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Microemulsions as topical delivery vehicles: ocular administration of timolol

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Abstract: The topical administration of timolol as an ion-pair with octanoate was achieved by use of an oil-in-water microemulsion containing lecithin as a surfactant. The microemulsion, a solution of the ion-pair and a solution of timolol alone were instilled in the conjunctival sac of rabbits.

A rapid method for the separation and determination of timolol in aqueous humour by HPTLC was used. The bioavailability of timolol from the microemulsion and the ion-pair solution was higher than that obtained from timolol alone. The areas under the curve for timolol in aqueous humour after administration of the microemulsion and the ion-pair solution were 3.5 and 4.2 times higher, respectively, than that observed after the administration of timolol alone.

Keywords: *Microemulsion; timolol–octanoate ion-pair; topical bioavailability; absorption prolongation.*

Introduction

In the last few years, several new preparations have been developed for ophthalmic use. Successful results were obtained with inserts and diffusion-controlled systems, although these preparations present some disadvantages, such as non-compliance, especially by elderly people [1]. Several colloidal systems, based on polymeric nanoparticles of alkylcyanoacrylate were investigated for controlled drug delivery [2]. In addition, liposomes of drugs were studied for human use to enhance ophthalmic absorption [3]; attempts were made to use other colloidal carriers, such as macromolecular complexes [4] and nanocapsules [5], as ophthalmic devices.

In previous work [6] the *in vitro* diffusion rates of timolol from microemulsions were studied in an attempt to prolong the release of the drug; octanoic acid was used as one of the oil components to increase the reservoir effect of the disperse oil phase and to form an ion-pair with the drug. Diffusion experiments through a lipoidal membrane showed that the presence of octanoate as a counter-ion increased the diffusion rate of timolol from solutions; microemulsions also showed a reservoir effect that was dependent on the concentration of octanoic acid in the internal phase [6].

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The present work is concerned with the administration of timolol to rabbits, using an oil-in-water microemulsion whose disperse phase contained octanoic acid. In addition, an aqueous solution of timolol as an ion-pair at pH 7.4 was administered *in vivo*.

The aims of the work were to examine the influence of the presence of the ion-pair on the corneal absorption of timolol in rabbits, and to observe the effect of the microemulsion on the prolongation of absorption of timolol.

The experiments involved the analysis of timolol in aqueous humour; for this purpose a suitable and rapid method for separation and quantitation of the drug was required.

Experimental

Materials

Timolol maleate was obtained from Sigma; octanoic acid, 1-butanol, isopropyl myristate (ISM), 1–4 dioxane (anhydrous), egg lecithin and Kieselgel HPTLC F_{254} plates (100 × 100 mm) were obtained from Merck; α-tocopherol was obtained from Roche; timolol base was prepared from timolol maleate; egg lecithin was purified as previously reported [6].

Apparatus

A capillary microviscometer (Schott Geräte), a laser light-scattering spectrometer (Malvern Type PCS 100), and a densitometer HPTLC/TLC Scanner (Camag) were used.

Methods

Formulations of timolol for ophthalmic administration

Solution of timolol alone: an isotonic, buffered solution (pH 7.4) of timolol 1.4% (m/v) was used.

Solution of timolol as an ion-pair: a 0.33% (m/v) isotonic solution of timolol at pH 7.4 was used; the molar ratio of timolol/octanoate was 1:10.

Microemulsion: isotonic phosphate buffer (pH 7.4), 40%, m/m; egg lecithin, 28.7%, m/m; ISM/octanoic acid (71.6:28.4, m/m), 16.4%, m/m; 1-butanol, 14.9%, m/m.

Preparation of microemulsions:

(1) In the absence of the drug — egg lecithin was dissolved in the oil phase (ISM-octanoic acid); the mixture was then added to the buffer with continuous shaking, and 1-butanol was added to the emulsion to obtain a clear system. α-Tocopherol (0.01%) was added to the microemulsion to prevent oxidation.

(2) In the presence of the drug — the microemulsion containing 2.6% (m/v) of timolol was prepared as previously described but timolol base was dissolved in the oily solution of lecithin.

The microemulsions could also be obtained by self-emulsification.

Stability tests. The microemulsion in the absence of the drug was tested for stability by means of repeated centrifuging and freeze–thaw cycles [7]. The microemulsion was centrifuged for 30 min at 13,000 rpm, then stored in a refrigerator at −20°C for 15 days and heated to 40°C [8]. After each cycle, the viscosity of the microemulsion was measured. Stability tests were repeated for 6 months. The same accelerated stability tests were performed on the microemulsion in the presence of the drug.

Mean radii. The determination was carried out by photocorrelation spectroscopy on the basis of the diffusion coefficient at 25°C. The sample was directly filtered on a 10-mm quartz cell (Helma) using membrane filters (Millipore 0.22 μm); the cell was then placed in a thermostatted water-bath (25°C ± 0.1°). The light source was a He–Ne laser at a wavelength of 632.8 nm; the absolute scattered intensity was measured at an angle of 90°.

Sterilization. All the formulations used for topical administration of timolol (solution of timolol alone, solution of the ion-pair, and the microemulsion) were sterilized by heating at 121°C for 30 min.

In vivo *experiments*

Physiological tolerance: The microemulsion in the absence of the drug was repeatedly instilled (4 times in 12 h) in the conjunctival sac of two groups of eight rabbits, which were examined for local irritation or inflammatory processes; the same tests were performed with the solution of octanoate only.

Topical administration and aqueous humour sampling: Three series of experiments were performed in which the solution of timolol alone, the solution of timolol as an ion-pair and the microemulsion containing the drug, were administered. Each series of experiments was carried out on three groups each of eight rabbits (of both sex) weighing 2.5–3.0 kg. In the case of the microemulsion and of the solution of timolol alone, 50 μl was instilled each time in the conjunctival sac of both eyes, by a micropipette; three drops were instilled at regular intervals during 4 min. The lower eyelid was held against the upper lid for a few seconds to prevent drainage. At fixed post-instillation times (5, 15, 25, 30, 40, 60, 90 and 120 min), aqueous humour samples were withdrawn from the anterior chamber using a hypodermic needle attached to a tuberculin syringe; the same syringe was also used for the opposite eye of each rabbit so that the aqueous humour of both eyes could be pooled.

Analysis of timolol by HPTLC and fluorescence quenching photometry

Analysis of timolol in aqueous samples: Increasing amounts (20, 25, 45, 50, 75, 90 and 100 μl) of a standard timolol aqueous solution (16.88 μg ml^{-1}) were diluted to 300 μl with double-distilled water, and extracted as previously described [9]. After extraction and evaporation of the organic solvent, the residue was redissolved in methanol and accurately applied as one single spot on a HPTLC plate. Separation was performed using dioxane–25% (m/m) aqueous ammonium hydroxide (98:2, v/v) as eluent, and the chromatogram was developed to a height of 8 cm in a pre-saturated chamber; the plate was then dried in an air stream at room temperature.

The areas of the resulting spots of the HPTLC plate were determined by fluorescence quenching photometry at 294 nm.

Analysis of timolol in aqueous humour: A series of 100–200 μl aqueous humour samples, withdrawn at different post-instillation times, were treated as described for aqueous solutions, and applied on the plates, alternating these solutions with standard solutions. Internal standard correction was used for samples withdrawn at post-instillation times longer than 40 min. The timolol concentration was determined from the resulting spots by fluorescence quenching photometry of the HPTLC plates at 294 nm, by means of the external standard method.

Results

Microemulsion stability

After 6 months of repeated shaking and freeze–thaw cycles [8], the system was still homogeneous and no phase separation could be observed; the viscosity was almost constant, its initial value corresponding to 24.8 ± 0.7 cP. After sterilization, no apparent change or phase separation took place in the microemulsion.

Mean radii

The mean radii of the microemulsion measured at 25° by laser photocorrelation spectrometry was 15 nm, with a polydispersion coefficient of 0.236.

Tolerance

After repeated instillations of the microemulsion in the absence of the drug, no inflammatory effects could be observed in the rabbits' eyes. The solution of octanoate did not cause inflammation.

Analysis of timolol in aqueous solutions

Standard solutions separated on different plates yielded almost identical shapes of calibration graphs, although variations in the absolute value of the integrated signal depended on the plate. A linear relationship was obtained between the logarithm of the integrated signal and the logarithm of the corresponding mass (μg) of timolol over a wide range. The regression equation was: $y = 0.9827585x + 5.12621$; $r = 0.999$.

Analysis of timolol in aqueous humour

The method allowed the determination of small amounts (0.2 μg ml^{-1}) of timolol in aqueous humour.

Topical administration

The results of the ophthalmic administration of timolol after topical instillation of the microemulsion, of the solution of timolol as ion-pair and of the solution of timolol alone, are reported in Table 1. In Fig. 1, the timolol aqueous humour concentrations versus post-instillation times are reported for the two solutions and for the microemulsion, refer to identical amounts of the drug. For each system the maximum peak corresponded to 15 min; the concentrations measured in the aqueous humour at this time were 3 and 4 times higher for the microemulsion and the ion-pair, respectively, than for timolol alone. The topical bioavailability of timolol in the microemulsion was enhanced; indeed, the area under the curve was 3.5 times higher for the microemulsion and 4.2 times higher for the solution of the ion-pair than that observed after administration of timolol alone. The trend of the curves was rather different, the times of absorption of timolol from the microemulsion being longer than those obtained from both solutions; detectable amounts of the drug were still present in the aqueous humour, 120 min after administration of the microemulsion.

Discussion

Microemulsions are clear, stable dispersions obtained by mixing oil, water, surfactant and co-surfactant [10]; the diameter of the droplets is always <100 nm. Microemulsions

Table 1
Concentrations of timolol in aqueous humour after topical administration of microemulsion, ion-pair solution, or timolol alone in solution

Time (min)	Timolol (μg ml^{-1})* Microemulsion†	Ion-pair‡	Aqueous solution§
5	18.5 (5.66)	7.7 (1.67)	2.4 (2.27)
15	24.9 (4.85)	8.2 (2.34)	4.5 (1.39)
25	14.3 (2.02)	7.4 (1.02)	3.2 (1.50)
30	7.5 (2.20)	3.7 (1.09)	2.9 (1.75)
40	7.3 (3.65)	1.8 (1.00)	1.4 (0.21)
60	6.5 (3.63)	0.6 (0.36)	0.2 (0.29)
90	2.2 (1.92)	—	0.4 (0.59)
120	1.6 (1.70)	—	—

*Values in parentheses are standard deviations (n = 6).
†o/w Microemulsion; timolol = 2.6% m/v. Instilled volume = 150 μl.
‡Aqueous solution of ion-pair (1:10) at pH 7.4; timolol = 0.33% m/v. Instilled volume = 300 μl.
§Aqueous pH 7.4 buffered solution of timolol alone; timolol = 1.4% m/v. Instilled volume = 150 μl.

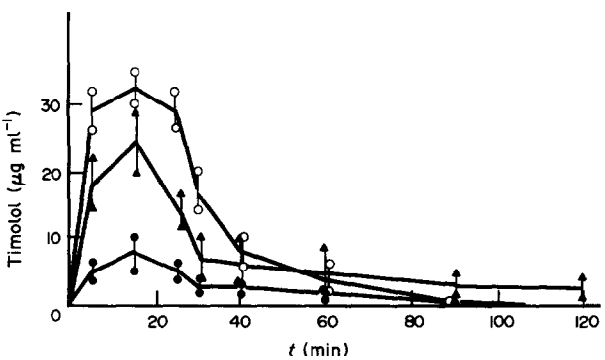

Figure 1
Aqueous humour concentration–time profiles following multiple instillations in rabbits' eyes: ●, timolol alone; ○, timolol as an ion-pair in solution; ▲, timolol as an ion-pair in microemulsion.

were suggested as controlled-release systems for the oral administration of drugs, for i.v. injection and for topical administration [11].

A microemulsion was administered to rabbits to enhance absorption by the cornea and to prolong the time of release of timolol. The microemulsion used in the present work had a physiological surfactant; its formation by self-emulsification as well as the small mean radii measured by photocorrelation spectroscopy showed the presence of a stable, disperse system. The microemulsion could be sterilized by heating at 121°C without compromising the physical and chemical properties of the therapeutic system; this result confirmed the stability of the microemulsion, as did the results of tests using repeated centrifuging and freeze–thaw cycles. The presence of a physiological substance, egg lecithin, as a surfactant, enabled a therapeutic device to be made with good biocompatibility.

The disperse phase of the microemulsion was devised as a reservoir of timolol as a lipophilic ion-pair with octanoate. Indeed, octanoic acid was added also to enhance the solubility of timolol in the oil phase [6]. A timolol concentration higher than that of the saturated aqueous solution was obtained in the microemulsion. At pH 7.4, octanoate and timolol were partitioned between the external phase and the disperse phase of the microemulsion; timolol as an ion-pair with octanoate is likely to be present in the aqueous phase.

The determination of timolol in aqueous humour was performed by HPTLC; this method allowed the rapid separation and determination of timolol in aqueous humour with satisfactory sensitivity, precision and accuracy.

The results *in vivo* suggested that the absorption of timolol from the microemulsion was higher than that from the reference solution. Even if inter-individual differences among the rabbits could be noted, a well defined trend was observed in the concentration–time profiles (Fig. 1). The area under the curve for the microemulsion was almost 3.5 times that of the solution, and the time of absorption was longer. The corneal absorption of timolol from the microemulsion was influenced by two factors: the presence of the lipophilic ion-pair in the external phase enhanced the bioavailability of timolol; and the reservoir effect of the disperse phase prolonged absorption. These results seemed to confirm the previous studies *in vitro* [6].

The solution of timolol as an ion-pair was prepared using a molar concentration of octanoate 10 times that of timolol. Previously [6], it was noted that at this molar ratio, the permeability coefficient of timolol in aqueous solution at pH 7.4 was independent of the concentration of the counter-ion. The results after topical administration of the solution of the ion-pair showed that the area under the curve was increased, compared with that of timolol alone, thus confirming the results *in vitro*. As expected, no prolongation in the absorption times could be noted.

Several authors postulated that the best prediction of the corneal penetration of a drug is its partition between octanol and water [9]; it is also assumed that the charged form of a drug, i.e. the ionized molecule, penetrates the cornea at a very low rate which in practice would be zero.

At pH 7.4, timolol as an ion-pair was more hydrophobic than the drug alone; consequently its o/w partition coefficient was increased [6]. Timolol applied to the cornea as an ion-pair, both in microemulsion and in aqueous solution, was quickly partitioned into the lipid-like epithelial layers of the cornea because of its high o/w partition coefficient [6]. The stroma presents itself as a rate-limiting factor for lipophilic substances, but the already diffused ion-pair was partly dissociated into timolol and octanoate so that the drug could diffuse quite quickly across the stroma. The tiny nanodroplets of the disperse phase remained on the cornea for some time and probably acted as a microreservoir of timolol, giving a sufficiently measurable prolongation of the time of absorption.

Several unwanted side-effects may occur when timolol is used in topical therapy for open angle glaucoma [12], to lower the intraocular pressure. Bradycardia or asthmatic phenomena [13] have often been noted in many patients, since systemic absorption has been shown to occur by conjunctival, nasal and pharyngeal absorption. The enhanced corneal absorption of timolol as an ion-pair might suggest the possibility of lowering the dose to be instilled in topical therapy; reduction of the systemic side-effects [14] might be obtained.

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