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Miotic effect and irritation potential of pilocarpine prodrug incorporated into a submicron emulsion vehicle

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Abstract

Pilocarpine prodrug, *O,O′*-dipivaloyl(1,2-ethylene) bispilocarpic acid diester, was introduced to a submicron emulsion vehicle in a dose equivalent to 0.5% pilocarpine base, and the formulation was studied in albino rabbits using miotic assay. Compared with pilocarpine HCl 0.5% solution delayed and prolonged miosis was observed after application of the prodrug emulsion. $AUC_{0-6\ h}$ values for the prodrug emulsion and pilocarpine solution were 9252 ± 1345 and $6845 \pm 1967\% \times$ min, respectively. The prodrug was also administered twice daily for 5 days in the form of aqueous solution or submicron emulsion in order to study ocular irritation. Irritation potential of the prodrug was significantly reduced when submicron emulsion was used as a vehicle.

Keywords: Pilocarpine; Prodrug; Submicron emulsion; Miotic effect; Irritation

1. Introduction

Ocular bioavailability of topically applied pilocarpine is only 0.1–3% (Chrai and Robinson, 1974; Lazare and Horlington, 1975) and the drug must be administered as eye-drops three to four times per day which impairs patient compliance (Kass et al., 1986). The poor bioavailability is attributed to the low lipophilicity of pilocarpine as well as to rapid loss of the drug from the precorneal area via drainage and conjunctival absorption. Eye-drops are formulated as aqueous solutions at pH 4–5.5, because this range of pH is optimal for chemical stability of pilocarpine. However, in such formulations the drug is present in ionized form what is not advantageous for the ocular penetration. The low bioavailability of pilocarpine can be improved by extending drug residence time in conjunctival sac. That was achieved by using viscosity increasing agents, nanoparticles or inserts (Mitra, 1993).

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Another approach to improve ocular bioavailability of pilocarpine is to enhance drug permeability across cornea by altering physicochemical properties of the drug. Lipophilicity, solubility and pK_a can be changed by prodrug derivatization. Prodrugs have been synthesized for several ophthalmic drugs: epinephrine, prednisolone, dexamethasone, prostaglandines as well as for pilocarpine, but dipivaloyl epinephrine, a prodrug of epinephrine, is the only commercially available prodrug (Balant et al., 1990; Schoenwald, 1993).

Prodrugs of pilocarpine were developed in the form of monoesters and diesters of pilocarpic acid (Bundgaard et al., 1986a,b; Järvinen et al., 1991a) or bispilocarpic acid (Järvinen et al., 1991b; Suhonen et al., 1996). These prodrugs are enzymatically converted to the active parent drug within the cornea or in the deeper tissue. The improved ocular penetration results from the more lipophilic character of these derivatives. The apparent corneal permeability measured in vitro was even seven times higher for a prodrug than for pilocarpine. The duration of the miotic effect was prolonged from 180 min up to 340 min when solutions containing pilocarpine or pilocarpine prodrugs were compared (Suhonen et al., 1996).

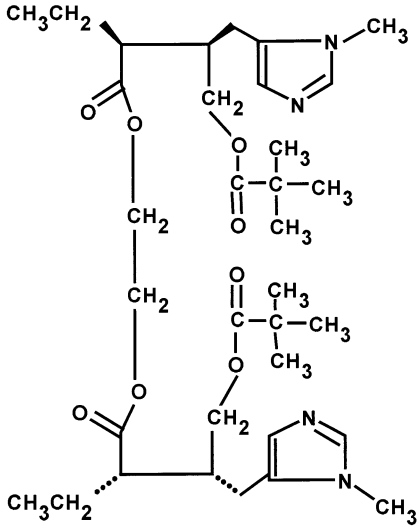

Fig. 1. Structure of the pilocarpine prodrug under investigation.

However, the prodrugs showed strong irritation effect (Saarinen-Savolainen et al., 1996), what could be explained by the amphiphilic character of their molecules. The effect was linearly correlated to the lipophilicity of the compound and was confirmed in vitro by liposome bilayer disruption and hemolysis test.

Submicron emulsions O/W type were proposed by several authors as formulations reducing toxicity and local drug irritation when used parenterally (Singh and Ravin, 1986; Prankerd and Stella, 1990). On the other hand increased pharmacological effect was obtained, when pilocarpine was topically delivered in the form of submicron emulsion. Naveh et al. (1994) observed reduction in the intraocular pressure for at least 24 h after a single dose application of pilocarpine in submicron emulsion of pH 5.0 composed of medium chain triglycerides as oily phase and egg phospholipids and Miranol MHT as emulgators. Such effect may be explained by prolonged residence time and partitioning of pilocarpine to the oily phase of the emulsion. In our studies we introduced a prodrug of pilocarpine to submicron emulsion in order to increase its bioavailability and reduce irritation.

2. Materials and methods

Synthesis and analysis of the prodrug, *O*,*O*′-dipivaloyl (1,2-ethylene) bispilocarpic acid diester (Fig. 1), have been described previously (Järvinen et al., 1995). Molecular weight of the compound is 647 Da and log P_{app} between octanol and phosphate buffer (pH 5.0) is 0.8 (Suhonen et al., 1996). The compound was used as a fumarate (mol. wt. 995 Da). Pilocarpine hydrochloride was purchased from Merck, Darmstadt, Germany.

Submicron emulsion and aqueous solution containing 1.2% (w/v) prodrug (equivalent to 0.5% w/v pilocarpine base) were prepared. The control aqueous solutions contained 0.5 or 2.0% (w/v) of pilocarpine hydrochloride. In all preparations the pH was adjusted to 5.0.

Submicron emulsion was prepared as described earlier (Benita and Levy, 1993). The preparation contained 10.0 g of soybean oil (Lipoid, Lud-

wigshafen, Germany), 1.2 g egg lecithin (Lipoid E 80, manufactured by Lipoid, Ludwigshafen, Germany) and 1.8 g glycerol (Odczynniki Chemiczne, Lublin, Poland) in water for injection up to 100 ml. The aqueous phase, containing lecithin, was combined with the oil at 70°C and the emulsion was stirred with a high shear mixer Ultra Turrax T25 (Janke & Kunkel, Staufen, Germany). Homogenization was performed using homogenizer 8.30 (APV Gaulin, Hilversum, Holland) and was followed by filtration in aseptic environment through a sterile Durapore filter 0.45 μm (Millipore, Bedford, USA). The prodrug was introduced by ex tempore technique—it was dissolved in the submicron emulsion.

Osmotic pressure of the emulsion, measured with a cryoscopic method (Knauer Automatic Osmometer, Bad Homburg, Germany) was 320 mOsm/kg. For the prodrug and pilocarpine solutions the values 310 and 302 mOsm/kg, respectively, were obtained.

In order to study short term stability of the prodrug in solution as well as in emulsion the preparations were stored for 48 h at 4°C and assayed chromatographically for the prodrug content. The separation was performed using HPLC method on Lichrospher 60 RP-select B column (Merck, Darmstadt, Germany) and a mixture of 0.02 mol/l phosphate buffer pH 4,5 and methanol (29:71) was used as a mobile phase. The detection was done at $\lambda = 215$ nm. The results demonstrated that no loss of the prodrug occurred during 48 h at 4°C. In the in vivo studies the prodrug formulations were stored before application not longer than for 10 h at 4°C.

Incorporation of the prodrug to the emulsion did not change the physical properties of the emulsion—stability of the system was confirmed visually and using laser diffractometer Mastersizer E (Malvern Instruments, Malvern, UK). The median particle diameter was 0.33 μm while 98.5% of the droplets were below 1.0 μm in diameter.

The bioavailability and irritation studies were done using albino rabbits weighing between 3.8–4.4 kg. The animals were kept on standard food, in daylight.

Miotic assay was performed in order to study bioavailability of pilocarpine from the prodrug submicron emulsion. This was compared with the miotic effect obtained after administration of 0.5 or 2.0% pilocarpine hydrochloride aqueous solution of the same pH (5.0). The test formulations (50 μl) were applied to the conjunctival sac. The pupil diameter was measured using pupillometer (Wessely's keratometer, Carl Zeiss-Jena, Germany), before drug application as well as every 30 min up till 6 h after drug installation. The prodrug emulsion or pilocarpine solution were applied to one eye while the other remained untreated and served as a control.

3. Results and discussion

Improved ocular penetration of the prodrug from aqueous solution in comparison to pilocarpine solution has been already proved (Suhonen et al., 1996). Thus in the present study the miotic effect obtained after application of the prodrug in submicron emulsion was studied and compared with the effect of 0.5 and 2.0% pilocarpine HCl solutions. Fig. 2 presents the plots of the mean changes in pupillary diameter as a function of time. Pilocarpine caused rapid miotic response peaking at 30 min or sooner, while the maximum response following the installation of the prodrug emulsion was between 120 and 240 min. $AUC_{0-6\ h}$ for the prodrug emulsion was $9252 \pm 1345\% \times$ min and was significantly (t-test, $P = 0.041$) larger than observed after application of the solution containing 0.5% pilocarpine HCl ($6845 \pm 1967\% \times$ min). The ocular bioavailability of the prodrug as measured by mean AUC is even higher than observed for 2.0% pilocarpine HCl solution ($8160 \pm 2903\% \times$ min), however, due to large standard deviations, the difference was not statistically significant ($P = 1.85$). Suhonen et al. (1996) studied the same prodrug and compared the miotic effect following application of 1.2% prodrug or 1.0% pilocarpine HCl, both in the form of aqueous solutions of pH 5.0. The prodrug solution produced prolonged miotic effect with $AUC_{0-\infty}$ value 1.6 times larger than obtained for the pilocarpine solution. Our studies did not compare the potency of the prodrug in solution and submicron emulsion but these two formulations seem to be comparable in efficacy.

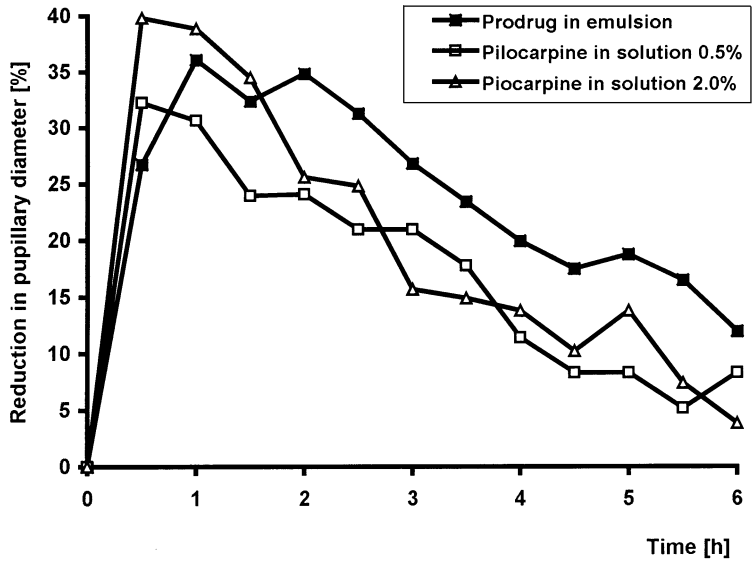

Fig. 2. Comparison of the miotic effect after ocular administration of the submicron emulsion containing 1.2% (w/v) pilocarpine prodrug (equivalent to 0.5% pilocarpine base) or aqueous solutions containing 0.5% (w/v) or 2.0% (w/v) of pilocarpine HCl (mean of measurements in six animals for each formulation).

In order to estimate whether submicron emulsion can assure sustained release of the prodrug distribution of the prodrug between aqueous and oily phase was determined using ultrafiltration technique. The prodrug concentration was measured in the aqueous phase separated by filtration of the emulsion through a centrifugal filtration unit Microcon 100 (Millipore, Bedford, USA). Approximately 72.2–80.7% of the total prodrug content was found in the aqueous phase while the rest was located in the oily phase and in the interphase. The result indicates that submicron emulsion is not probably a very efficient depot vehicle for the prodrug, since the majority of the compound is present in the external phase.

For the purpose to study irritation the prodrug of pilocarpine was applied onto rabbit eye in the form of submicron emulsion or aqueous solution, both containing 1.2% (w/v) of the prodrug. The volume of the preparation installed in the conjunctival sac was 50 μl. The application was done twice daily for the subsequent 5 days. Thirty and 60 min after each delivery conjunctiva and cornea were examined. Intensity of lacrimation and frequency of eyelid closure as well as general behavior of the animals were also observed. Ocular changes were graded according to the five-scores OECD scale (OECD, 1993). After the last application the pupil was also observed using ophthalmoscope and the fluoresceine test was performed in order to detect damage of the cornea. The test was done 4 h after the drug application: two drops of sodium fluoresceine aqueous solution were introduced to the conjunctival sac and after 10 min the eye was rinsed with an access of isotonic saline. The cornea was observed using a magnifying glass and blue light illumination.

Table 1 presents results of the irritation study. All observed changes were estimated maximally for one or two scores in the five-grades scale, which means that the irritation was not very severe. In Table 1, the frequency, not degree, of irritation is shown. Conjunctival erythema and edema were observed after the first application of the prodrug solution in all six eyes in the test group. In contrast, in the emulsion treated group only one rabbit demonstrated corneal edema and two rabbits showed erythema on the first day, while 5 days of treatment resulted only in increased frequency of erythema. In the course of

Table 1
Frequency of the ocular changes observed after application of the prodrug in the form of submicron emulsion or aqueous solution[a]

Observation	Formulation	Day of application					
		$t=0$	1	2	3	4	5
Corneal cloudiness, but iris can be easily observed	Solution	0	1	0	3	5	3
	Emulsion	0	0	0	0	1	1
Slight conjunctival and corneal erythema	Solution	0	6	6	6	6	6
	Emulsion	0	2	2	3	4	4
Slight conjunctival edema	Solution	0	6	6	6	6	6
	Emulsion	0	1	0	0	1	1
Lacrimation and longer eyelid closure	Solution	0	6	6	6	6	6
	Emulsion	0	0	0	0	0	0

[a] The numbers of eyes demonstrating changes, in the test group of six, are presented.

the experiment, corneal cloudiness was observed in one, three or even five eyes treated with the solution but only in one eye treated with the prodrug in emulsion. However, the prodrug did not cause corneal epithelial damage when applied for 5 days in either solution or emulsion form, what was demonstrated by fluoresceine test.

The most evident difference between both formulations was observed in lacrimation intensity and eyelid closure. Application of the prodrug in the form of solution was always followed by lacrimation and longer than normal time of eyelid closure while such a reaction was absent after application of the emulsion.

It can be concluded that irritation potential of the prodrug was reduced by incorporating it to a submicron emulsion vehicle. When submicron emulsion is applied, the contact of epithelium with the prodrug is probably not so immediate and the eye is not directly exposed to the maximal prodrug concentration as it may be in the case of a solution. Although not more than 30% of the prodrug was found in the internal phase of emulsion but a significant portion of the prodrug may be also encapsulated in phospholipid micelles also present in the emulsion and finally concentration of free molecules interacting with the membrane may be significantly reduced. The reduced surface potential of the drop applied in the form of emulsion may be another reason for lower irritation. While the drop of an aqueous solution goes very fast to the conjunctival sac resulting in high local concentration and consequently in conjunctival erythema and swelling, the emulsion covers the whole corneal surface more evenly and does not cause irritation due to high local concentration of the prodrug.

Submicron emulsion may be a suitable vehicle for lipophilic ophthalmic prodrugs such as pilocarpine prodrugs, whose clinical use is limited due to their significant irritation potential. It is possible to obtain submicron emulsion with a prodrug, containing natural phospholipids as emulgator. The pilocarpine prodrug in such formulation showed prolonged pharmacological effect and its irritation potential was reduced to such a level that it should not be an obstacle for medical application of such derivative. However, the long-term stability of the formulation must be studied before it can be considered as a formulation for the ophthalmic prodrug.

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