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Increased partitioning of pilocarpine to the oily phase of submicron emulsion does not result in improved ocular bioavailability

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Abstract

Submicron emulsions containing pilocarpine as ion-pair with mono-dodecylphosphoric acid were prepared. Physical stability of these preparations was confirmed during 4 months of storage at 4°C. Approximately 50% of the drug was found in the aqueous phase of emulsion separated using an ultrafiltration technique, while the rest was present in the oily phase and interphase. The miotic effect observed in rabbits after application of the ion-pair in aqueous solution or in submicron emulsion was the same; indicating that the drug distribution into the oily phase of the colloidal vehicle does not improve ocular bioavailability. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pilocarpine ion-pair; Submicron emulsion; Miotic effect

Low bioavailability of pilocarpine observed after the application of eye-drops can be improved by extending drug residence time in the conjunctival sac or by increasing lipophilicity of the drug by ion-pair or prodrug formation (Cavalli et al., 1995a,b; Suhonen et al., 1996). Submicron emulsions with pilocarpine or pilocarpine prodrug were formulated and measurements of the miotic effect were performed (Sznitowska et al., 2000). Prolonged activity of the drug applied in such vehicle was confirmed, although increased bioavailability was not as evident when compared to the aqueous solutions. In order to improve drug bioavailability in the current study, pilocarpine was incorporated into submicron emulsion in a more lipophilic form, namely an ion-pair with mono-dodecylphosphoric acid as a counter-ion (Pil-IP). The ion-pairs (with mono-octyl- and mono-decylphosphoric acid) have previously been studied by Cavalli et al. (1995b) and improved bioavailability was shown in albino rabbits from aqueous solution as well as from colloidal lipospheres; however, studies on submicron emulsions have not been previously reported. The aim of our study was an examination of the physical stability

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of such a preparation as well as in vivo evaluation of bioavailability in comparison with an aqueous solution.

Soya-bean oil (10% w/w) submicron emulsions containing 1.2% w/w egg lecithin were prepared in a standard manner described in details elsewhere (Zurowska-Pryczkowska et al., 1999). Lecithin (Lipoid E 80, Lipoid, Ludvigshafen, Germany) was dispersed in water–glycerol (2.25% w/w) mixture, soya-bean oil (Lipoid, Ludvigshafen, Germany) was added and the emulsion was stirred with a high-shear mixer (Ultra-Turrax, Janke&Kunkel, Staufen, Germany) and homogenized at 500 bar using a high-pressure homogenizer (APV Gaulin, Hilversum, Holland). The emulsion was filtered aseptically using a 0.45 μm Durapore filter (Millipore, Bedford, MA). Emulsion containing 20% w/w soya-bean oil was also prepared.

Pil-IP was prepared by mixing mono-dodecylphosphoric acid (Lancaster, Morecambe, UK) with an aqueous solution of pilocarpine base (Merck, Darmstadt, Germany). The molar ratio of pilocarpine and the counter ion was 1:1 (1.7:2.1 by weight). Pil-IP was incorporated to the emulsion using de novo and ex tempore techniques: Pil-IP was dissolved in part of the water used for emulsion preparation or in the prepared emulsion. The final pH values in de novo and ex tempore prepared emulsions were 6.24 and 6.35, respectively and were not corrected. The emulsions were homogenous, however, the color was creamier in comparison with a drug-free preparation.

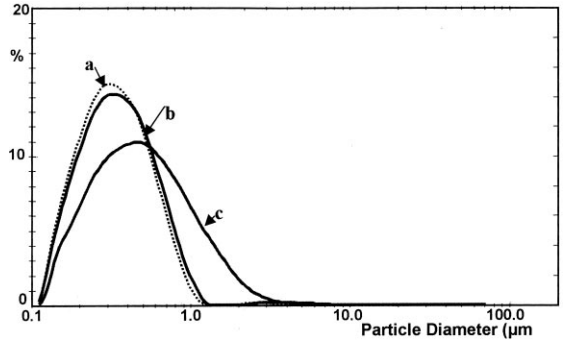

Fig. 1. Distribution of oily droplet sizes in drug-free submicron emulsion (a) and emulsions containing 2% Pil-IP, counted as hydrochloride: (b) emulsion prepared using ex tempore and (c) de novo techniques.

The distribution profile of oil droplet diameters was measured employing a laser diffractometer (Mastersizer E, Malvern Instruments, Malvern, UK) and compared with the emulsion without drug (Fig. 1). In the drug-free formulation, the median diameter was 0.33 μm, and the diameter of 90% of the oil droplets was smaller than 0.64 μm. When Pil-IP was added to the submicron emulsion (ex tempore method) the droplet sizes did not change, while in the de novo prepared formulations the oil droplet size increased: the median diameter was 0.47 μm, and the diameter of 90% of the oil droplets was smaller than 1.28 μm. After 4 months storage no significant change in droplet sizes, pH, or visual appearance was observed. These formulations were physically more stable than formulations containing pilocarpine hydrochloride and the size distribution profile was favorable. These observations confirm that pilocarpine cation electrostatically bound to an organic ion does not influence the structure of the interphase film as much as it does in the systems containing pilocarpine salts, e.g. hydrochloride (Zurowska-Pryczkowska et al., 1999).

In contrast to physical stability, chemical stability of the system was poor. After 4 months of storage at 4°C significant degradation was detected: the amount of isopilocarpine was 3.3% and pilocarpic acid 7.6% in respect to the total pilocarpine content. The degradation was even faster than in preparations containing Pil-HCl (Zurowska-Pryczkowska et al., 1999) and resulted from a pH value which was beyond the optimal pH range 4–5.5. This observation indicates that neither chemical modification nor increased drug incorporation in the internal oily phase of the emulsion (Table 1) inhibits pH dependent degradation of pilocarpine.

Drug distribution between the aqueous and oily phase was determined using an ultrafiltration technique. The pilocarpine concentration was measured in the aqueous phase separated by filtration of the emulsion through a centrifugal filtration unit Microcon 100 (Millipore, Bedford, USA). The analysis was performed using an HPLC method described elsewhere (Zurowska-Pryczkowska et al., 1999). The amount of the

Table 1
The effect of the chemical form of pilocarpine on drug distribution between emulsion phases and bioavailability from aqueous solution and submicron emulsion[a]

Formulation	Total dose determined in aqueous phase (%)	t_{max} (h)	E_{max} (%)	$t_{20\%}$ (h)	K_{el} (%h^{-1})	AUC_{0-6h} (%min)
Pil-IP emulsion	40.4	0.5	52.1 ± 11.1	3.2 ± 1.0	7.76	9713 ± 2704
Pil-IP solution		0.5	53.3 ± 12.4	3.3 ± 1.8	7.62	9589 ± 2520
Pil-HCl solution		0.5	41.8 ± 7.0	2.8 ± 0.9	5.95	7629 ± 2528
Pil-HCl emulsion[b]	104.9	1.0 (0.5–1.0)	50.8 ± 9.0	4.3 ± 1.3	6.18	9994 ± 2178

[a] Pharmacokinetic parameters characterize the miotic effect observed after application to rabbit's eye of preparations containing 2.0% w/w pilocarpine counted as hydrochloride salt. The abbreviations are explained in the text.
[b] Data reported by Sznitowska et al., 2000.

drug found in the aqueous phase was greatly reduced in comparison to the emulsion containing pilocarpine hydrochloride (Table 1). Pil-IP distributes to the oily phase and interphase, thus less than half of the total content was found in the aqueous phase. We have been studying the phase distribution of pilocarpine in the form of a base, hydrochloride, oleate and prodrug (Sznitowska et al., 1999, 2000) and among those chemical forms Pil-IP shows the highest distribution to the oily phase or/and interphase of the submicron emulsion. Incorporation of the drug in the internal oily phase of the emulsion may influence the kinetics of transcorneal drug absorption. Higher bioavailability of pilocarpine from such a formulation can be expected.

The bioavailability studies were done using rabbits weighing between 3.8 and 4.4 kg. The animals were kept on a standard food, in daylight. The formulations were freshly prepared.

Miotic assay was performed to study the bioavailability of pilocarpine from the submicron emulsion. It was compared with the miotic effect obtained after administration of Pil-IP in solution and with pilocarpine hydrochloride in aqueous solution or submicron emulsion of the same pH (6.5). All formulations studied contained 2% w/w pilocarpine as the hydrochloride. The test formulations (50 μl) were administered to the conjunctival sac of each animal. Pupil diameter was measured using pupillometer (Wessely's keratometer, Carl Zeiss-Jena, Germany), before drug application as well as every 30 min up to 6 h after drug installation. The test preparations were applied into one eye while the other was not treated and served as a control.

Fig. 2 presents the plots of the mean changes in pupillary diameter as a function of time. The calculated pharmacokinetic parameters are presented in Table 1. These values were also compared with the parameters obtained for submicron emulsion containing 2% pilocarpine HCl (pH 6.5) (Sznitowska et al., 2000). Pilocarpine in the form of ion-pair, irrespective of the vehicle, caused a rapid miotic response, peaking at 30 min or sooner. This finding is in contrast to our previous results because emulsion containing Pil-HCl of-

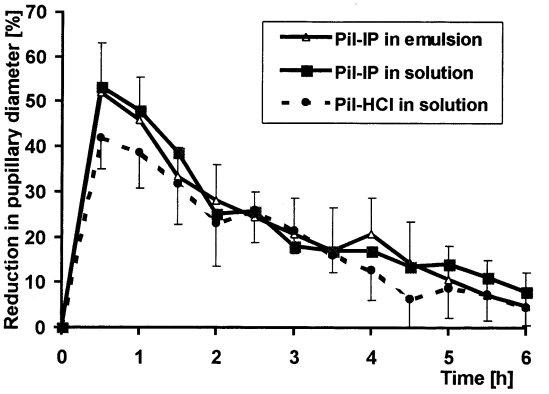

Fig. 2. Plot of change in pupillary diameter as a function of time following administration of Pil-IP in aqueous solution or submicron emulsion in comparison with Pil-HCl in solution. All preparations contain 2% pilocarpine as the hydrochloride. Error bars are shown for Pil-IP in emulsion and Pil-HCl in solution.

fered longer duration of maximal effect, E_{max}, up to 1 h (Table 1). When Pil-HCl solution and Pil-IP solution are compared it may be concluded that Pil-IP offers increased pilocarpine bioavailability — which can be demonstrated by a larger E_{max} and AUC_{0-6h}. However, a statistically significant difference (Student t-test, $P < 0.05$) can only be shown for the E_{max} value. Emulsion vehicle did not cause a further increase of bioavailability, although such effect was observed for Pil-HCl or for other chemical forms of pilocarpine (Sznitowska et al., 2000). Neither prolongation of the miotic effect was observed as indicated by t_{max} and $t_{20\%}$ values.

It is concluded that sustained action of pilocarpine incorporated in submicron emulsion can not be correlated with drug ability to be encapsulated in the oily internal phase. Better bioavailability of pilocarpine from emulsion in comparison to the solution was not obtained either for Pil-IP or for pilocarpine prodrug (Sznitowska et al., 1999) irrespective of their improved distribution to the oily phase.

Only a moderate increase of pilocarpine transcorneal penetration was observed in our studies when the drug was applied as an ion-pair, which is in contrast to results reported by Cavalli et al. (1995b) who demonstrated more than two times greater miotic effect of pilocarpine ion pair with mono-decylphosphoric acid used as a counter-ion when compared with Pil-HCl. However, those authors also did not observe a further increase of the AUC value when colloidal vehicle — lipospheres — were used. Thus colloidal lipophilic vehicles, such as submicron emulsions or lipospheres, do not contribute significantly to the increased bioavailability of the in the lipophilic derivatives of pilocarpine and inadequate precorneal residence time of the formulation is the most probable reason for such phenomenon. However, the advantage of the emulsion vehicle in prolongation of the miotic response for Pil-HCl was shown in a previous article (Sznitowska et al., 2000), which suggests that in the case of the hydrophilic forms, even slight prolongation of the drug contact with the cornea may increase penetration.

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