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Influence of dissolution rate of sparingly soluble drugs on corneal permeability in vitro

Mikael Bisrat, Christer Nyström and Peter Edman

Department of Pharmaceutics, Biomedical Center, Uppsala University, Box 580 S-751 23 Uppsala (Sweden)

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Summary

The effects of particle size and dissolution rate of sparingly soluble drugs on their permeability coefficients were studied using excised pig cornea. Two wet sieve fractions, $\ll 5$ and 10–15 μm, of griseofulvin and prednisolone acetate were tested. The results show that there is no significant difference between the permeability coefficients of the two fractions. The dissolution rates of both sieve fractions were greater than the permeability rates and thus did not represent a rate-limiting step. Prednisolone acetate was fully hydrolyzed during passage through the cornea and only prednisolone was detected in the receiving compartment. The deviation from linear steady-state permeation at around 200 min suggests that the enzymatic process which hydrolyzes prednisolone acetate is saturable.

Introduction

A major impediment to ocular therapy is incomplete absorption and thus difficulty in achieving optimal drug concentration at the intraocular site. Normally less than 10% of the instilled dose is absorbed ocularly (Burstein and Anderson, 1985). This low bioavailability is due to low corneal permeability of drugs and a rapid drainage from the site of application (Chrai et al., 1973, 1974). One of the most promising ways of overcoming these bioavailability problems is to use prodrugs. The cornea consists of both lipophilic (endothelium and epithelium) and hydrophilic (stroma) layers which results in poor permeation of drugs with extreme partition coefficients (Schoenwald and Ward, 1980; Schoenwald and Huang, 1983). By virtue of their high lipid solubility, prodrugs penetrate the cornea readily and their permeability was shown to be much higher than the parent compound (Mandell et al., 1978). Prodrugs are hydrolyzed in the epithelium and their metabolites released into the anterior chamber (Anderson et al., 1980).

Owing to their lipophilicity, prodrugs can be used as suspensions. Suspensions are widely used in ocular drug therapy for administering sparingly soluble drugs such as steroids. However, instillation of a suspension into the eye is not a simple process and is associated with several difficulties. The particle size and size distribution of the sus-

Correspondence: M. Bisrat, Department of Pharmaceutics, Biomedical Center, Uppsala University, Box 580 S-751 23 Uppsala, Sweden.

pended drug should be well defined because of the risk of irritation and subsequent excessive tearing and drainage. The dissolution rate of the drug has also been claimed to be an important parameter since it determines the amount of drug actually in solution and thus available for transport through the cornea during the short residence time in the eye (Hui and Robinson, 1986). Although the range of particle size that could be used for ocular suspensions is limited, it is well known that a decrease in particle size leads to an increased surface area and thus an increase in dissolution rate. Further, previous studies (Bisrat and Nyström, 1988) have shown that the surface specific dissolution rate of a sparingly soluble drug increased with a decreasing particle size and that this was especially pronounced for particle size below approx. 5 μm.

Several physiological and physical factors determine the ocular bioavailability of drug. The aim of this paper was therefore to study, in a well defined model, the effect of particle size on the ocular permeability of sparingly soluble drugs.

Experimental

Materials

Microsized griseofulvin (Glaxo, U.K.) and prednisolone acetate (Allergan, U.S.A.) were used. Griseofulvin is an anti-fungal drug and is not used in ocular therapy but was chosen here as a model substance since it is non-polar and not metabolized. Its physico-chemical properties have been studied in our department. Prednisolone acetate on the other hand is widely used as a prodrug in suspended form to treat various inflammatory diseases of the eye.

The corneas used in this study were obtained from pigs (Yorkshire & Swedish Laundrace, Farmek, Uppsala), aged 6–7 months.

Methods

Preparation of sieve fractions

Excess griseofulvin or prednisolone acetate was added to a measured volume of glutathione bicarbonate Ringer's (GBR) solution, stirred for about 3 min and then treated in an ultrasonic bath for the same period of time to disperse the materials properly. These suspensions were sieved (precision test sieve with circular openings, Veco, The Netherlands) and sieve fractions in the range to $\ll 5$ μm and 10–15 μm were prepared.

TABLE 1

Primary characteristics of test materials

Materials	Density, ρ (g/cm^3)	Solubility (34–35 °C) C_s (μg/ml)	Wet sieve fraction (–) (μm)	Size distribution by weight [a]	
				d_v (μm)	σ (μm)
Griseofulvin	1.44	12.8	< 5	3.5	1.1
			10–15	9.6	3.3
Prednisolone acetate	1.28	18.0 [b]	< 5	3.2	1.1
			10–15	7.5	2.1

[a] Arithmetic normal distribution characterized by arithmetic mean and standard deviation.
[b] Estimated from literature value (Chiou, 1975).

Primary characterization of test materials

The primary characteristics of the untreated test materials and the wet sieve fractions are given in Table 1.

Density. The density was measured with an air comparison pycnometer (Beckman Mod. 930, USA). The results are the mean values of three determinations (Table 1).

Solubility. The solubility of griseofulvin in the media, a glutathione bicarbonate Ringer's (GBR) solution, was determined by adding an excess of drug to 500 ml of the media. The suspensions were agitated for 48 h at a constant temperature of 34–35 °C. After centrifugation and filtration through a 0.22 μm filter, the supernatant was assayed spectrophotometrically (Hitachi Model U-3200, Japan) at 295 nm.

Mean particle size and size distribution. The numbers of particles in 14 size classes were recorded by means of a Coulter Counter TA II and from these values the mean particle sizes and the particle size distributions of each fraction were calculated according to Nyström et al. (1985a).

The Coulter Counter was fitted with an aperture tube of 30 μm for the sieve fraction ≪ 5 μm and 70 μm for the fraction 10–15 μm. These aperture tubes were chosen to cover adequately the entire size distribution by weight.

Preparation of media

Glutathione bicarbonate Ringer's (GBR) solution was prepared in two parts. Part I consisted of sodium chloride (12.4 g/l), potassium chloride (0.716 g/l), monobasic sodium phosphate monohydrate (0.206 g/l) and sodium bicarbonate (4.908 g/l). Part II consisted of calcium chloride dihydrate (0.230 g/l), magnesium chloride hexahydrate (0.318 g/l) and oxidized glutathione (0.180 g/l) (O'Brien and Edelhauser, 1977; Camber, 1985). Both parts were stored cold and were used within 2 weeks to avoid microbial growth. Equal volumes of parts I and II were mixed prior to use.

Preparation of excised cornea

The excised eyes were packed in ice and transported quickly from the slaughterhouse to the laboratory. To facilitate dissection, the eye was placed in a specially designed holder. The cornea and a small ring of sclera tissue were carefully removed with the help of a pair of scissors and mounted in a perfusion apparatus (Camber, 1985). During this process, great care was taken to avoid damaging the cornea, and the epithelium was frequently moistened with GBR solution. After the cornea had been positioned in the apparatus, 1 and 6 ml GBR solution was added to the donor and receiving compartments, respectively. The apparatus was ready for use less than 1 h after slaughter of the pigs.

Corneal permeability

The perfusion apparatus, made of acrylic plastic, was placed on an electric hot plate adjusted to maintain a solution temperature of 34–35 °C. The temperature was checked periodically. The solutions in both compartments were agitated by bubbling a mixture of 95% oxygen and 5% carbon dioxide at a speed of approx. 3 bubbles/s through them, which also maintained a constant pH of 7.65 (Camber, 1985).

A portion of the GBR solution on the epithelial side (donor compartment) was replaced with an equal volume of stock suspension to give a final concentration of 50 or 80 μg/ml for griseofulvin and 150 μg/ml for prednisolone acetate. Samples of 200 μl were withdrawn from the receiving compartment every 40 min over a period of 4 h for analysis. Each volume was immediately replaced with an equal volume of GBR solution.

The apparent permeability coefficient (P_{app}) in cm/s was determined according to Eqn 1 (Kim et al., 1971; Schoenwald and Ward, 1980; Camber, 1985)

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot 60 \cdot A \cdot C_0} \quad (1)$$

where Q represents the total amount permeated (μg), t is the time (min), 60 corresponds to the conversion of minutes to seconds, $\Delta Q/\Delta t$ denotes the permeability rate (i.e. steady-state flux) (μg/min), A is the corneal surface area (in this study 1.33 cm^2), and C_0 represents the initial concentration of dissolved drug (μg/ml) in the donor compartment.

Partition coefficient

The octanol-water partition coefficients of the test materials were taken from the literature (Leo et al., 1971).

Methods of assay

Spectrophotometry. The amounts of griseofulvin and prednisolone permeated were determined spectrophotometrically (Hitachi Model U-3200, Japan). The UV absorbances were measured at 295 and 242 nm for griseofulvin and prednisolone acetate/prednisolone, respectively. Corrections for unspecific absorbance for compounds released from the cornea at the different time intervals were made at the given wavelengths.

HPLC determination. The amount of prednisolone permeating the excised pig cornea was also determined using HPLC. Samples withdrawn from the endothelial side were assayed using a variable-wavelength UV detector which was operated at 242 nm. The HPLC analyses were made with a reverse-phase 5 μm Lichrosorb RP-8 col-

umn (125 × 4 mm). The mobile phase consisted of methanol and water (1 : 1 v/v) and the flow rate was 1 ml/min. The retention times of prednisolone acetate and prednisolone were about 8.9 and 5.0 min, respectively. Separations were performed isocratically at room temperature.

Stability of prednisolone acetate

The stability of prednisolone acetate at 34–35 °C was studied as follows.

Stability in solution. A known amount of prednisolone acetate was dissolved in GBR solution and stored at a temperature of 34–35 °C for a period of 240 min. Thereafter samples were analysed by HPLC as described above.

Stability in suspension. A given volume of stock suspension was added to a volume of a GBR solution equilibrated in a water-bath at 34–35 °C. Samples (200 µl) were withdrawn every 40 min for 4 h and the volume immediately replaced by GBR solution. The sample was diluted with methanol and then analysed by HPLC.

Stability in the perfusion model. The perfusion apparatus was assembled as described above. Thereafter, 90 µl of the GBR solution in the donor compartment was replaced by an equal volume of prednisolone acetate stock suspension. Samples (100 µl) were withdrawn from the donor compartment, every 40 min for a period of 240 min and immediately replaced by 100 µl of GBR solution. The sample was diluted with methanol and then analyzed by HPLC.

Results

Particle size distribution of test materials

The efficiency of the wet sieving preparation is apparent from the mean particle sizes by weight and standard deviations presented in Table 1. However, all fractions of both griseofulvin and prednisolone acetate contained substantial amounts of particles smaller than the lower size limits used. Therefore the mean particle sizes by weight were close to or smaller than the lower nominal limits of each sieve fraction prepared.

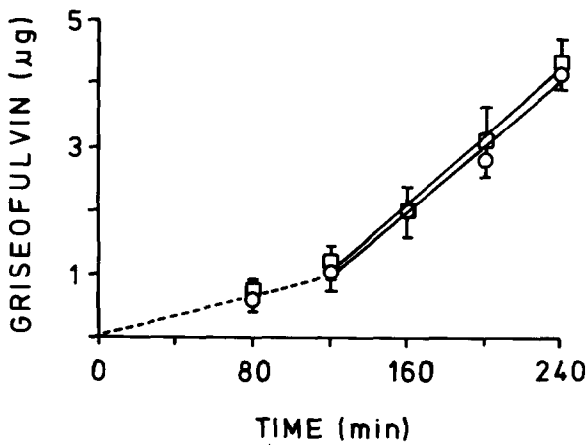

Fig. 1. Permeability of griseofulvin across excised pig cornea for wet sieve fraction (○) ≪ 5 µm and (□) 10–15 µm. The initial drug concentration used was 50 µg/ml. The points were fitted by regression analysis and each point represents the mean of 6 determinations. The vertical bars indicate the standard deviation.

Corneal permeability of griseofulvin

To determine the effect of particle size on the permeability through excised pig cornea, a study was conducted using two sieve fractions ≪ 5 µm and 10–15 µm, and two different concentrations (50 and 80 µg/ml) of griseofulvin.

Fig. 1 represents a cumulative plot of the amount of griseofulvin permeating the membrane (Q) vs time (t) for the sieve fractions used. The initial concentration in the donor compartment is 50 µg/ml.

A lag phase is observed before steady-state conditions are achieved. The lag time, obtained by extrapolation, for griseofulvin is 82 min. It represents the time required for drug to reach steady state in the cornea. After the lag time the concentration of griseofulvin in the receiving compartment increased linearly with time. The data points closely fit the least-square regression line once steady-state has been reached. This increase in the amount permeated is similar for both concentrations. The steady-state flux, i.e. permeability rate, is obtained from the slope of the linear portion of Fig. 1. The apparent permeability coefficient ($P_{app.}$) in cm/s was obtained by substituting the permeability rate ($\Delta Q/\Delta t$) value in Eqn 1.

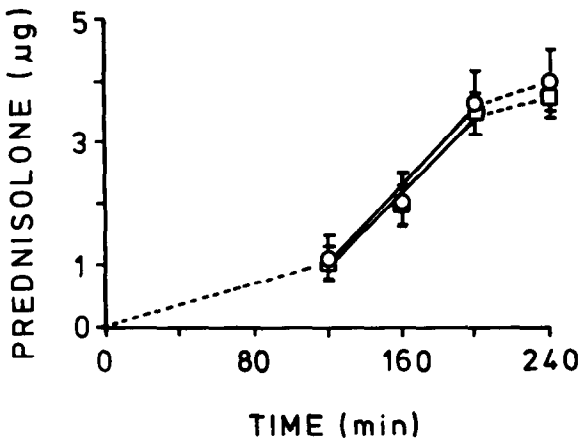

Fig. 2. Permeability of prednisolone acetate across excised pig cornea for wet sieve fraction (○) ≪ 5 µm and (□), 10–15 µm. The initial concentration used was 150 µg/ml. The points were fitted by regression analysis and each point represents the mean of 6 determinations. The vertical bars indicate the standard deviation.

The permeability coefficients of the two sieve fractions and the different concentrations of griseofulvin used are listed in Table 2.

Corneal permeability of prednisolone acetate

The plot of amount permeated vs time for the two sieve fractions of prednisolone acetate is shown in Fig. 2.

Only prednisolone could be detected in the receiving compartment, indicating that prednisolone acetate was fully hydrolyzed during passage through the cornea. The lag time for prednisolone acetate is 88 min. After the lag time the concentration of prednisolone on the endothelial side, steady state flux, increased linearly with time up to approx. 200 min. Thereafter a deviation from a linearity was observed.

The slope of the linear portion of the plot (permeability rate) was substituted in Eqn 1 to obtain the permeability coefficient of prednisolone acetate. The permeability coefficients of the different particle sizes of prednisolone acetate are given in Table 2.

As mentioned earlier the amount of prednisolone acetate/prednisolone permeating pig cornea was also determined by HPLC. The amount permeating was plotted vs time, as described previously and the permeability rate obtained was used to calculate the permeability coefficient (Table 2).

Stability of prednisolone acetate

To ensure that prednisolone acetate did not hydrolyse before passing through the cornea, three types of stability studies were undertaken.

The amount (percentage) of prednisolone acetate hydrolyzed in a solution kept at 34–35 °C for 240 min is shown in Table 3. In this study, maximally 31% of the total amount was hydrolyzed.

Table 3 also lists the amounts hydrolyzed in a suspension, maintained at 34–35 °C for 240 min. At most (after 240 min) 22% was hydrolyzed.

The stability of prednisolone acetate in the donor compartment of the perfusion apparatus was analysed every 40 min up to 4 h (Table 3). In this case, we could detect no prednisolone, since the amount of prednisolone hydrolyzed was also capable of permeating through the cornea. Fur-

TABLE 2

Permeability coefficients and some physico-chemical data of prednisolone acetate and griseofulvin

Materials	Sieve fractions (µm)	Concentration (µg/ml)	Permeability coefficient (cm/s) (×10^6)	Partition coefficient	Molecular weight
Griseofulvin	< 5	50	25.2	150	352.77
		80	25.9		
	10–15	50	26.1		
		80	26.6		
Prednisolone acetate	< 5	150	21.9	250	402.5
	10–15	150	21.1		
	< 5 [a]	150 [a]	19.6 [a]		

[a] HPLC data.

TABLE 3

Stability of prednisolone acetate

System	Time (min)	% Hydrolyzed
Solution	240	31
Suspension	0	0
	40	2.96
	80	5.26
	120	10.02
	160	13.93
	200	17.76
	240	21.71
Suspension (in donor compartment)	0–240	0

ther, the small amounts of prednisolone which could have been present in the withdrawn samples were undetectable due to dilution of the sample with methanol before analysis.

Discussion

The studies with this perfusion model did not reveal any significant difference in the permeability coefficients of the two fractions of griseofulvin or prednisolone acetate suspensions (Table 2; Figs 1 and 2).

The corneal permeability of drugs in this model is dependent on (a) concentration of dissolved drug molecules in the media, (b) diffusion of drug through the media, (c) partition (transfer) of drug from the media to the cornea (epithelium), and (d) permeation of drug through the cornea.

For any given concentration of drug, the last three parameters are not affected by the particle size of the suspension. Thus, for a given drug, only the concentration of the drug molecules in the media would affect the permeability of the different particle sizes used.

Although the surface specific dissolution rate (μg/min per cm^2) of sparingly soluble drugs increases with decreasing particle size especially for particles below approx. 5 μm (Bisrat and Nyström, 1988) Figs 1 and 2 show that the increase in dissolution rate due to the decrease in particle size has no influence on the permeability coefficient. This is due to the fact that in the perfusion model the permeability rate rather than the dissolution rate represents the rate-limiting step.

This could also be demonstrated by comparing the dissolution and permeability rates of the different fractions of griseofulvin (Table 4). The dissolution rate of the finer fraction is 7.0 μg/min and the permeability rate 0.02563 μg/min and for the fraction 10–15 μm the values are 2.8 μg/min and 0.02645 μg/min, respectively. The dissolution rate is thus 270 (sieve fraction < 5 μm) and 106 (sieve fraction 10–15 μm) times faster than the corresponding permeability rates and thereby cannot represent a rate-limiting step.

TABLE 4

Comparison of some physico-chemical parameters of two sieve fractions of griseofulvin

Sieve fraction (μm)	Harmonic mean diameter by weight [a] (μm)	Surface to volume shape [b] (–)	Specific surface area by weight [c] (cm^2/g)	Available surface area [d] (cm^2)	Surface specific dissolution rate [e] (μg/min per cm^2)	Maximum dissolution rate [f] (μg/min)	Permeability rate [g] (μg/min)
< 5	3.15	9.7	21 400	1.07	6.5	7.0	0.02563
10–15	7.93	9.7	8 500	0.43	6.5	2.8	0.02645

[a] Harmonic mean diameter by weight measured using the Coulter counter TAII.
[b] Surface to volume shape factor (Heywood, 1954).
[c] Calculated from harmonic mean diameter by weight and surface to volume shape factor according to Allen (1981).
[d] Surface area available when using 50 μg griseofulvin.
[e] Earlier reported data (Nyström et al., 1985b).
[f] Product of d and e.
[g] Slope of the permeated drug vs time plot (Fig. 1).

Samples taken from the donor compartment at the end of the experimental period contained, for all drug combinations tested, drug amounts higher than the drugs' saturation concentration (solubility). The amount of dissolved drug was thus constant during the entire experiment and was independent of particle size and initial concentration (i.e. total concentration composed of both dissolved and suspended drug). Since the experiments were performed under sink conditions, the driving force according to Fick's first law was constant. Thus, higher initial concentrations of griseofulvin (80 μg/ml) did not increase the amount of permeated drug on the endothelial side.

An important feature of prodrugs used as ocular delivery forms is their conversion to the parent pharmacologically active drug during passage through the cornea. It is well known that the cornea, particularly the epithelium, contains highly active enzymes including, for example, esterases (Redell et al., 1983). Prednisolone acetate is thus fully hydrolyzed during the passage through the cornea, and only prednisolone could be detected in the receiving compartment.

The prednisolone acetate is rapidly absorbed by the epithelium owing to its high partition coefficient and thereafter cleaved enzymatically to give the hydrophilic compound prednisolone, with a relatively high solubility in the stroma, facilitating the flux through that layer. However, it is noteworthy that after 200 min, the permeation curve deviates from a straight line. This may be ascribed to the very high uptake of prednisolone acetate in the epithelial layer. As the concentration of the suspension in the donor compartment is high (150 μg/ml) and since the partition coefficient of prednisolone acetate is high, the concentration of drug will be high in the superficial cell layers. Most of the esterase activity in the epithelium is due to butyrylcholinesterase, and as prednisolone acetate is not the optimal substrate, the tissue may become saturated. In this range of substrate concentrations, the reaction (deesterification) is zero order. Under these conditions the rate of production of prednisolone will be constant.

This study demonstrates that the particle size of sparingly soluble drugs does not influence the corneal permeability irrespective of whether they are prodrugs or stable drugs. Hui and Robinson (1986) claimed that an increase in total surface area would give an increased concentration of drug in the aqueous humor and therefore, they suggested that drug suspensions should be as fine as possible to optimize the ocular bioavailability. However, the results from this study suggest that the particle size has no significant influence on the ocular permeation.

The differences seen in vivo with the different particle sizes are obviously not linked to dissolution rate, and presumably other parameters such as clearance rate, individual tear flow or other physiological mechanisms govern the ocular bioavailability of sparingly soluble drugs.

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