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Research paper

Studies on the effect of pilocarpine incorporation into a submicron emulsion on the stability of the drug and the vehicle

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Abstract

In order to obtain a novel ocular formulation with a potential for prolonging pilocarpine activity, the drug (2.0%) was incorporated into a submicron emulsion containing soya-bean oil and lecithin as emulgator. The effect of drug incorporation into the emulsion on its physical stability and on the other hand, the potential of the vehicle to reduce drug degradation at pH higher than 5.0 was studied. The pH was adjusted to 6.5 or 5.0 and the physicochemical stability of the formulations was observed. The mean diameter of oily particles in the resulting emulsions measured by a laser diffractometer was 0.6-0.7 μm and this was larger than in a drug-free emulsion where a 0.33 μm value was measured. The formulations were physically stable for 6 months at 4°C, but progressing chemical degradation of pilocarpine was noted at pH 6.5. At that pH nearly 8% of pilocarpine was degraded to isopilocarpine and pilocarpic acid, both in the emulsion and in the solution. Thus, it may be concluded that pilocarpine in submicron emulsion is not protected against degradation. The presence of pilocarpine changes the physical stability of the vehicle since the formulation was easily destabilised during autoclaving or at room temperature. In the presence of higher concentration of lecithin (2.4%) or co-emulgators (poloxamer 2.0% or Tween 80 0.5%) the mean droplet size in the emulsions was the same as in a drug-free system. However the emulsions containing poloxamer were not stable during storage. Viscosity of pilocarpine emulsions can be increased by addition of methylcellulose or sodium carmellose (1.0%), but an intensive creaming occurs in these systems. Pilocarpine base is less suitable for emulsion preparation than hydrochloride salt, and emulsions prepared at pH 5.0 show the most satisfying stability.

Keywords: Pilocarpine; Submicron emulsions; pH; Chemical stability; Particle sizes; Poloxamer; Tween 80; Methylcellulose; Sodium carmellose

1. Introduction

Submicron emulsions with pilocarpine could be beneficial for prolonging pharmacological effect of the drug, when used as eye drops in glaucoma patients. Naveh et al. [1] have already reported a prolonged reduction of the intraocular pressure after administration of pilocarpine as submicron emulsion, and according to the non-published data, such formulation is already under clinical trials. The pH of the emulsion was adjusted to 4.8 which was optimum for pilocarpine stability. However, such value is not the most favourable for drug bioavailability, since higher absorption can be observed from neutral and slightly alkaline formulations, when the drug is in a non-ionized form. Higher pH could also be beneficial for the physicochemical stability of the emulsion vehicle. There are reports in the literature, that susceptibility of a drug to degradation may be reduced in submicron emulsions [2] and this makes it reasonable to investigate if it is possible to prepare submicron emulsions with pilocarpine at pH higher than 5.0.

In our work we have studied how submicron emulsion as a vehicle influences chemical stability of pilocarpine, and on the other hand what is an effect of the drug on the physical stability of submicron emulsions. Emulsions containing 2% (w/w) pilocarpine (as hydrochloride) whose pH was

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5.0 or 6.5 were prepared and their stability was observed during 6 months. The following technological factors varied in the course of emulsion preparation: chemical form of the drug and method of drug introduction to the emulsion, pH value and presence of additional excipients, i.e. poloxamer or polysorbate 80 as co-emulgators and methylcellulose and sodium carmellose as viscosity increasing agents.

2. Materials and methods

2.1. Materials

Pilocarpine base and pilocarpine hydrochloride (extra pure) were purchased from Merck (Darmstadt, Germany) and Lipoid E80 (lecithin) as well as soya-bean oil – from Lipoid (Ludwigshafen, Germany). Synperonic F68 (poloxamer) was purchased from Boehringer Ingelheim (Heidelberg, Germany), Tween 80 (Polyoxyethylenesorbitan monooleate) from Sigma (St. Louis, MO), Methocel MC from Fluka (Buchs, Switzerland) and sodium carmellose from Loba Feinchemie (Germany). Glycerol was obtained from Pollena-Strem (Dabrowa Gornicza, Poland) and sodium hydroxide from ZCh (Oswiecim, Poland) (10% w/v solution was used).

Components of the mobile phase for HPLC analysis, methanol (POCh, Lublin, Poland) and triethylamine (Fluka, Buchs, Switzerland), were distilled directly before use. Phosphoric acid was obtained from POCh (Lublin, Poland).

2.2. Methods

The emulsions contained the following quantities of ingredients (% w/w): pilocarpine hydrochloride 2.0, egg lecithin (Lipoid E-80) 1.2, glycerol 2.3, soya-bean oil 10.0, water to make total 100.0 g. In order to study factors influencing the stability of the system pilocarpine hydrochloride was replaced by pilocarpine base (1.7% w/w), lecithin content was increased up to 2.4% and additional ingredients were added: poloxamer (2% w/w), polysorbate 80 (0.5% w/w) methylcellulose or sodium carmellose (1% w/w).

The emulsions were prepared according to a standard procedure [3]. Lecithin was dispersed in a mixture of water and glycerol at 40°C and next filtration was performed using 0.45 μm filter (cellulose esters, Millipore, Bedford, MA). Soya-bean oil was filtered through 0.5 μm teflon filter (Millipore, Bedford, MA) and both phases were combined at 70°C. The emulsion was stirred for 10 min using high-shear mixer (Ultra Turrax, Janke & Kunkel, Staufen, Germany) at the speed 20 500 rpm, and subsequently homogenized in 8 cycles (500 bar) using high-pressure homogenizer (APV Gaulin, Hilversum, Holland). With the aid of 10% sodium hydroxide solution, the pH was adjusted either to 5.0 or to 6.5 and the emulsion was filtered through polivinylidene fluoride 0.45 μm filter (Millipore, Bedford, MA).

Pilocarpine hydrochloride was introduced either to the aqueous phase (de novo method) or to the ready emulsion, before pH adjustment (ex tempore method). When pilocarpine base was used, the emulsion was prepared using only ex tempore method and pH was adjusted to 6.5.

The composition of emulsions was also modified using co-emulgators - poloxamer (2.0% w/w) or Tween 80 (0.5% w/w). These agents were dissolved in water and glycerol, next lecithin and pilocarpine HCl were added and the next steps were the same, as described above. In order to study the effect of viscosity increasing agents on stability of pilocarpine emulsions methylcellulose or sodium carmellose, 5.0% (w/w) solutions containing 2.3% glycerol were mixed in the ratio 1:4 with the de novo prepared pilocarpine submicron emulsion.

Physicochemical stability of the emulsions was studied during storage at temperature 4°C for a period up to 6 months, as well as after autoclaving at 121°C for 15 min. Chemical stability of pilocarpine in the emulsions was compared with its stability in aqueous solutions containing 2.0% (w/v) pilocarpine HCl and 0.47% NaCl, while pH was adjusted to 5.0 or 6.5.

The drug distribution between aqueous and oily phase was evaluated. Five hundred microliters of the emulsion was placed in a Microcon-100 (100 000 NMWL filter) centrifugal filtration unit (Millipore, Bedford, MA) and subjected to centrifugation at 2000 × g for 10 min. The ultrafiltrate was assayed for pilocarpine HCl content. Drug concentrations in the aqueous phase and in the whole emulsion were compared.

2.3. Methods of analysis

The appearance of the emulsions was evaluated visually. Laser diffractometer (Mastersizer E, Malvern Instruments, Malvern, UK) was used for measurement of sizes of the internal phase particles. Calculations were done using the Mie theory model of light scattering. Additionally, the presence of droplets larger than 5 μm was confirmed using binocular microscope. Chemical degradation of pilocarpine was evaluated employing a method described in USP XXIII for pilocarpine hydrochloride. The analysis was performed using C18 column (Vydac, CA) and HPLC apparatus (Merck-Hitachi). The mobile phase was a mixture of methanol and triethylamine/phosphoric acid buffer pH 3.0 (98:2); detection was done at 215 nm. The samples were prepared by dilution of the analyzed preparations with methanol.

Viscosity of the emulsions was measured at 20°C using Hoeppler Viscosimeter model BH (VEB Prüfgerate-Werk, Medingen/Dresden, Germany).

3. Results

Submicron emulsions with pilocarpine, containing soya-bean oil, lecithin and glycerol were formulated. As the

Table 1

Influence of technological factors on droplet size distribution in submicron emulsions, with pilocarpine and changes of pH and droplet sizes during storage[a]

Pilocarpine form	Lecithin (%)	Additional agent	$t = 0$			$t = 6$ months (4°C)		
			pH	Droplet sizes μm		pH	Droplet sizes μm	
				d(0.5)	d(0.9)		d(0.5)	d(0.9)
Method of preparation: ex tempore								
Pil-base	1.2	–	6.50	0.62	1.12	6.44	0.82	3.69
Pil-HCl	1.2	—	6.50	0.82	1.68	6.32	0.83	1.93
Method of preparation: de novo								
Pil-HCl	1.2	–	6.50	0.73	1.55	6.40	0.74	1.48
			5.00	0.66	1.21	4.90	0.66	1.23
Pil-HCl	2.4	–	6.50	0.43	1.03	Ns	Ns	Ns
			5.00	0.38	0.78	Ns	Ns	Ns
Pil-HCl	1.2	Poloxamer 2%	6.50	0.36	0.74	6.40	12.51	39.70
			5.00	0.36	0.74	4.96	9.20	43.09
Pil-HCl	1.2	Tween 80 0.5%	5.00	0.36	0.77	5.00	0.36	0.72
Pil-HCl	1.2	MC 1%	6.50	0.78	1.47	6.45	0.83	3.89
			5.00	0.65	1.23	5.16	0.69	1.78
Pil-HCl	1.2	CMCNa 1%	6.50	0.75	1.44	6.46	0.79	1.90
			5.00	0.71	3.01	5.39	0.68	2.60

[a]MC, methylcellulose; CMCNa, sodium carmellose; d(0.5), the volume median diameter; d(0.9), the limit diameter of 90% particles; ns, not studied.

major fraction of the oily phase particles was larger than 0.5 μm, the emulsions could not be sterilized through a 0.22 μm filter. Alternatively, aseptic procedure was applied when possible and filtration was done using 0.45 μm filters. Sterility of the preparations was confirmed. The resulting emulsions were white and homogenous. The primary pH of a drug-free emulsion was 5.3 and dropped below 5 when pilocarpine HCl was added or raised to 8.0 when pilocarpine base was added, thus the value required adjustment to pH 5.0 or 6.5.

In drug-free emulsions, the mean particle diameter was 0.35 μm and the size of 90% particles was smaller than 0.69 μm. Table 1 presents characteristics of droplet sizes in all emulsions prepared with pilocarpine. In comparison with a drug-free emulsion, larger droplet sizes were observed in the emulsions containing pilocarpine. However, emulsions prepared with co-emulgators, poloxamer or polysorbate 80, had nearly the same droplet sizes as the emulsions without pilocarpine. This was also the case for the formulation containing double portion of lecithin, i.e. 2.4% w/w. The distribution profile of oily droplets sizes in relation to the emulsion composition is presented in Fig. 1. It was observed that the mean droplet size was practically the same, irrespective of the chemical form of pilocarpine, final pH of the emulsion and method of pilocarpine introduction to the emulsions. Viscosity agents, methylcellulose or sodium carmellose, added to the pilocarpine emulsions, did not influence the size distribution pattern. The viscosities of a drug-free emulsion and the one containing pilocarpine were 1.28 and 1.25 cP, respectively. When the viscosity agents were added, the viscosities of both preparations were 7.3–7.5 cP and this parameter remained constant during storage.

Total concentration of pilocarpine HCl in the emulsion containing 1.2% w/w lecithin was 20.3 mg/ml, but in its aqueous phase, collected after centrifugal ultrafiltration, approximately 10% higher concentration was measured, i.e. 22.8 mg/ml. These values indicate that partitioning to the oily phase is negligible – when such assumption is done it may be calculated that after phase separation 99.0–102.7% of the total drug content was found in the aqueous phase. The same results were obtained for the preparations adjusted either to pH 5.0 or to 6.5.

Physical stability of the emulsions was observed during storage at temperature 4°C for up to 6 months. Besides slight creaming, no other visual changes were observed.

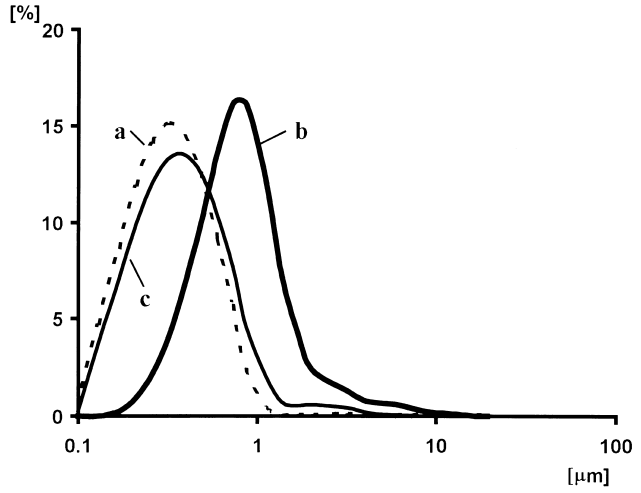

Fig. 1. Distribution of oily droplets sizes in submicron emulsions at pH 6.5: (a) drug-free emulsion (b) emulsion containing 2% pilocarpine HCl prepared de novo (c) emulsion containing 2% pilocarpine HCl and 2% poloxamer.

Table 2
Chemical stability of pilocarpine in emulsions and solution stored at 4°C for 6 months

Pilocarpine form	Method of preparation	pH	Time of storage (months)	Products of degradation (%)*		
				iso-Pil	Pil-acid	iso-Pil acid
Pil-base	Ex tempore	6.5	0	5.4	8.4	0
			6	6.5	13.7	0.8
Pil-HCl	Ex tempore	6.5	0	0.7	0	0
			6	2.0	5.7	0
Pil-HCl	De novo	6.5	0	0.7	0.5	0
			6	1.7	5.2	0
		5.0	0	0.5	0	0
			6	0.6	0	0
Pil-HCl	solution	6.5	0	0.5	0	0
			6	1.7	5.5	0

*Counted as a ratio of a peak response due to the degradation product to the peak corresponding to pilocarpine.

The intensity of the process was very pronounced in the emulsions containing viscosity agents where two separated phases were formed: the upper phase was still emulsion and the lower – almost clear, viscous solution. However, no breakage of the emulsion occurred in these preparations, and after short mixing, the system was homogenous again. The mean droplet size of the oily phase was not changed (Table 1). Only small changes of droplet sizes were noticed after 6 months in emulsions prepared with pilocarpine salt, irrespective of their pH and composition, with the exception, however, of the formulations containing poloxamer. In such cases a serious increase of the droplet sizes occurred, although visually the emulsions were not changed. Only slight increase of the droplet sizes could be noticed in emulsion prepared using pilocarpine base.

The emulsions were easily destabilized when subjected to autoclaving – due to coalescence oily drops could be seen on the surface of all emulsions. The process was slower in emulsions prepared using pilocarpine base, where destabilization occurred not immediately but within the following 30–50 h. After autoclaving, pH of the emulsions dropped from 5.0 to 4.6 and from 6.5 to 6.3 and the mean droplet size increased up to 1.7–2.0 μm. After autoclaving of drug-free emulsions the same pH decrease occurred, but neither visual changes nor changes in droplet size were observed.

Small amounts of isopilocarpine (0.5–0.7%) were detected in emulsions analyzed 2–5 days after preparation, the same degradation was found in solutions at the corresponding pH, as well as in a standard solution prepared ex tempore. During storage for 6 months, only very slight increase in isopilocarpine content was observed at pH 5.0 but the degradation progressed at 6.5, irrespective of the vehicle – aqueous solution or emulsion (Table 2). At pH 6.5 after 6 months of storage, nearly 10% of the degradation products, both isopilocarpine and pilocarpic acid, was found. The extent of degradation was much larger when pilocarpine base was used for preparation of the emulsions – more than 10% of the drug was degraded in these formulations only 2–3 days after preparation, and 20%, 6 months after. In the latter case, isopilocarpic acid was also detected. The pilocarpine base used for preparation of the emulsion contained not more than 1.0% isopilocarpine.

4. Discussion

While preparing submicron emulsions with pilocarpine, the stability of both, the active agent and the vehicle, must be taken into consideration and this brings up the technological problem of an appropriate pH. The drug is stable at pH 4–5 and its stability drops down largely at higher pH [4,5]. However, submicron emulsions are physically and chemically the most stable at pH 6.5–8.0 what is related to the rate of hydrolysis of triglycerides and zeta potential, whose negative value is significantly reduced at lower pH [3,6,7]. It seems obvious then, that due to the above discrepancies, it is difficult to compromise between stability of the drug and the vehicle. One other reason makes the problem more complicated and worthy of researching – increased bioavailability of pilocarpine could be achieved when the drug is present in the form of a base, since non-ionized species diffuse easier through the lipophilic cornea [8]. The above mentioned stability problems do not allow preparation of eye drops at pH > 5, but at pH 5 practically all drug is ionized. Biphasic systems, like submicron emulsions may offer, however, a protective environment for a drug sensitive to degradation in aqueous solutions [2], and the study was designed to investigate if this is the case for submicron emulsions with pilocarpine.

pH 6.5 has been chosen as the pH value compromising between emulsion stability, drug stability and maximal bioavailability and pH 5.0 served as a control. It may be calculated that 30.9 and 1.4% pilocarpine is non-ionized at pH 6.5 and 5.0, respectively. Preliminary studies showed that in emulsions at pH 8.5, when 97.8% of the drug was non-ionized, a very fast degradation of pilocarpine base occurred (more than 10% of the drug was degraded during

1 week at 4°C) and such formulations were not further investigated.

Submicron emulsions were prepared according to well known technology [3], using high-pressure homogenization to achieve appropriate reduction in oily particle sizes below 1 μm. Pilocarpine content in all formulations under investigation was 2.0% (counted as pilocarpine HCl), besides those with increased viscosity, as they were diluted in the course of preparation and the final pilocarpine HCl concentration was 1.6%.

When pilocarpine salt was used, emulsions were prepared using de novo or ex tempore technology but pilocarpine base was introduced only ex tempore. Under visual inspection, the emulsions were white and homogenous, irrespective of the method chosen and pH value. Our studies show that due to the presence of pilocarpine, sizes of the internal phase droplets increase seriously and the mean droplet diameter is more than twice of that in pilocarpine emulsions than in a drug-free formulation (Table 1, Fig. 1). Particle size distribution in the pilocarpine loaded emulsions shows a significant 'tailing' towards coarse particles. Droplets as large as 20 μm in diameter were present, what was confirmed by inspection under microscope. Particle size distribution remained unchanged in comparison with a drug-free emulsion, when lecithin concentration was increased up to 2.4%, as well as when poloxamer or Tween 80 were introduced as co-emulgators. It may be concluded that pilocarpine causes changes at the interphase boundary saturated with phospholipids, and only supplementary surfactant is able to protect the system against such changes. This conclusion may be supported by a fact that Naveh et al. [1] formulated with pilocarpine, using lauroamphodiacetate as a co-surfactant, emulsions with a mean diameter 115 nm (PCS measurements).

In spite of the increased droplet sizes, the emulsions were physically stable for 6 months at 4°C – no other changes than creaming were observed visually and the mean diameter of oily droplets remained unchanged (Table 1). Surprisingly, intensive coalescence occurred in emulsions containing poloxamer. In these systems, a large increase in measured droplet sizes was observed in spite of the satisfying initial particle size distribution. However, the appearance of these emulsions did not indicate destabilization. When poloxamer was added to emulsion without pilocarpine, the system was stable during storage and no change in particle size distribution was noted. The reason for the observed incompatibility of poloxamer and pilocarpine in emulsion is not known. However, Tween 80 was compatible with the system and on the basis of the droplet sizes after 6 months of storage the preparations containing this co-surfactant can be selected as the most satisfying.

As the bioavailability of pilocarpine may be prolonged when viscosity of eye drops is increased, methylcellulose or sodium carmellose in concentration 1.0% were introduced to the emulsions. A homogenous system was obtained. However, during storage an intensive creaming occurred. In spite of that, no changes in the sizes of the oil droplets were noticed and after shaking, the emulsions were homogenous. The reason for the observed reversible separation of the system into two phases (emulsion and viscous solution) is not known – the phenomenon can probably not be explained by a difference in density (density of methylcellulose 1% solution is 1.0012 g/cm^3). No changes in the viscosity were found after 6 months of storage. Such results indicate that viscosity of the emulsions may be increased with traditionally used agents, however, the intensity of the creaming makes the preparations aesthetically unacceptable. There is no difference between systems containing either methylcellulose or sodium carmellose, although one can speculate that due to the difference in ionization their effect on emulsion stability may be different.

The stability studies were performed at 4°C, because at room temperature visual changes like intensive creaming, changes in colour or droplets of oil on the surface occurred very quickly, as soon as within 4–5 weeks. All emulsions, independently of the pH, were also destabilised when subjected to autoclaving. Such results prove that pilocarpine largely decreases physical stability of submicron emulsions, which otherwise can be autoclaved or stored at room temperature [6].

Chemical stability of the drug in the prepared emulsions was evaluated, using a HPLC method which allows the separation of pilocarpine from its degradation products: isopilocarpine, pilocarpic acid and isopilocarpic acid. We compared the stability of the drug in emulsions at pH 6.5 with emulsions at pH 5.0 and aqueous solution at pH 6.5 (Table 2). At pH 6.5, degradation was much faster than at pH 5.0, no matter what type of a vehicle was used: emulsion or solution. When pilocarpine base was used for preparation of emulsion, the degradation was the most significant – over 10% of the drug was degraded at pH 6.5. The temperature during emulsion preparation (70°C) does not seem to be a factor determining the stability, at least on a laboratory scale, because the same amounts of degradation products were found in emulsions prepared ex tempore and de novo.

Since no difference could be seen between drug degradation in emulsion and in solution at the same pH, one may conclude that incorporation of pilocarpine or its salt to emulsion, does not result in increased stability of the drug. Naveh et al. [1] estimated that at pH 4.8, as much as 5.9% of the total pilocarpine was distributed to the oily phase of the emulsion. Our studies showed that practically all drug was present in the aqueous phase, irrespectively of the pH value (5.0 vs. 6.5), however, in our system soya-bean oil was used while the other authors used more hydrophilic oil – medium chain triglycerides (MCT). Solubility of pilocarpine base in water is so good ($>$1:30) that its partitioning to the oily phase is low, even lower in the presence of micelle forming agent – lecithin. Due to the relatively good solubility of pilocarpine base in water, elevation of pH from 5.0 to 6.5 leads to decreased ionization of the drug, but does not increase significantly its partitioning to the oily phase of

emulsion. In this way, chemical degradation of pilocarpine is not diminished in the studied formulations.

5. Conclusions

It may be concluded that the submicron emulsion system does not change the rate of pilocarpine degradation and, as for solution, pH 5, must be chosen as the most favourable for chemical stability of the drug. Our studies show that at such pH, the system is also physically stable during 6 months when refrigerated. Although pH 5 may promote hydrolysis of triglycerides, but only slight pH changes observed during storage, indicate that the process is rather slow. An increase in the oily droplet occurs when pilocarpine is introduced to the emulsion containing 1.2% lecithin, but this effect is not observed when additional amount of lecithin or another co-surfactant is used. Our studies revealed, however, that poloxamer is not compatible with the system. Pilocarpine salt rather than base should be used for preparing emulsions. Their viscosity can be increased with methylcellulose or sodium carmellose, but intensive creaming is observed in such preparations.

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