, batch 42; 1,2-^3H-fluorometholone, lot TRQ.974, batch 21474; and 6,7-^3H-prednisolone 21-acetate, lot TRQ.915, batch 20806.

[2] Solvent systems specific for each steroid and suggested by the manufacturer were used; methylene chloride–acetone (4:1) was suggested by Stahl (10) for general steroid use and also was used.

[3] Progesterone (Aldrich, lot 021957), hydrocortisone (Sigma, lot 25C-0319), prednisolone (Sigma, lot 23C-1900), desoxycorticosterone (Aldrich, lot 031847), testosterone (Aldrich, lot 112457), cortexolone (Aldrich, lot 06181), triamcinolone acetonide (Sigma, lot 26C-0211), dexamethasone (Alcon RPA 5399), dexamethasone acetate (Sigma, lot 83C-1700), fluorometholone (Farmila, lot 56/015), and prednisolone acetate (Organon, lot 92305).

[4] The silica gel was scraped from the origin to the solvent front (Quanata/Gram, Fairfield, N.J. LQDT prescored 20 × 20-cm TLC plates) in 1-cm increments. Every centimeter of silica gel was placed in a separate vial, scintillation fluid was added, and the vials were counted.

[5] Visual location of spot with the short wavelength of a UV lamp (Ultra-Violet Products, San Gabriel, Calif.).

[6] Less than 1.5% total radioactivity was recovered in distillate.

[7] The block system and corneal holder were purchased from Mr. Harold Eick through the cooperation of Dr. H. F. Edelhauser, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wis.

[8] This solution differed from the glutathione bicarbonate Ringers solution used in Ref. 12 in that sodium bicarbonate was replaced with 0.77 g of sodium biphosphate/liter to maintain the pH at 7.2 ± 0.2 during the experiment.

The concentration of steroid[9] used in each experiment was significantly lower than the drug's reported solubility in water at 25 or 37° (15). The specific activities of each steroid test solution[10] were adjusted so that nanogram levels could be detected on the internal side. This condition required relatively high specific activities for practically insoluble steroids. The block system containing the excised cornea was placed on a hot plate[11] preadjusted to maintain the solution temperature within 35–37°. The temperature was periodically measured to assure that the solution temperature remained within the specified range. The block system and the hot plate were surrounded by Styrofoam for insulation against temperature fluctuations.

As soon as the solutions were added to each side, circulation of fluids was induced by slowly bubbling air at a rate of approximately two or three bubbles per second. Samples of 0.1 ml were taken[12] from the internal side. The first sample was withdrawn within 1 min of adding the solution to the external side. This sample represented the zero-time sample and also served as a control. If the conjunctiva was accidently perforated during the surgical procedure, then fluid would be exchanged rapidly between compartments within this 1st min. Under these conditions, the zero-time sample would yield a counting rate well above background and indicate that the kinetic data were invalid. Subsequent samples were taken every 0.5 hr for 4 hr.

All samples were immediately placed into a scintillation vial[13] containing 10 ml of scintillation solution[14]. A like volume of solution was removed and discarded from the external side at each time increment. Thus, the cornea remained in hydrostatic equilibrium on both sides of the system. All vials containing tracer and scintillation solution were dark adapted for 18 hr or more and counted[15]. The external standardization method (16) was used to determine counting efficiencies and, therefore, permitted the quantity of steroid present in each sample to be calculated.

Partition Coefficient—All partition coefficients are expressed as averaged log octanol–water and were taken from Leo *et al.* (17). For dexamethasone acetate, no value was listed; however, the acetate ester could be estimated readily from the parent molecule, dexamethasone (17, 18). An octanol–water system was chosen because of the large volume of data that has been generated for correlation studies (17, 18).

RESULTS

All corneas were judged clear at the end of each 4-hr experiment[16]. Figure 1 represents a typical plot of data for the penetration of dexamethasone acetate and dexamethasone across an excised cornea. A short lag time was observed, representing the time required for drug to reach steady state in the cornea. Following the lag time, the data followed a linear relationship. The individual experiments yielded highly linear correlations as judged by the Pearson r (>0.975). In addition, no trends in nonlinearity were observed with time. This result indicated that the steady-state permeability rate was constant with time as required by Eqs. 1–3.

The transfer of substances across membranes by simple diffusion has been described (20) as a simplification of Fick's law according to:

$$\frac{dq}{dt} = \frac{DA(PC)}{T}(C_E - C_I) \qquad \text{(Eq. 1)}$$

where dq/dt is the permeability rate or the rate of drug quantity penetrating the cornea at time t, D is the diffusion coefficient of steroid through the excised cornea (square centimeters per second), A is the area of the absorbing surface of the cornea, T is the thickness of the cornea, (PC) is the partition coefficient (ratio of the drug concentration in the barrier membrane to the *in vitro* testing solution), C_E is the drug concentration on the external side, and C_I is the drug concentration on the internal side.

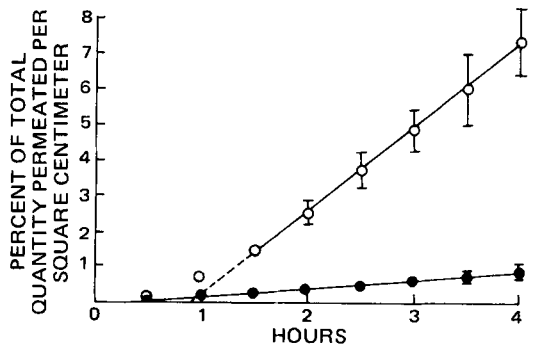

Figure 1—*Permeability of dexamethasone acetate* (○) *and dexamethasone* (●) *across an excised rabbit cornea. Each point represents a mean of four determinations. The vertical bars indicate 1* SD; *bars that are absent were smaller than the circle. The slope of the line represents the steady-state permeability rate.*

For these experiments, samples taken from the internal side were considered negligible in drug concentration when compared to the external side[17]. Therefore, C_I was eliminated from the equation, and C_E was considered a constant through the time course of the experiments. Therefore, Eq. 1 can be rewritten as:

$$\frac{dq}{dt} = \frac{DA(PC)}{T} C_E \qquad \text{(Eq. 2)}$$

When q is plotted *versus* t, the linear portion of the plot represents the steady-state permeability rate. The least-squares slope of each individual experiment was determined and averaged. Each averaged slope value was divided by an averaged value[18] of A, 1.089 cm^2. To compare the results from each steroid experiment, each averaged slope value was also divided by the C_E value used for each steroid. Consequently, the final value represented a permeability coefficient $[D(PC)/T]$ with units of square centimeters per second. When expressed in logarithmic form, this relationship becomes:

$$\log (P_{\text{perm}}) = \log \frac{D}{T} + \log (PC) \qquad \text{(Eq. 3)}$$

Figure 2 illustrates the parabolic nature of the data when the logarithms of permeability and partition coefficients are plotted against one another. The data could be best represented by a second-order power series[19]:

$$\log (P_{\text{perm}}) = -0.28 (\log P)^2 + 1.7 \log P - 7.0 \qquad \text{(Eq. 4)}$$

where $\log (P_{\text{perm}})$ is the logarithm of the permeability coefficient (centimeters per second), and $\log P$ is the logarithm of the octanol–water partition coefficient.

An optimum $\log P_0$ value of 2.9 was found by setting $d \log (P_{\text{perm}})/d \log P$ equal to zero and solving for $\log P_0$ (21). Figure 2 predicts a decrease in permeability once a partition coefficient of 2.9 is reached. According to Flynn and Yalkowsky (22), limited solubility is often responsible for the parabolic shape of many structure–activity curves. As a consequence, the solubility of progesterone was determined carefully under the exact test conditions of the permeability experiments (same solution, temperature, *etc.*). A solubility of 14.1 ± 1.46 μg/ml (n = 3) was obtained for progesterone. Therefore, to be certain that solubility would not influence the results, a concentration of 0.90 μg/ml, which was greater than 1 log unit below the drug's solubility, was chosen for study.

If the progesterone results are excluded from consideration, the remaining 10 steroids approach a plateau, as predicted by the kinetic model

[9] The concentration (micrograms per milliliter) of each steroid placed into the external compartment was: prednisolone, 16.7; hydrocortisone, 267; testosterone, 25; progesterone, 0.90; desoxycorticosterone, 133; dexamethasone, 83.3; dexamethasone acetate, 1.33; triamcinolone acetonide, 8.33; prednisolone acetate, 8.33; cortexolone, 267; and fluorometholone, 1.17.

[10] The specific activity (microcuries per milligram) of each radioactive steroid tracer was: prednisolone, 236; hydrocortisone, 15.4; testosterone, 119; progesterone, 151.1; desoxycorticosterone, 26.5; dexamethasone, 41.2; dexamethasone acetate, 2554; triamcinolone acetonide, 353; prednisolone acetate, 603; cortexolone, 12.1; and fluorometholone, 931.

[11] Model 4812, 15 × 15 cm, Cole Parmer, Chicago, Ill.

[12] Eppendorf pipettor, Brinkmann Instruments, Westbury, N.Y.

[13] With Polyseal core liners, Kimble, Toledo, Ohio.

[14] Handifluor Scintillar, Mallinckrodt, St. Louis, Mo.

[15] LS-230 liquid scintillation counter, Beckman Instruments, Fullerton, Calif.

[16] Each cornea was judged clear if typewritten print could be identified through the cornea at the end of each experiment. The clarity of the cornea is related to its hydration level; a normal cornea measures 78% (19). As the hydration level increases, the thickness of the cornea also increases; however, linear steady-state plots of drug penetrating the cornea generally result as long as the hydration level is below 85%. These latter corneas are cloudy and typewritten print cannot be read through them. Measurements were carried out for fluorometholone, prednisolone acetate, and progesterone and were 82.2 ± 0.6% (n = 4), 82.6% (n = 1), and 82.6 ± 0.8% (n = 4), respectively.

[17] After 4 hr, samples taken from the internal side were 3% or less of the concentration on the external side, with the exception of dexamethasone acetate which was 7.4%.

[18] Dr. H. Edelhauser, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wis., personal communication.

[19] Nonlinear regression was performed using the BMDX85 program on a CDC6400 computer.

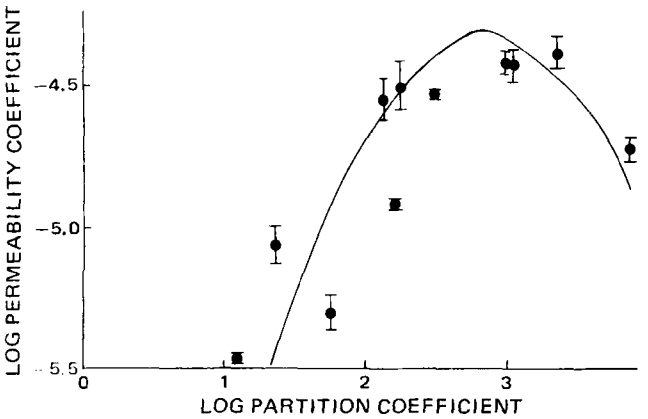

Figure 2—*Computer-generated curvilinear relationship between the log permeability coefficient of 11 labeled steroids and their respective log octanol–water partition coefficients. From left to right, the steroids are prednisolone, hydrocortisone, dexamethasone, fluorometholone, triamcinolone acetonide, prednisolone acetate, cortexolone, desoxycorticosterone, dexamethasone acetate, testosterone, and progesterone. The vertical bars represent 1* SD.

of Yalkowsky and Flynn (23). However, the value obtained for progesterone is statistically different ($p < 0.05$) from desoxycorticosterone, dexamethasone acetate, and testosterone, all of which are associated with maximum permeability. To determine if the carbon-14 label resided with progesterone during the experiment, an aliquot was taken from the external side following each experiment and chromatographed on silica gel thin-layer plates. Results showed that 95% of the label was associated with the progesterone molecule.

DISCUSSION

Equation 3 requires that the data follow a linear relationship with respect to the logarithms of permeability and partitioning coefficients. However, Fig. 2 shows a linear increase in log (P_{perm}) but begins to level off with log PC for cortexolone, desoxycorticosterone, and dexamethasone acetate. As discussed by Sinkula and Yalkowsky (24), the increase in permeability cannot go on indefinitely with an increase in the hydrophobicity of the homolog series; several reasons were given for the decline. Strictly speaking, progesterone and testosterone do not belong to the corticosteroid class of compounds; they lack a 17α-hydroxyl group. Therefore, if they are deleted from Fig. 2, the kinetic model best describes the remaining data.

The parabola in Fig. 2 identifies a specific optimal partition coefficient for steroids, expressed as log P_0, and predicts that a decreased intrinsic corneal penetration results if the optimal partitioning behavior is either decreased or increased. The kinetic model (23), which suggests that a plateau is a more precise relationship, identifies only a lower limit above which an intrinsic optimal penetration is theoretically predicted. Regardless of whether the parabola or the kinetic interpretation best describes the results, the data can be useful in the design of optimally permeable ophthalmic drugs. For practical purposes, optimal penetration can be considered to occur when $d \log (P_{perm})/d \log P$ begins to approach zero, which in Fig. 2 is represented by a log P of about 2.5–3.0.

Optimal penetration is desirable because a more rapid penetration rate leads to higher peak concentrations and lower quantities lost to nasolacrimal drainage. This condition permits a lower dose to be administered without sacrificing drug activity and promotes a lowered potential for systemic side effects. Even though the kinetic model predicts a plateau, ophthalmic bioavailability would not always be expected to follow the same relationship with an increase in log P. According to Eq. 2, the penetration rate is a function of the drug concentration as well as the partition coefficient. However, as molecular modification produces a more hydrophobic analog, aqueous solubility decreases. Also, the residence time of nondissolved drug particles in the eye is limited; therefore, expulsion of the particles by the eye may take place before solubilization may occur.

Under these conditions, the partition coefficient and solubility would tend to cancel one another in terms of increased penetration. Consequently, improved ophthalmic bioavailability would reach an upper limit or perhaps decrease if a low enough tear solubility is produced. If the parent drug is very soluble, however, then molecular modification may reduce solubility as partitioning is improved. But because the administered therapeutic dose never approaches its tear solubility, bioavailability is enhanced. Dipivalylepinephrine represents an example of this type.

The data generated in this study showed that the addition of an acetate functional group to the 21-hydroxy position of prednisolone and dexamethasone improved the log partitioning by 2 and 1.7 times over each parent drug. Based on permeability calculations, prednisolone and dexamethasone esters penetrated the excised cornea 7.4 and 8.2 times faster than the respective parent drugs. The observed improvement in *in vitro* permeability may not necessarily extrapolate to a significant improvement in ophthalmic bioavailability. However, when tested *in vivo*, a large difference in ophthalmic bioavailability was observed for dexamethasone acetate in comparison to dexamethasone.

In a study by Kupferman *et al.* (25), 0.1% petrolatum base ointments of ^{14}C-dexamethasone and ^{14}C-dexamethasone acetate were dosed in volumes of 50 μl to normal rabbits. After sacrificing animals at fixed times, the drug was assayed in aqueous and corneal tissue samples. The area under the corneal dose–time curve was 4.5 times greater for the acetate. Aqueous humor levels were detected for the ester but not for the parent drug. This latter study established, to a limited extent, a correlation between the *in vitro* corneal permeability model and an improvement in *in vivo* ophthalmic bioavailability.

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