International Journal of Pharmaceutics, 39 (1987) 113–120
Elsevier

IJP 01315

Ocular bioavailability of pilocarpic acid mono- and diester prodrugs as assessed by miotic activity in the rabbit

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(Received 23 January 1987)
(Modified version received 25 March 1987)
(Accepted 21 April 1987)

Key words: Pilocarpine prodrug; Miosis; Ocular bioavailability; Structure/response correlation

Summary

Following topical ophthalmic dosing of rabbits with pilocarpic acid diester and monoester prodrug solutions, significant biological activity was observed. The response, measured as pupillary diameter, vs time profiles, showed a slightly longer time requirement for attainment of maximal activity, a plateauing region of sustained response, and a longer duration of action as compared to pilocarpine. Several monoesters were capable of maintaining durations of action 1.5 times that of pilocarpine while the diesters were active for up to 2.25 times as long, and from half the dosing concentration. The profile shapes eliminate the early spiking response seen with higher doses of pilocarpine. The bioavailability, as assessed by response, of the prodrugs relative to pilocarpine is a balance between 3 factors: prodrug lipophilicity, the kinetics of conversion from diester to monoester to pilocarpine, and ocular clearance or elimination rates. The increased bioavailability (response vs time) of the diesters is primarily a result of their lipophilicity, with an optimum being seen. For the monoesters, the increase is dependent on the rate of the monoester to pilocarpine conversion. A linear correlation has been established between the monoester structures and the activities observed following their dosing, through the use of the Taft σ^* values for the alcohol alkyl moieties. For the diesters, an inverted V-shaped correlation exists between the partition coefficients of the prodrugs and their relative bioavailabilities, as calculated from response data. In both cases, considerable predictability of response from prodrug structure should be possible.

Introduction

Pilocarpine (1) (Scheme 1) a direct-acting cholinergic agent, is used topically in the eye to control the elevated intraocular pressure associated with glaucoma. It is widely used in spite of significant delivery problems. The drug shows low bioavailability due to poor absorption coupled with rapid elimination from its site of action in the eye. The resulting short duration of action necessitates its frequent administration (3–6 times/day). Such treatment regimens often lead to poor patient compliance, a contributing factor in inadequate pressure control and deterioration of vision (Norell and Granstrom, 1980; Norell, 1982). In addition, many patients experience undesirable

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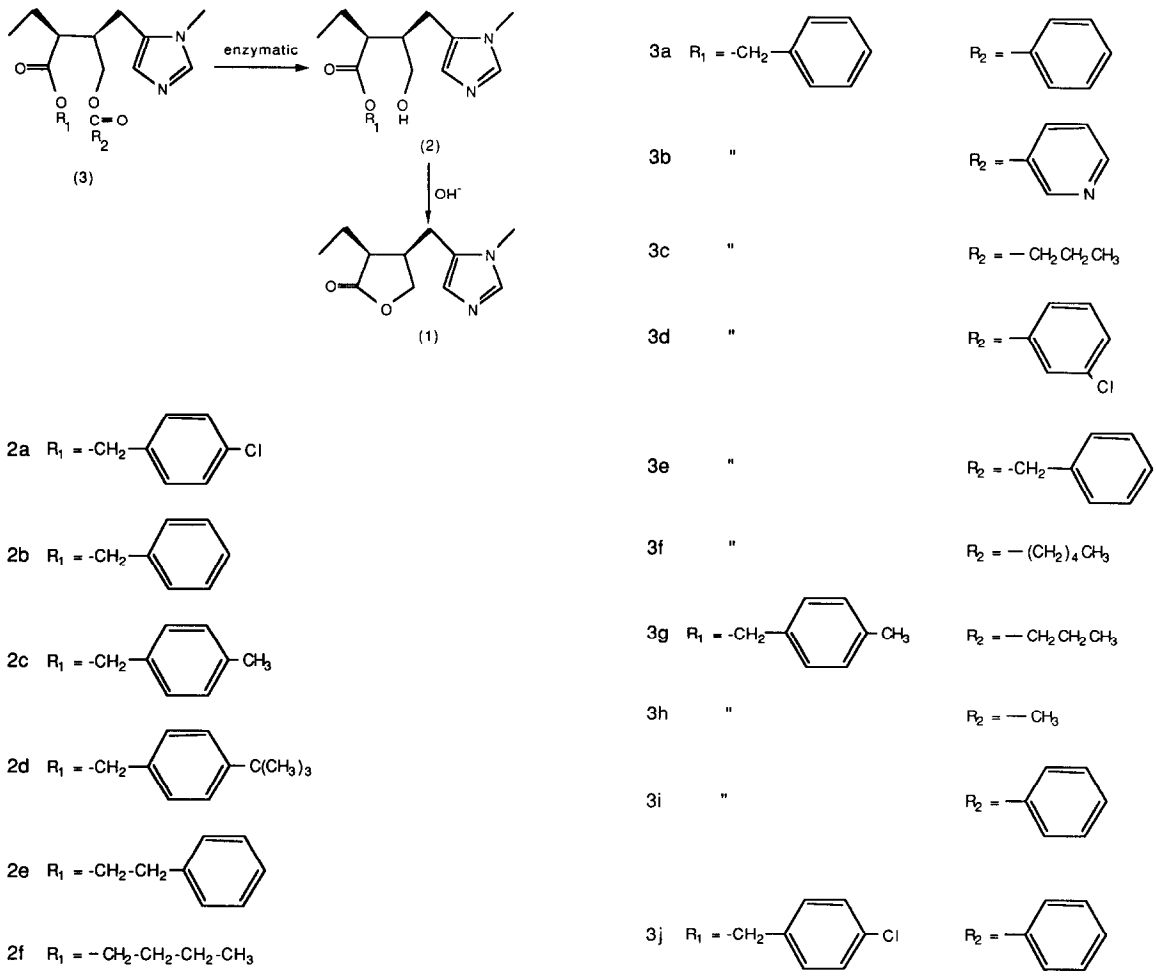

Scheme 1.

side effects such as myopia and miosis (Brown et al., 1976) as a result of the transient peaks of high drug concentration found in the eye following the frequent administration of massive amounts of pilocarpine. Also, since most of the applied drug is not productively absorbed and is available for systemic absorption from the naso lacrimal duct (Patton and Francoeur, 1978, Salminen et al., 1984), concerns about systemic toxicity arise.

One approach to overcome these delivery problems is the use of prodrug forms of pilocarpine. By altering the physicochemical properties of pilocarpine (e.g., lipophilicity), it appears likely that improvements in the rate and extent of absorption as well as duration of action are possible. In a preliminary paper (Bundgaard et al., 1985), we presented some early results to support this concept. Selected monoester (2) and diester (3) prodrugs of pilocarpic acid were shown to have greater bioavailablity and longer durations of miotic activity in rabbits than pilocarpine.

A previous report (Bundgaard et al., 1986a) demonstrated that the prodrug monoesters undergo a quantitative cyclization to pilocarpine in aqueous solution. However, the kinetics of the cyclization reaction prohibit the formulation of ophthalmic solutions having both acceptable shelf-life and pH. This problem is solved by blocking the free hydroxyl group in the pilocarpic acid monoesters with a second ester. The resultant

diesters are substantially more stable in aqueous solution but the second esters are easily and rapidly cleaved in the presence of the esterases located in ocular tissues (Bundgaard et al., 1986b) (see Scheme 1). Thus solution formulations can be prepared that have very long shelf-lives with "bioactivation" of the cyclization scheme occurring upon dosing.

The present work is a further evaluation of the observed biological activity as assessed by miotic response after topical ophthalmic dosing with solutions of numerous mono- and diester prodrugs. The relative bioavailability of each prodrug has been determined from response data and correlations are made, where appropriate, between the observed biological activities and the physicochemical properties of the prodrugs.

Materials and Methods

Materials

Preparation of the prodrugs has been described previously (Bundgaard et al., 1986a and b). The pilocarpine nitrate was purchased from Sigma Chemicals Co., St. Louis, MO. The rabbits used were male New Zealand Whites, purchased from Small Stock Industries, Inc., Pea Ridge, AR. No special diet was followed.

Solution preparation

Each prodrug or drug solution was prepared by dissolving the required amount in 1 ml of distilled water made isotonic with sodium chloride, and adjusting the final pH to 4.75 with 0.1 N HCl. The solutions were prepared equimolar to a 0.5%, 0.25% or a 0.125% solution of pilocarpine nitrate, depending on solubility and observed activity.

Miotic response

Evaluation of the observed miotic response following dosing of the prodrugs or drug was performed in adult male rabbits weighing 2.2–2.6 kg. Each rabbit selected for a particular study was placed in an upright position in a plastic restraining box located in a small quiet room with dim lighting. The animal was left in this environment for varying lengths of time from 0.5–2 h each day for 3 days in order to acclimate the rabbit to the restraints and the environment.

To perform each evaluation, the rabbit was set up as above, acclimated to the environment, and then the pupillary diameter of the eye to be used was measured with a cathetometer (Eberbach Corp., Ann Arbor, MI.) positioned 1 m from the corneal surface. Measurements were taken every 5 min until a steady baseline could be reached. The eye was then dosed with 25 μl of a drug solution, administered with a lambda pipet (Fisher Scientific, St. Louis, MO.). The drug was dosed onto the upper portion of the exposed globe and allowed to collect in the lower cul-de-sac. Since the rabbit's natural response to the dosing was to blink, no manual mixing or massage was utilized. The pupillary diameter was measured and recorded every 5 min with 3 quick consecutive measurements being taken and averaged for each time point, until the diameter returned to the baseline value.

The studies were set up in a 4×4 or a 5×5 cross-over design with pilocarpine nitrate being used as a control for pharmacological response in each study. Both eyes of the rabbits were used and a 48-h wash-out time was allowed for each eye between dosings.

Results and Discussion

Miosis, pupillary constriction, is one of the biological responses elicited by pilocarpine in the eye. It results from the direct action of the pilocarpine molecule on the muscarinic cholinergic receptors on the sphincter pupillae muscle of the iris (Ohara, 1978). The iris is not directly attached to the trabecular meshwork, the main site for drainage of aqueous humor, hence its state of contraction or relaxation does not necessarily affect outflow facility. Rather it is the ciliary body that primarily regulates outflow through the meshwork (Havener, 1983). The receptors on the iris and on the ciliary body are identical however, so miosis should give a reasonable measure of activity in the ciliary body.

Structure–activity relationship studies with pilocarpine analogs have shown 4 probable bind-

ing sites on the receptor; sites for the carbonyl and ether oxygens, a nucleophilic site for the protonated imidazole nitrogen, and a site for the ethyl group attached to the lactone (Aboul-Enein and Al-Badr, 1982). Optimal activity requires therefore, a protonatable imidazole (but not formally quaternized), correct stereochemistry of the ethyl group (isopilocarpine is inactive), and an intact γ-lactone ring. As a result the prodrugs, which all possess open lactone rings, are themselves inactive. They must undergo cyclization to pilocarpine without isomerization in order to be active.

A precise correlation has not been established for the rabbit between miosis and intraocular pressure drop, a result of facilitated outflow of aqueous humor through the trabecular meshwork. However, studies in beagles (Whitley et al., 1980) show a close correlation with intraocular pressure reduction lagging slightly behind in time relative to the miotic effect. Miosis is therefore used as an indication of biological response.

Monoester prodrugs

Exemplary miosis–time profiles following single topical doses of selected monoester prodrugs and pilocarpine nitrate are given in Fig. 1. The pilocarpine profile shows a rather rapid onset of action with maximal response being achieved in 20 min. This is followed by a continual drop in response over the remainder of the 120-min duration of action. A longer duration can be achieved by increasing the dose, but the maximal response will be likewise increased; the corresponding drug concentrations will enter a toxic range. As illustrated in Fig. 1, the monoester prodrugs can increase the duration of action 50% while avoiding the early spiking concentrations and the associated toxicities. The profiles for the prodrugs show a slower onset of action with a maximal response occurring after 40–50 min. A region of plateauing response is then observed, lasting for approximately 90 min followed by a significant drop in activity back to the baseline pupillary diameter. The duration of action is extended to 3 h.

Calculated areas under the response (miosis–time) curves, AURC, for the monoester prodrugs and pilocarpine nitrate are given in Table 1 along with some physicochemical data. The monoester prodrugs generally tend to have greater bioavailability in terms of AURC values than pilocarpine nitrate. The pilocarpic acid butyl ester (2f), however, elicited no response. Since the prodrugs must cyclize to pilocarpine in order to show activity, it was assumed that either the rate of cyclization for compound 2f was too slow or the generated pilocarpine did not reach the receptor. On the

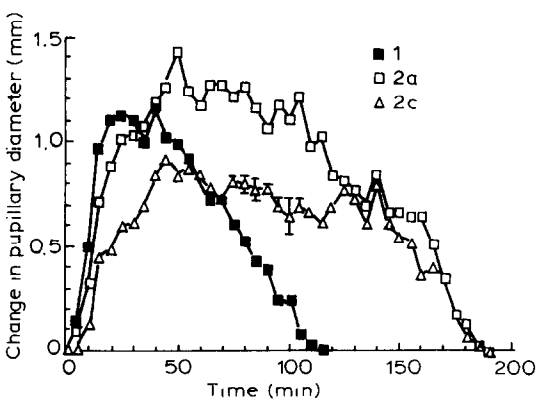

Fig. 1. Response, given as the change in pupillary diameter, as a function of time following a 25-μl topical ophthalmic dose of a monoester prodrug or pilocarpine nitrate. All solutions were prepared equimolar to 0.5% pilocarpine nitrate. Error when shown is S.E.M.

TABLE 1

AURC values and physicochemical data for selected monoester prodrugs (as the free base) and pilocarpine nitrate

Compound	AURC (mm min)	k_{chem} (min^{-1}) [a,c]	PC [b,c]
2a	160 (7.5, 6)	0.0231	347
2b	137 (11.2, 4)	0.0139	66
2c	110 (3.6, 4)	0.009	204
2d	90 (2.0, 4)	0.008	3310
2e	24 (16.4, 4)	0.003	186
2f	0	0.0008	145
1	77 (4.2, 14)	–	0.7

Data are from single 25-μl topical doses at a molar concentration equivalent to 0.5% pilocarpine nitrate. Numbers in parenthesis are S.E.M. and *n*. [a] Rate constant for the cyclization to pilocarpine at pH 7.40 and 37°C. [b] Partition coefficients between *n*-octanol and 0.05 M phosphate buffer at pH 7.40 and 22°. [c] From Bundgaard et al., 1986a.

basis of the partition coefficient data in Table 1, the prodrugs would all be expected to transport across the cornea and gain access to the receptor area. No correlation was found between AURC and the partition coefficient values which would explain the negligible AURC value for compound 2f.

A linear correlation was found, however, between the AURC values and log k for the cyclization reaction. This relationship is shown in Fig. 2 where the AURC values are seen to increase as the rate of cyclization increases. A least-squares linear regression of the data gives Eq. 1:

$$\text{AURC} = 157.3 \log k + 423.8 \quad (1)$$

This line intercepts the abscissa at a point corresponding to a cyclization rate constant of 0.002 min^{-1} or a half-time of cyclization of 5.7 h. No measurable activity should be seen for prodrugs with rate constants less than this value (e.g. compound 2f). A balance exists between the rate of cyclization to pilocarpine and the rates of clearance of the prodrugs from the eye. A slowly converting prodrug such as 2f is cleared from the eye before it can generate a significant concentration of pilocarpine. The enhanced bioavailability of the faster converting prodrugs over pilocarpine is a function of enhanced partitioning and corneal transport (to be published in a later report).

Eqn. 1 can be combined with a relationship

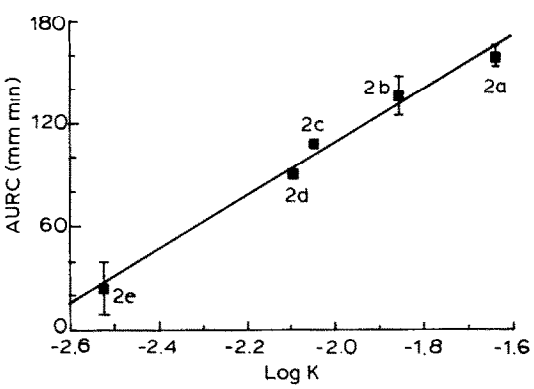

Fig. 2. AURC vs the log of the rate constant for the chemical conversion of the dosed monoester prodrug to the active species, 1, at pH 7.40 and 37 °C. Error is S.E.M.

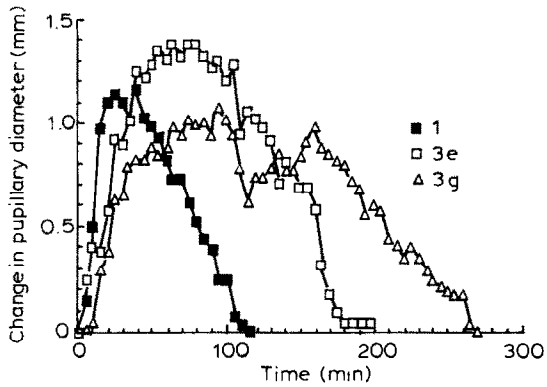

Fig. 3. Response, given as the change in pupillary diameter, as a function of time, following a 25-μl topical ophthalmic dose of a diester prodrug or pilocarpine nitrate. The prodrugs, prepared from their fumarate salts, were equimolar to 0.25% pilocarpine nitrate, while the pilocarpine data are from a 0.5% solution.

discovered between log k and the Taft substituent constant values (σ^*) for the R_1 promoieties (Bundgaard et al., 1986a), to give an equation relating activity to structure (Eqn. 2):

$$\text{AURC} = 225\ \sigma^* - 30.8 \quad (2)$$

From this equation it is now possible to predict the biological response of potential monoester prodrugs from the Taft σ^* values of the proposed R_1 substituent groups.

Diester prodrugs

The pilocarpic acid diester prodrugs generated even greater miotic response than the monoesters. Due to lower solubility and increased potency, the solutions used for these studies were at molar equivalents to 0.25% or 0.125% pilocarpine nitrate. Representative response profiles for several diesters and the pilocarpine nitrate reference are given in Fig. 3. The diesters, at the reduced concentration, show greater activity than the monoesters at a higher dosing concentration. The duration of action of the diesters is as much as 2.25 times that of pilocarpine, with an onset of action and time of maximal response only slightly later than the monoesters. The maximal responses were quite similar to the monoesters, so an even longer dura-

tion of action has now been achieved, again with avoidance of high early concentrations and with half the initial dosing concentration.

The AURC values for these compounds and for several other diesters are presented in Table 2 along with the dosing concentrations used. All derivatives show improvement in bioavailability over pilocarpine (Table 1) with the exception of 3b.

In order to compare the effects of the R_2 substituents on the response data, the relative activity, A_{rel}, is defined by Eqn. 3 to be:

$$A_{rel} = \frac{\text{diester AURC}}{\text{corresponding monoester AURC}} \quad (3)$$

where the corresponding monoester is the prodrug formed after enzymatic removal of the R_2 moiety. Had the AURC values been AUC values, ie. concentration instead of response, A_{rel} would be equivalent to F_{rel}, the relative bioavailability.

$$F_{rel} = \frac{\text{diester AUC}}{\text{corresponding monoester AUC}} \quad (4)$$

Since the AURC data in Table 2 are from various dosing solution concentrations and the monoester data in Table 1 are from 0.5% data, a normalization is required. A simplistic approach would be to assume a linear relationship between dosing concentration and response, thus a doubling of the dosing concentration should double the AURC. However, response is not a linear function of drug concentration at the receptor site. In addition, intraocular pilocarpine concentrations (the active species) may or may not be linearly related to the diester dosing concentrations because of the transport and bioconversion processes that must first occur. As a result, the normalization approach used first involves a conversion of the response data to effective aqueous humor pilocarpine concentrations through the use of the equation:

$$R = \frac{R_{max} \times C^s}{(RC_{50})^s + C^s} \quad (5)$$

where R is response, R_{max} and RC_{50} are the maximal response and concentration at half-maximal response respectively, C is the concentration of drug and s is a sensitivity factor (Wagner, 1968). The values used for R_{max}, RC_{50} and s, (2.49 mm, 0.98 μg/ml, and 1.792 respectively) had been determined for aqueous humor pilocarpine concentrations and their corresponding miotic responses (Mitra, 1983).

The concentration values were then normalized in a non-linear fashion to what would be expected if 0.5% equivalent solutions were used for the dosing instead of 0.125% or 0.25%. This amounted to a multiplication factor of 3.60 for the 0.125% data and 1.85 for the 0.25% data. These values were determined from a comparison of pilocarpine concentration values in aqueous humor following dosing of rabbits with pilocarpine solutions of differing concentrations (Mitra, 1983). Since these factors were determined for pilocarpine solutions, their use for diester data assumes (1) that the enzymatic conversion of diester to monoester is not rate-controlling, and (2) that the absorption and transport of the esters and pilocarpine are mechanistically the same. In intraocular disposition studies in rabbits, the rates of diester to monoester enzymatic conversion were shown to be sufficiently great so that no diester crossed the cornea intact and none could be found in intraocular tissues following dosing (Mosher, 1986). These results were further substantiated

TABLE 2

AURC values for selected diester prodrugs as fumarate salts following a single 25-μl topical dose

Compound	Conc. (%) *	AURC (mm min)
3a	0.24	245 (24.4)
3b	0.25	55 (8.5)
3c	0.125	89 (10)
3d	0.25	131 (35)
3e	0.25	163 (7.3)
3f	0.125	71 (5.2)
3g	0.25	174 (7.8)
3h	0.50	106 (9.5)
3i	0.50	250 (25.2)
3j	0.125	114 (13.7)

Numbers in parenthesis are S.E.M. with $n = 4$.

* The prodrug solution was equimolar to this concentration of pilocarpine nitrate.

with transport studies across the isolated corneas of rabbits, where no transport of the diesters could be observed but substantial amounts of the corresponding monoesters appeared in the receptor cells (Mosher, 1986). Comparisons of the diester dosing concentrations with the apparent Michaelis parameters, k_m and V_{max}, for the enzymatic conversion of diester to monoester (Bundgaard, 1986b; Mosher, unpublished data) also indicated extremely rapid bioconversion rates, limited by V_{max} for the dosing concentrations. It was concluded from these studies that corneal transport, not the rate of enzymatic bioconversion, is rate-limiting in determining the appearance of monoester inside the eye. The corneal transport studies also showed the monoesters and pilocarpine to both follow Fickian diffusion (the diester were converted to monoesters and not transported) and precorneal loss studies with the esters showed no anomalous behavior relative to pilocarpine (Mosher, 1986). The normalization was therefore felt to be valid for the correlations drawn. The 0.5% equivalent monoester response (AURC) data was also converted to pilocarpine concentration (AUC) data with equation 5, thereby eliminating any effects from the monoester to pilocarpine conversion kinetics when the diester and monoester data were compared.

Equation 4 was used with the calculated diester and monoester AUC data to generate apparent F_{rel} values for the diesters. Correlations were attempted between the apparent F_{rel} values and physicochemical parameters of the diester prodrugs. A general trend toward increasing bioavailability was observed as the rate of enzymatic conversion (Bundgaard et al., 1986b; Mosher, unpublished data) from diester to monoester increased but the correlation was poor. When F_{rel} was plotted against the log of the partition coefficients (PC) of the diester prodrugs an interesting relationship was found. This plot is given in Fig. 4 and shows that an optimal partition coefficient exists around log PC = 4.2–4.4, where the relative bioavailability is at a maximum. Both larger and smaller partition coefficients lead to a reduction in bioavailability. The broken line in the figure is only intended for visualization of what this trend might be.

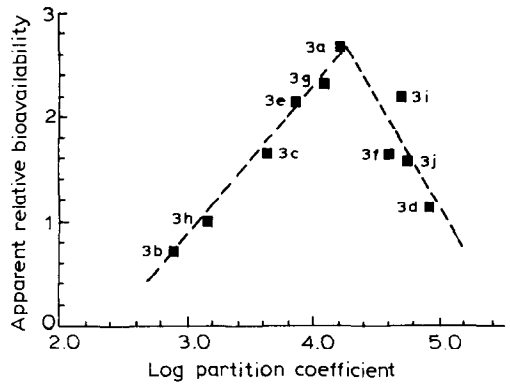

Fig. 4. Apparent relative bioavailability of the diester prodrugs as a function of the log of their *n*-octanol/pH 7.40 buffer partition coefficients. The line depicts a possible trend.

Contrary to the results of the monoesters, the diesters show a balance between bioavailability and lipophilicity. The rates of enzymatic conversion to the monoesters are sufficiently great such that intraocular elimination pathways for the diester play a negligible role in their overall bioavailability.

References

Aboul-Enein, H.Y. and Al-Badr, A.A., Prognosis of the pilocarpine receptor sites. *Meth. Find. Exptl. Clin. Pharmacol.*, 4 (1982) 321–329.

Brown, H.S., Meltzer, G. Merrill, R.C., Fisher, M., Ferre, C. and Place, V.A., Visual effects of pilocarpine in glaucoma. *Arch. Ophthalmol.*, 94 (1976) 1716–1719.

Bundgaard, H., Falch, E., Larsen, C., Mosher, G.L. and Mikkelson, T.J., Pilocarpic acid esters as novel sequentially labile pilocarpine prodrugs for improved ocular delivery. *J. Med. Chem.*, 28 (1985) 979–981.

Bundgaard, H., Falch, E., Larsen, C. and Mikkelson, T.J., Pilocarpine prodrugs I. Synthesis, physicochemical properties and kinetics of lactonization of pilocarpic acid esters. *J. Pharm. Sci.*, 75 (1986) 36–44.

Bundgaard, H., Falch, E., Larsen, C. Mosher, G.L. and Mikkelson, T.., Pilocarpine prodrugs II. Synthesis, stability, bioconversion and physicochemical properties of sequentially labile pilocarpic acid diesters. *J. Pharm. Sci.*, 75 (1986b) 775–783.

Havener, W.H., *Ocular Pharmacology*, 5th edn., Mosby, St. Louis, MO, 1983, p. 326.

Mitra, A.K., *Passive and Facilitated Transport of Pilocarpine Across the Corneal Membrane of the Rabbit*, Ph.D. Thesis, University of Kansas, 1983.

Mosher, G.L., *Theoretical and Experimental Evaluation of Pilocarpine Prodrugs for Ocular Delivery,* Ph.D. Thesis, University of Kansas, 1986.

Norell, S.E. and Granstrom, P.A., Self medication with pilocarpine among outpatients in a glaucoma clinic. *Br. J. Ophthalmol.,* 64 (1980) 137–141.

Norell, S.E., Pilocarpine t.i.d. — how is it taken? *Pharm. Int.,* 3 (1982) 123–125.

Ohara, K., Action sites of some cholinergic agonists on the isolatled iris sphincter muscles of rabbits. *Jpn. J. Ophthalmol.,* 22 (1978) 280–285.

Patton, T.F. and Francoeur, M., Ocular bioavailability and systemic loss of topically applied ophthalmic drugs. *Am. J. Ophthalmol.,* 5 (1978) 225–229.

Salminen, L., Urtti, A. and Periviita, L., Effects of ocular pigmentation on pilocarpine pharmacology in the rabbit eye. I. Drug distribution and metabolism. *Int. J. Pharm.,* 18 (1984) 17–24.

Wagner, J.G., Kinetics of pharmacological response 1. Proposed relationship between response and drug concentration in the intact animal and man, *J. Theoret. Biol.,* 20 (1968) 173–201.

Whitley, R.D., Gelath, K.N. and Gumm, G.G., Dose response of topical pilocarpine in the normotensive and glaucomatous beagle. *Am. J. Vet. Res.,* 41 (1980) 417–424.