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Communications to the Editor

Pilocarpic Acid Esters as Novel Sequentially Labile Pilocarpine Prodrugs for Improved Ocular Delivery

Sir:

Although pilocarpine (1) is widely used as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma, the drug presents significant delivery problems. Its ocular bioavailability is very low; only 1–3% or less of an instilled pilocarpine dose gains access to the internal eye structures.[1-3] This poor bioavailability has been predominantly attributed to rapid loss of the drug from the precorneal area via drainage, nonproductive conjunctival absorption and vasodilation due to the drug in conjunction with poor permeability across the corneal membrane.[4-6] The poor ability of pilocarpine to permeate the cornea may most likely be ascribed to the low lipophilicity of the drug. Because of the low bioavailability, a large ophthalmic dose is required to enable an effective amount of pilocarpine to reach the inner eye receptors and affect an intraocular pressure reduction over a suitable duration. This in turn gives rise to concern about systemic toxicity since most of the applied drug is then available for systemic absorption from the nasolacrimal duct.[7,8] The systemic absorption of pilocarpine may lead to undesired side effects, e.g., in those patients who display sensitivity to cholinergic agents.

Another delivery problem associated with pilocarpine is its short duration of action. Upon instillation into the eye, the duration of lowering of the intraocular pressure caused by pilocarpine lasts only for about 3 h. As a consequence, the frequency of administration is at an inconvenient 3–6 times/day. Patient compliance with such treatment regimens is poor, and failure to comply is likely to contribute to inadequate pressure control and deterioration of vision.[9,10] Furthermore, the frequent administration of massive amounts of pilocarpine is associated with transient peaks of high drug concentration in the eye, which in turn results in undesirable side effects such as myopia and miosis.[11]

Table I. Properties of Various Pilocarpic Acid Esters 2

compd	R	σ^{*a}	$t_{1/2}$,[b] min	log P[c]
2a	C_2H_5	0.00	510	0.58
2b	n-C_4H_9	−0.12	820	1.58
2c	n-C_6H_{11}	−0.23	1105	2.56
2d	$C_6H_5CH_2$	0.75	50	1.82
2e	4-$ClC_6H_4CH_2$	0.87	30	2.54
2f	4-$(CH_3)C_6H_4CH_2$	0.59	77	2.31
2g	4-(t-C_4H_9)$C_6H_4CH_2$	0.52	87	3.52
2h	$C_6H_5CH_2CH_2$	0.27	227	2.16
2i	2-$(CH_3)C_6H_4CH_2$	0.62	139	2.27
2j	$C_6H_5CH(CH_3)$		475	2.08

[a] The Taft polar substituent constant σ^* refers to R minus CH_2. [b] Half-lives of cyclization to pilocarpine at pH 7.40 and 37 °C. [c] Apparent partition coefficients between octanol and 0.05 M phosphate buffer solution of pH 7.40; the log P value for pilocarpine was found to be −0.15.

These shortcomings of pilocarpine may probably be overcome by the prodrug approach. To be successful, a pilocarpine prodrug should exhibit a high lipophilicity in order to enable a more efficient penetration through the corneal membrane, should posses sufficient aqueous solubility and stability for formulation as eye drops, should be converted to the active parent drug within the cornea or once the corneal barrier has been passed, and finally should lead to a controlled release and hence prolonged duration of action of pilocarpine. In this paper we report that pilocarpic acid esters, especially pilocarpic acid diesters, may be promising pilocarpine prodrugs with these desirable attributes.

Pilocarpic Acid Monoesters. A series of alkyl and aralkyl esters of pilocarpic acid (**2**)[12] was synthesized by reacting the sodium salt of pilocarpic acid (obtained by hydrolysis of pilocarpine with sodium hydroxide[13]) with the appropriate alkyl or aralkyl halide in dimethylformamide solutions. The pilocarpic acid esters **2** were shown to function as prodrugs of pilocarpine (**1**) both in vitro and in vivo. In aqueous solution the esters undergo a quantitative and apparent specific-base-catalyzed lactonization to pilocarpine.[14] The cyclization most likely involves

(1) Asseff, C. F.; Weisman, R. L.; Podos, S. M.; Becker, B. *Am. J. Ophthalmol.* **1973**, *75*, 212.
(2) Chrai, S. S.; Robinson, J. R. *Am. J. Ophthalmol.* **1974**, *77*, 735.
(3) Patton, T. F. *J. Pharm. Sci.* **1977**, *66*, 1058.
(4) Lee, V. H. L.; Robinson, J. R. *J. Pharm. Sci.* **1979**, *68*, 673.
(5) Chrai, S. S.; Patton, T. F.; Mehta, A.; Robinson, J. R. *J. Pharm. Sci.* **1973**, *62*, 1112.
(6) Thombre, A. G.; Himmelstein, K. J. *J. Pharm. Sci.* **1984**, *73*, 219.
(7) Patton, T. F.; Francoeur, M. *Am. J. Ophthalmol.* **1978**, *85*, 225.
(8) Salminen, L.; Urtti, A.; Periviita, L. *Int. J. Pharm.* **1984**, *18*, 17.
(9) Norell, S. E.; Granström, P.-A. *Br. J. Ophthamol.* **1980**, *64*, 137.
(10) Norell, S. E. *Pharm. Int.* **1982**, *3*, 123.
(11) Brown, H. S.; Meltzer, G.; Merrill, R. C.; Fisher, M.; Ferre, C.; Place, V. A. *Arch. Ophthalmol.* **1976**, *94*, 1716.

(12) Elemental analysis (C, H, and N) for all new compounds were within 0.4% of theoretical values. ^1H NMR and IR spectra were consistent with the assigned structures.
(13) By performing the hydrolysis at 0–4 °C, the simultaneous formation of isopilocarpic acid sodium salt was depressed. The product obtained contained about 10% of this isomer and proved satisfactory as a starting material for the synthesis of optically pure pilocarpic acid esters.
(14) The rates of cyclization were followed by HPLC methods, including a procedure capable of separating pilocarpine and isopilocarpine: Bundgaard, H.; Hansen, S. H. *Int. J. Pharm.* **1982**, *10*, 281.

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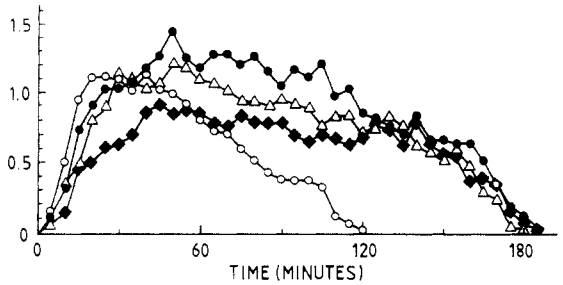


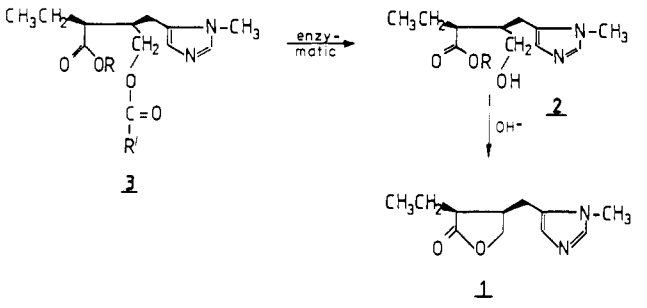
Figure 1. Plots of the average observed changes in pupillary diameters (ΔPD, in millimeters) as a function of time following the instillation of 25 μL of isotonic aqueous solutions (pH 4.75) of pilocarpine nitrate (○), **2e** (●), **2d** (△), and **2f** (♦) in equimolar concentrations (18.4 mM, corresponding to 0.5% pilocarpine nitrate equivalent). Four rabbits were used in the crossover study. The standard deviations for the data points were <15%.

preequilibrium ionization of the hydroxy group and intramolecular nucleophilic attack of alkoxide ion on the ester carbonyl moiety. In accordance with this mechanism, which is similar to that previously suggested for the cyclization of 4-hydroxybutyric acid esters to α-butyrolactone,[15] the rate and extent of the lactonization were found to be unaffected by the presence of human plasma or rabbit tissue eye homogenates at pH 7.4 and 37 °C.

As it appears from the rate data obtained (Table I), the various esters differ greatly in their rates of cyclization. Except for the sterically hindered 2-methylbenzyl and α-methylbenzyl esters, the variation of the rates of lactonization of these ester derivatives could be fully accounted for in terms of polar effects exhibited by the alcohol portions of the esters. The following correlation was found between the half-time (in minutes) of pilocarpine formation from these esters at pH 7.4 and 37 °C and the Taft polar substituent constant σ*, the latter referring to R in RCH_2OH for the alcohols: $\log t_{1/2} = 1.44\sigma^* + 2.73$ ($n = 8$; $r = 0.998$). It is readily evident that by appropriate variation of the alcohol portion of the esters there are ample possibilities to vary and predict the rate of ring closure and hence to control and modify the rate of pilocarpine generation.

The ocular bioavailability and pharmacological activity of the prodrugs were assessed in terms of the miotic activity resulting from topical ocular instillation in male albino rabbits. The miotic activity was quantitated by measurement of pupillary diameter as a function of time as previously described.[16] The studies were of a 4 × 4 or 5 × 5 crossover design, and pilocarpine was employed in each crossover as a pharmacological activity reference. The compounds were administered as aqueous solutions (25 μL), made isotonic with NaCl and with pH being adjusted to 4.75. The changes observed in pupillary diameter from the base-line value as a function of time for several prodrugs and pilocarpine are shown in Figure 1. It is readily seen that the effect–time course for the prodrugs is significantly altered in comparison to pilocarpine in that the onset of effect is delayed, the duration of effect is lengthened from 2 h to about 3 h without increasing the peak intensity of the miotic response and the extent of ocular bioavailability, as evaluated in terms of areas under the curves, is increased. Analysis of the data for all the derivatives revealed that the main physicochemical property influencing the magnitude and duration of the miotic activity for the pilocarpic acid esters was the rate of the chemical prodrug-to-drug conversion, the differences in the

Scheme I



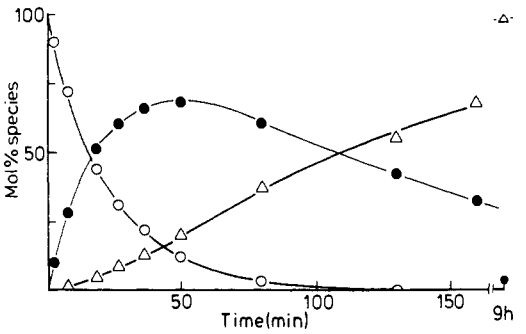


Figure 2. Time course for *O*-benzylpilocarpic acid 4-methylbenzyl ester (**3**, R′ = C_6H_5, R = 4-$(CH_3)C_6H_5CH_2$) (○), pilocarpic acid 4-methylbenzyl esters **2f** (●), and pilocarpine (△) during incubation of the *O*-benzoyl derivative in 75% human plasma (pH 7.4) at 37 °C. The concentration of each compound at various times was determined by HPLC after deproteinization of the plasma samples with 5 parts of ethanol. The half-life of **3** is 16 min whereas the half-time of conversion of **2f** to pilocarpine is 80 min.

lipophilicity (see Table I) of the derivatives being less important. Increased rates of cyclization of the esters resulted in increased activity and hence in increased bioavailability of the parent pilocarpine. The pilocarpic acid esters per se had no miotic activity.

While some of these pilocarpic acid esters possess promising properties in terms of pilocarpine delivery, they suffer from inadequate solution stability at physiological pH. Although the stability of the monoesters is improved at low pH (e.g., pH 3), premade or ready-to-use solutions with acceptable shelf-lives require intolerably low pH. This problem can, however, be overcome by esterification of the free hydroxy group in the monoesters.

Pilocarpic Acid Diesters. The pilocarpic acid diesters (**3**) obtained[17] proved to be highly stable in aqueous solutions even at pH 5–6 (the shelf-lives of such solutions being greater than 5 years at 25 °C) and, most significantly, to be subject to facile enzymatic hydrolysis at the *O*–acyl bond. It has thus been demonstrated that in the presence of human plasma[18] or rabbit eye tissue homogenates pilocarpine is formed from these derivatives in quantitative amounts through a sequential process involving enzymatic hydrolysis of the *O*–acyl bond followed by the spontaneous lactonization of the intermediate pilocarpic acid ester (Scheme I, Figure 2). Thus, the half-times for the conversion of various *O*-benzoyl and *O*-butyryl esters (**3**) to

(15) Bundgaard, H.; Larsen, C. *Int. J. Pharm.* **1980**, *7*, 169.
(16) Mitra, A. K.; Mikkelson, T. J. *Int. J. Pharm.* **1982**, *10*, 219.
(17) Compounds **3** were prepared by treating the monoesters **1** with the appropriate acid chloride in toluene solutions in the presence of potassium carbonate. The compounds were isolated as crystalline fumarate or nitrate salts.
(18) Human plasma has previously been shown to be a good model of ocular tissue enzymes for ester hydrolysis: (a) Hussain, A.; Truelove, J. E. *J. Pharm. Sci.* **1976**, *65*, 1510. (b) Anderson, J. A.; Davis, W. L.; Wei, C.-P. *Invest. Ophthalmol. Vis. Sci.* **1980**, *19*, 817. (c) Redell, M. A.; Yang, D. C.; Lee, V. H. L. *Int. J. Pharm.* **1983**, *17*, 299.

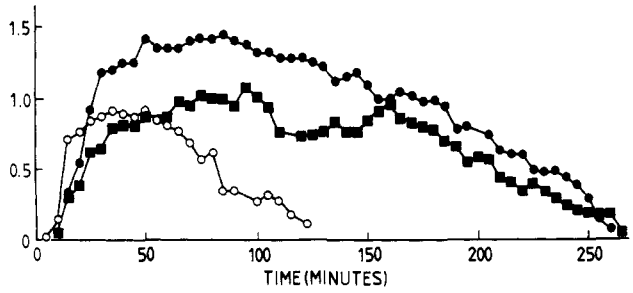


Figure 3. Plots of the average observed changes in pupillary diameter (ΔPD, in mm) as a function of time following the installation of 25 μL of isotonic aqueous solutions (pH 4.75) of pilocarpine nitrate (○), **3a** (R′ = C_6H_5, R = $C_6H_5CH_2$) (●), and **3b** (R′ = *n*-C_3H_7, R = 4-$(CH_3)C_6H_5CH_2$) (■) in equimolar concentrations (9.2 mM, corresponding to 0.25% pilocarpine nitrate equivalent). Four rabbits were used in the crossover study. The standard deviations for the data points were <15%.

pilocarpic acid monoesters (**2**) were found to be 3–17 min in 75% human plasma solutions at 37 °C, which may be compared to half-times of about 5×10^3 h in pH 7.4 buffer solutions at 37 °C. Besides solving the stability problem of the pilocarpic acid monoesters, the cascade latentiated derivatives or pro-prodrugs were found to possess even better ocular delivery characteristics (enhanced absorption and longer lasting pilocarpine activity) than the monoesters (Figure 3). In comparing Figures 1 and 3, the concentration difference should be noted. As can be seen from Figure 3 the activity profiles produced by some diesters are not very dissimilar to those attainable from an apparent zero-order delivery device. In addition, the *O*-acylation step gives further possibilities of varying the physicochemical properties (e.g., lipophilicity) of the prodrugs. Although the pilocarpic acid diesters prepared are very lipophilic at physiological pH (e.g., log *P* for the *O*-benzoyl ester of **2d** being 4.22 at pH 7.4), the basic character of the imidazole moiety in the compounds ($pK_a \sim 7.0$) allows the preparation of sufficiently water-soluble salts, e.g., nitrates. Studies are in progress to delineate the main factors being responsible for the greater and prolonged activity of the diesters.

In conclusion, pilocarpic acid esters are shown to be potentially useful prodrugs of pilocarpine with the aim of improving its ocular delivery characteristics. The diesters are of particular interest since they combine an enhanced bioavailability and greatly prolonged duration of activity with a high stability in eye-drop formulation. The pro-prodrug or sequential cascade concept utilized in the design of these derivatives, involving an enzymatic cleavage mechanism prior to the occurrence of a chemical (nonenzymatic) reaction, may also be of more general interest in prodrug design.

Registry No. 1, 92-13-7; **2a**, 96914-10-2; **2b**, 92598-80-6; **2c**, 96914-11-3; **2d**, 92598-82-8; **2e**, 92598-89-5; **2f**, 92598-93-1; **2g**, 92598-99-7; **2h**, 92598-86-2; **2i**, 92598-92-0; **2j**, 92598-81-7; **3**, 92598-94-2; **3a**, 92598-84-0; **3b**, 92598-95-3; sodium pilocarpate, 92598-79-3.

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