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KINETICS OF CORNEAL DRUG UPTAKE STUDIED BY CORNEAL PERFUSION IN SITU I. EVALUATION OF SYSTEM AND UPTAKE OF ETHYL p-AMINOBENZOATE IN RABBITS

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SUMMARY

An in situ corneal perfusion system which enables quantitation of corneal drug uptake is described. By determining the decline in drug concentration in the perfusion apparatus as a function of time, it is possible to measure drug clearance from the system thereby obtaining a measure of corneal drug uptake. Using this method, the clearance from the system of ethyl p-aminobenzoate by rabbit corneas was investigated. The in situ perfusion system provides reproducible results and obviates many of the problems associated with isolated, in vitro corneal studies. This method can be utilized to study the effect of physical–chemical drug properties on corneal uptake, as well as in ocular pharmacokinetic modeling and dosage form evaluation.

INTRODUCTION

The cornea comprises the anterior one-sixth of the globe of the eye and is the membrane through which topically applied drugs pass in order to reach the interior of the eye. Although numerous in vitro investigations of corneal drug transport have been conducted (Donn et al., 1959; Ehlers and Ehlers, 1966; Green and Otori, 1970; Maurice, 1972; McCarey et al., 1973; O'Brien and Edelhauser, 1977; and Hull et al., 1974) the basic mechanism(s) of corneal drug uptake is still not well understood.

Benson (1974) has written an extensive literature review of corneal permeability and has considered various studies which attempt to explain the mechanism(s) involved. By and large, these studies have been in vitro investigations using isolated corneas from various animal species. Due to the complex nature of the corneal membrane, removal from its natural environment, causes changes in the properties of the membrane (Mishima

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and Kudo, 1967). Much effort, therefore, has been devoted to finding methods of maintaining the integrity of isolated corneas so that meaningful drug permeability studies can be conducted.

It seems reasonable that permeability studies conducted on the cornea in situ should avoid many of the problems associated with maintaining the integrity of isolated corneas. This report describes an in situ corneal perfusion system, its mathematical basis, and preliminary results on the corneal uptake of ethyl p-aminobenzoate. The data show that this perfusion system provides reproducible results and suggest that it should be a useful method to investigate corneal drug uptake under a variety of conditions.

MATERIALS AND METHODS

Ethyl p-aminobenzoate, obtained from J.T. Baker Chemical Co., was used as received. All other chemicals were of either reagent or analytical grade and were used as received. Ethyl p-aminobenzoate solutions were prepared (2×10^{-3} M) in isotonic Sorenson's phosphate buffer at pH 7.38. Solutions were prepared fresh for each experiment.

All rabbits were male New Zealand albinos, 58–66 days old. Prior to experimentation rabbits were housed in standard laboratory rabbit cages and allowed food and water ad libitum. In preparation for experimentation, rabbits were injected intramuscularly with a combination of ketamine hydrochloride (Ketaset, Bristol Laboratories), 35 mg/kg and xylazine (Rompun, Haver-Lockhart Laboratories), 5 mg/kg. Following this initial injection an i.v. infusion was started which delivered ketamine at a rate of 1.4 mg/min and xylazine at a rate of 0.20 mg/min. Intravenous infusions were administered into a marginal ear vein. This procedure was found to be successful in maintaining anesthesia for periods as long as 10 h.

A diagrammatic representation of the perfusion apparatus is shown in Fig. 1. The apparatus is a single piece of glass approximately 6 cm in length with a 13 mm teflon-

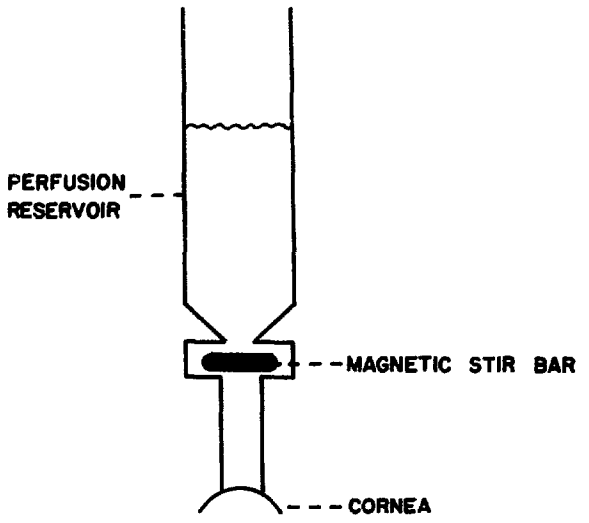

Fig. 1. Diagrammatic representation of in situ corneal perfusion system.

coated magnetic stir bar built into the system. The portion below the stir bar which was affixed to the cornea has an inner diameter of 6 mm.

Rabbits were anesthetized and placed on their side with the head horizontal such that one eye was exposed to the perfusion system. The lower rim of the perfusion system was coated with a thin film of a surgical adhesive (Aron Alpha, Toagosei Chemicals, Japan) and lowered onto the cornea. Care was taken that no adhesive came in contact with the area of the cornea being perfused. Once the apparatus was firmly in place, a magnetic stir plate was suspended directly above the system. The stirring rate was maintained just below the speed which would cause the solution to form a vortex. Such a procedure should minimize the thickness of a diffusion layer although such a layer would probably need to be accounted for in a more complex mathematical treatment. Although the total capacity of the apparatus is 4–5 ml, 2 ml of ethyl p-aminobenzoate solution was used in these experiments. Ten microliter samples were removed from the system at 0.25, 0.5, 1.0, 1.75, 2.5, 3.25, 4.0, 5.0 and 6.0 h after initiation of the experiment; therefore, the volume of the system changed by less than 5% during the course of an experiment. The system was run at ambient temperature (21 ± 1°C) and kept covered between sampling times in order to avoid evaporation. Future experiments will evaluate the influence of temperature on corneal drug uptake. After 6 h, rabbits were sacrificed and the perfusion apparatus was removed. Each sample was diluted with 1 ml of buffer, mixed, and read at 285 nm on a Cary 118 spectrophotometer.

To ensure that no protein or other foreign matter coming off the cornea would interfere with the spectrophotometric assay, the system was also run with only buffer. No interfering absorbance readings at 285 nm were obtained when this procedure was carried out for 6 h. It was also determined that ethyl p-aminobenzoate was not adsorbed onto the glass of the perfusion apparatus nor by the stir bar.

DISCUSSION

Assuming a well stirred system with a uniform concentration throughout, the rate of mass flux in the system can be described by:

$$-\frac{dCsVs}{dt} = Cls \cdot Cs \tag{1}$$

where Cs is the concentration of drug in the system, Vs is the volume of fluid in the reservoir and Cls is a clearance parameter describing the loss of drug from the system. Eqn. 2:

$$\int_{C\overset{\circ}{s}}^{Cs} \frac{dCs}{Cs} = -\frac{Cls}{Vs}\int_{0}^{t} dt \tag{2}$$

is the rearranged integral form of Eqn. 1 which can then be put into logarithmic form suitable for plotting;

$$\log Cs = \log C\overset{\circ}{s} - \frac{Clst}{Vs(2.303)} \tag{3}$$

TABLE 1

CORNEAL UPTAKE OF 2×10^{-3} M ETHYL p-AMINOBENZOATE IN RABBITS USING IN SITU CORNEAL PERFUSION

Experiment	Slope	Correlation coefficient	Clearance (ml/min)
1	-2.61×10^{-4}	0.91	1.20×10^{-3}
2	-2.97×10^{-4}	0.87	1.37×10^{-3}
3	-2.57×10^{-4}	0.97	1.18×10^{-3}
4	-2.53×10^{-4}	0.94	1.17×10^{-3}
		mean ± SE	$1.23 \quad 10^{-3} \pm 4.71 \times 10^{-5}$

By plotting the decline in concentration of the perfusate as a function of time, it is possible to determine the clearance of drug from the system. This clearance parameter is a measure of corneal drug uptake. Since the clearance parameter should be constant for a particular drug under a given set of experimental conditions, it can be seen that the slope of the decline in concentration with time is inversely proportional to the volume of drug solution contained in the reservoir. This theoretical relationship is similar to that originally described by Teorell (Teorell, 1937) for the transfer of drugs between compartments in the body.

The log absorbance versus time data was treated by linear regression analysis to determine the clearance parameter described by Eqn. 3 [1]. Four separate experiments using different rabbits were performed. Experiments were carried out for a period of 6 h. The results of these experiments are shown in Table 1 which illustrates the reproducibility of the in situ corneal perfusion system for calculating the uptake of drugs by the cornea.

Once the distribution volume in the interior of the eye is known for a particular drug, it will be possible to use the clearance parameter to calculate the rate of change of drug concentration within the eye assuming that all other precorneal disposition factors have been accounted for. Such distribution volumes can be experimentally determined using the method of Conrad (Conrad and Robinson, 1977). Furthermore, by manipulating the volume contained in the perfusion system, one can control the rate of decline in concentration in the perfusion system. By operating the system with a sufficiently small volume, clearance parameters for drugs which undergo very slow uptake can also be determined. This technique is currently being investigated.

Some of the properties which significantly affect the penetration of drugs into and through the cornea include molecular size, configuration, concentration, oil/water partition coefficient, degree of dissociation, surface activity, osmotic pressure, and reactivity with corneal tissue (Marzulli et al., 1967). With few exceptions, these factors have not been studied systematically, yet are essential to an overall understanding of ophthalmic

1 Although one may expect to see an initial lag time in the concentration vs. time profile, it must be very short since no lag time was observed with an initial sample taken at 15 min. This data was therefore treated in a monoexponential fashion.

drug therapy. The system described herein provides a convenient method for quantitating many of these factors and a basis upon which to assess the effect of chemical modification of drugs on their corneal uptake properties.

Furthermore, a recent ophthalmic pharmacokinetic study (Himmelstein et al., 1978) has shown that it is possible to mathematically model the distribution of drugs in the eye following their topical application. One term which is essential to such a model is the clearance of drug from the precorneal area of the eye due to corneal uptake.

In addition, differences in drug penetration in the eye as a function of age have been considered theoretically (Patton and Robinson, 1976) and demonstrated experimentally (Friedman and Patton, 1976). Structural changes in the corneal membrane as a function of age have the potential to contribute to the differences observed. The effects of such changes on drug penetration into the eye must be quantitated in order for appropriate dosage modifications to be made.

CONCLUSION

The results of the present investigation show that the in situ corneal perfusion system can provide useful information in quantitating and comparing the corneal uptake of drug substances. The method offers the advantage over isolated corneal studies in that the cornea is not removed from the eye so that problems in maintaining the integrity of the membrane are avoided.

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