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Solubilization of tropicamide by hydroxypropyl-β-cyclodextrin and water-soluble polymers: in vitro/in vivo studies

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Abstract

1% (w/v) aqueous solutions of tropicamide (TR), a poorly water-soluble mydriatic-cycloplegic drug, are usually obtained by adjusting the pH to ~5.0, at the expense, however, of ocular tolerance and bioavailability. The capacity of hydroxypropyl-β-cyclodextrin (HP-β-CD) to solubilize TR in pH 7.4 0.02 M phosphate buffer was investigated in the absence and presence of hydrophilic polymers (PVP, CMC and HPMC). Approximately 3.5% (w/v) HP-β-CD was required to solubilize 1% (w/v) TR in pH 7.4 buffer at room temperature. The required amount was reduced to 0.9% (w/v) by heating at 120°C in the presence of 0.1% (w/v) HPMC. Mydriatic activity tests in rabbits showed an improved bioavailability and maximal mydriatic response for two CD formulations, with and without HPMC, when compared to standard 1% (w/v) TR eyedrops, buffered at pH 5.0. The improved in vivo behaviour of the CD formulations are likely due to their physiological pH, resulting in a reduced irritant effect, although an effect of HP-β-CD on corneal permeability cannot be dismissed a priori.

Keywords: Tropicamide; Hydroxypropyl-β-cyclodextrin; Solubilization; Polyvinylpyrrolidone; Carboxymethylcellulose; Hydroxypropyl methylcellulose; Ophthalmic vehicles; Mydriatic effect; Rabbits

1. Introduction

Tropicamide (TR) is a synthetic mydriatic and cycloplegic drug, commonly used for refractive examinations as 1% (w/v) solution (Hardman et al., 1996). The main pharmaceutical drawback of this drug is its poor water solubility (570 mg/100 ml at 25°C), which can be further decreased by the ingredients used in ophthalmic vehicles (buffers, tonicity-adjusting agents, preservatives, etc.) (Saettone et al., 1988). Since TR is a weak base, its solubility increases as the pH of the

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solution decreases. A 1% (w/v) TR solution in water can be obtained by adjusting the pH to a relatively low value (~ 5.0); this, however, makes it irritant and decreases its ocular bioavailability due to induced lacrimation (Lee and Robinson, 1986). For optimal eye tolerance and activity, the pH value of a 1% (w/v) ophthalmic solution of TR should be set at or near 7.4, which represents physiological conditions. The solubility of tropicamide in pH 7.4 0.02 M phosphate buffer (460 mg/100 ml), however, is even lower than in water.

The solubility of TR might be improved by the use of cyclodextrins (CDs), a group of cyclic oligosaccharides capable of forming inclusion complexes with many drugs. Complexation by CDs affects many physico-chemical properties of drugs, such as solubility and stability (Duchêne and Wouessidjewe, 1990). In ophthalmic preparations, co-administration of CDs has been reported to increase corneal penetration, ocular absorption and the efficacy of poorly water-soluble drugs like dexamethasone (Kristinsson et al., 1996), cyclosporin (Kanai et al., 1989), acetazolamide (Loftsson et al., 1994b), etc. These positive results are attributed to the ability of CDs to increase the aqueous solubility of lipophilic drugs without modifying their molecular structure, i.e. without affecting their intrinsic ability to permeate biological membranes. It is thought that CDs act as true carriers by keeping the hydrophobic drug molecules in solution, and by delivering them to the surface of the biological membrane, e.g. the corneal epithelium, where they partition. The relatively lipophilic epithelium has a low affinity for the hydrophilic CD molecules, which therefore remain in the aqueous membrane exterior, i.e. the tear fluid.

The addition of small portions of water-soluble polymers has been reported to enhance the effect of CDs (Loftsson et al., 1994a), thus allowing reduction of the CD amount required for drug solubilization.

Aim of the present work was to investigate the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD), alone or in association with different water soluble polymers, on the solubility of TR in pH 7.4 buffer. HP-β-CD was selected on account of its good tolerance by albino and pigmented rabbit eyes (Jansen et al., 1990) and of its lack of toxicity to the human corneal epithelium (Saarinen-Savolainen et al., 1998). The tested polymers, all commonly used as viscosity enhancers in ophthalmic formulations, were polyvinylpyrrolidone (PVP), carboxymethylcellulose sodium salt (CMC) and hydroxypropyl methylcellulose 4000 (HPMC). The mydriatic effect of 1% (w/v) TR solubilized by HP-β-CD, both in the presence and absence of HPMC, was evaluated in albino rabbits using commercial 1% (w/v) TR eyedrops as reference.

2. Materials and methods

2.1. Materials

Tropicamide was provided by TCI (Tokyo, Japan). 2-Hydroxypropyl-β-cyclodextrin, DS 0.61 (HP-β-CD) was purchased from Roquette (Lestrem, France). Polyvinylpyrrolidone (PVP) (Kollidon® 17PF, BASF, Ludwigshafen, Germany) and medium viscosity carboxymethylcellulose sodium salt (CMC) were purchased from Sigma (St. Louis, MO). Hydroxypropyl methylcellulose 4000 (HPMC) was obtained from Prodotti Gianni (Milan, Italy). All other chemicals used were of pharmaceutical or analytical grade. Double distilled water was used throughout the study.

2.2. Solubility studies

Solubility studies on TR were performed by the method of Higuchi and Connors, 1965. An excess drug (400 mg) was added to 5 ml portions of pH 7.4, 0.02 M phosphate buffer, each containing a variable amount of HP-β-CD (0–20% (w/v)). The solubility of TR was also assessed after adding to the solutions 0.1% (w/v) HPMC, 0.25% (w/v) PVP or 0.25% (w/v) CMC. These polymer concentrations were indicated as optimal in aqueous cyclodextrin solutions (Loftsson et al., 1994a).

The suspensions were mechanically shaken in a water bath at 25°C until equilibrium was reached (at least 3 days). Alternatively, they were placed in an autoclave in sealed containers, heated to 120°C

for 20 min and then allowed to equilibrate in a shaking bath at 25°C for 6 days. Separate experiments showed that this period of time was sufficient, since longer equilibration times (up to 12 days) did not result in further drug precipitation.

The samples were filtered through a 0.45 μm membrane filter (Millex-HA filter units, Millipore) and suitably diluted with pH 7.4 phosphate buffer for analysis. The TR content was determined by UV spectrophotometry (Philips PU8700 Spectrophotometer) at 256 nm. The presence of HP-β-CD and of the polymers did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate; the coefficient of variation associated with each measurement was never $> 3\%$.

2.3. Rheological measurements

Measurements were performed at 30°C using a Rheomat -115 apparatus equipped with coaxial cylinders (Contraves A.G., Zurich, Switzerland).

2.4. Mydriatic activity tests

The test solutions (4% (w/v) HP-β-CD and 1% (w/v) HP-β-CD plus 0.1% (w/v) HPMC, both containing 1.0% (w/v) TR and 0.01% (w/v) benzalkonium chloride as preservative) were made appropriately isotonic with NaCl (H. Roebling micro-osmometer, Berlin, Germany) then were filtered through 0.2 μm filters (Millipore Millex®-GS) prior to use.

Mydriatic activity tests were carried out on male, non anaesthetised New Zealand albino rabbits (2.0–2.5 kg, Pampaloni Rabbitry, Fauglia, Italy), selected on the basis of their similar response to light intensity and to the mydriatic activity of TR. The animals were used and treated as prescribed in the publication 'Guide for the care and use of laboratory animals' (NIH Publication No. 92–93, revised 1985), were allowed to move their heads freely, and their eye movements were not restricted. The overall procedure and experimental details suggested by Smolen and Schoenwald (1974) and also reported in a previous paper (Saettone et al., 1982) were followed throughout. Each solution was tested on groups of at least nine rabbits; the administered dose was in all cases 10 μl. Commercial 1% (w/v) aqueous TR eyedrops (Visumidriatic 1%, Visufarma s.r.l., Rome, Italy) were used as a reference.

3. Results and discussion

3.1. Solubility studies

The phase solubility diagrams of TR in aqueous HP-β-CD solutions at pH 7.4, with and without addition of polymers (PVP, HPMC or CMC) are shown in Fig. 1. These diagrams refer to unheated solutions: under these conditions the solubility of TR increased linearly as a function of HP-β-CD concentration, but remained unaffected by the addition of polymers. This results in overlaying of the diagrams, which are of A_L type, showing linear patterns (Higuchi and Connors, 1965). The stability constant of the complex drug/CD, assuming a 1:1 stoichiometry ($K_{1:1}$), was calculated from the slope of the line using the Higuchi and Connors equation:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the intrinsic solubility of TR at pH 7.4 (0.0161 mol/L). The value calculated for $K_{1:1}$ was 34.5 M^{-1}.

According to the phase solubility diagram in the absence of polymers (Fig. 2), the amount of

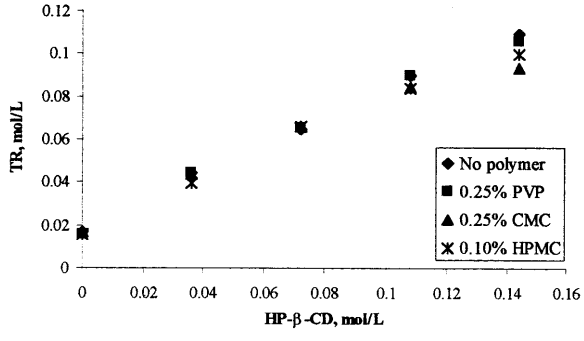

Fig. 1. Phase solubility diagrams of TR in aqueous unheated HP-β-CD solutions at pH 7.4, in the presence or absence of polymers (mean of three experiments, CV < 3%, error bars omitted for clarity).

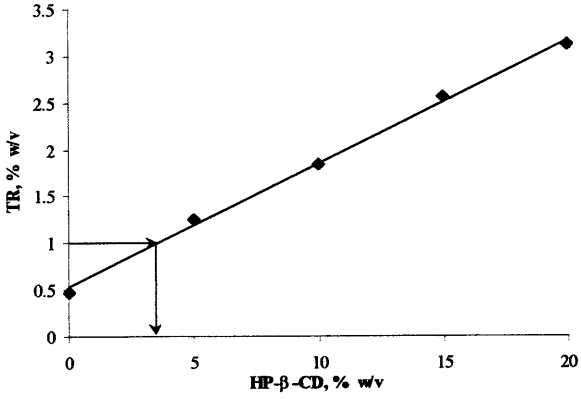

Fig. 2. Solubility of TR (% w/v) in aqueous unheated HP-β-CD solutions at pH 7.4.

HP-β-CD needed to solubilize 1% (w/v) TR in pH 7.4 buffer was ~3.5% (w/v). A 4% (w/v) CD concentration was used in the formulation for in vivo studies, an excess of ~10% being considered necessary to ensure the stability of the solution during storage.

Although this HP-β-CD concentration was relatively low, a further reduction was desirable for a variety of reasons, including toxicological considerations, isotonicity adjustment, drug bioavailability and production costs. According to recent reports (Loftsson and Järvinen, 1999; Savolainen et al., 1998) water-soluble polymers, when activated by heating, may enhance drug solubilization induced by CDs. Accordingly, phase solubility studies were carried out by heating the solutions in autoclave at 120°C for 20 min. It is worth noting that these heating conditions are usually employed to sterilize pharmaceutical preparations.

The results showed that the solubilizing effect of HP-β-CD was unaffected by heating, while it was increased in the presence of polymers. The solubility of TR in 5% (w/v) aqueous HP-β-CD solutions at pH 7.4, heated in autoclave with and without polymers, is illustrated in Fig. 3. PVP did not increase the solubilizing effect of HP-β-CD, whereas CMC, and particularly HPMC, were remarkably effective. According to Brackman and Engberts (1993) and Loftsson and Järvinen (1999), these polymers may interact with drug/CD

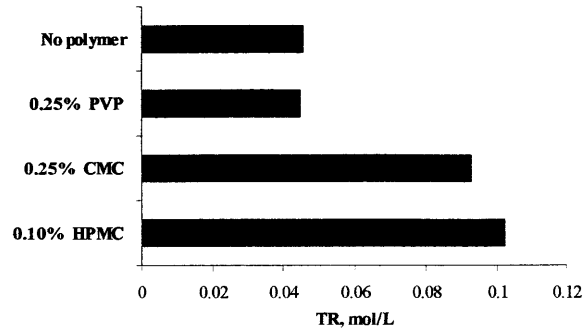

Fig. 3. Effect of polymers on TR solubility in aqueous, pH 7.4, 5% (w/v) HP-β-CD solutions, after heating at 120°C (mean of three experiments, CV < 3%).

complexes in a similar way as with micelles, forming drug–CD–polymer aggregates or a co-complex, i.e. a complex between several drug/CD complexes and a polymer chain. As reported by Fridriksdóttir et al. (1997), these macromolecular clusters show higher stability constants than the simple drug/CD complexes, which accounts for the higher drug solubility.

Since HPMC was most effective in increasing drug solubility after heating, a phase solubility diagram of TR in HP-β-CD solutions (0–20% (w/v)) in pH 7.4 buffer containing 0.1% (w/v) HPMC, heated in autoclave at 120°C for 20 min,

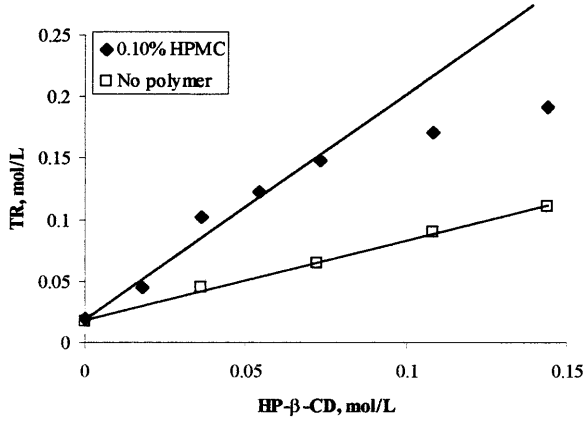

Fig. 4. Phase solubility diagram of TR in aqueous, pH 7.4, HP-β-CD solutions containing 0.1% (w/v) HPMC, after heating at 120°C (mean of three experiments, CV < 3%, error bars omitted for clarity). The phase solubility diagram of the drug in the absence of HPMC is also shown for comparison.

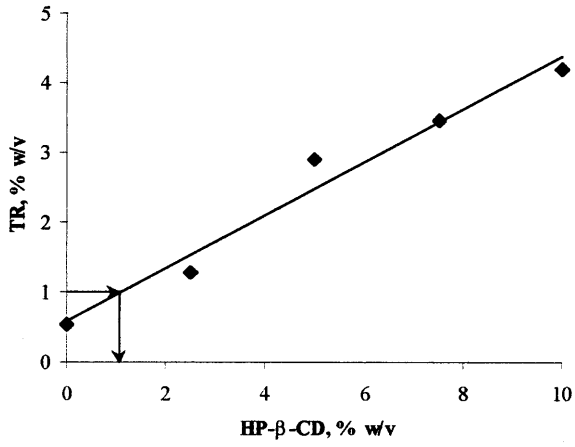

Fig. 5. Solubility of TR (% w/v) in aqueous, pH 7.4, HP-β-CD solutions containing 0.1% (w/v) HPMC, after heating at 120°C.

was constructed (Fig. 4). Under these conditions HPMC alone (in the absence of HP-β-CD) was inactive, whereas it was synergic with HP-β-CD in enhancing TR solubility. Moreover, the presence of HPMC changed the type of phase solubility diagram from A_L to A_N type, showing a negative deviation from linearity. The apparent stability constant of the complex ($K_{1:1}$), calculated from the initial linear portion of the diagram by the Higuchi and Connors equation, was 90.2 M^{-1}. This value was more than 2.5-fold higher than the one obtained in the absence of polymer, or when the polymer was present but the solution was not heated.

As a consequence of the improvement of the drug/CD complexation obtained by adding 0.1% (w/v) HPMC and heating the solutions at 120°C for 20 min, less CD was required to dissolve a given amount of drug. As clearly shown in Fig. 5, the amount of HP-β-CD needed to solubilize 1% (w/v) TR in pH 7.4 buffer containing 0.1% (w/v) HPMC was 0.9% (w/v). A 10% excess CD (i.e. 1%) was used in the solution tested for mydriatic activity. Both CD solutions (with and without HPMC) did not show any cristallization after 6-month storage at room temperature.

3.2. Mydriatic activity tests

The results of the mydriatic activity tests on albino rabbits, summarized in Table 1, indicate that the two CD solutions, containing 4% (w/v) HP-β-CD (2) and 1% (w/v) HP-β-CD plus 0.1% (w/v) HPMC (3), improved to a small but statistically significant extent the bioavailability of TR when compared to the reference solution, RS (1).

The bioavailability increases were associated with an increased duration ($\sim$60 min) of mydriatic effect and by a significantly increased I_{max} (maximal mydriatic response) value. The improved effect of the CD formulations with respect to the reference solution might be attributed to their physiological pH value (7.4 versus 5.0 for RS), resulting in reduced irritation and lacrimation, hence in prolonged retention at the absorption site. Another, albeit less likely reason for the increased TR bioavailability from solutions 2 and 3 might reside in the ability of HP-β-CD to enhance corneal permeability.

The TR formulation 3, when compared with 2, delayed the peak time from 90 to 120 min. This delay cannot be ascribed to viscosity effects, since the CD formulations showed very similar Newton-

Table 1
Summary of the mydriatic activity parameters in rabbits of ophthalmic solutions containing 1% (w/v) TR

Formulation	Peak time (min)	I_{max} (mm ± SEM)	Duration (min)	AUC (cm^2 ± SEM)	Relative AUC
(1) RS[a]	90	2.3 ± 0.09	360	131.9 ± 3.2	1.00
(2) TR 1%+HP-β-CD 4%	90	2.7 ± 0.12[b]	420	150.8 ± 6.0[b]	1.14
(3) TR 1%+HP-β-CD 1%+HPMC 0.1%	120	2.8 ± 0.09[b]	420	174.9 ± 3.8[b]	1.33

[a] RS, reference solution: commercial 1.0% TR eyedrops, pH 5.0.
[b] Significantly different from the RS value ($P<0.05$).

ian viscosities (1.72 and 1.78 mPa.s for 2 and 3, respectively). The delayed peak time might reasonably be attributed to the higher stability constant of formulation 3, resulting in a slower release mode of TR from the TR-CD-HPMC aggregate or co-complex.

4. Conclusions

The results of this study indicate the capacity of HP-β-CD to increase TR solubility in pH 7.4 buffer, through formation of an inclusion complex. The TR solubilizing effect of HP-β-CD was increased by heating at 120°C in the presence of some water-soluble polymers. While PVP was poorly active, CMC and particularly HPMC were particularly effective in assisting TR solubilization by HP-β-CD. As a consequence, in the presence of 0.1% (w/v) HPMC less HP-β-CD (0.9 versus 3.5% (w/v)) was required to solubilize 1% (w/v) TR.

Mydriatic activity tests in rabbits showed an improved bioavailability and maximal mydriatic response for two pH 7.4 formulations containing TR solubilized by HP-β-CD (alone or associated with HPMC) when compared to standard 1% (w/v) TR eyedrops, buffered at pH 5.0. The in vivo data indicated a slightly superior performance (shorter time to peak) for the 4% (w/v) HP-β-CD formulation, when compared to the 1% (w/v) one containing 0.1% (w/v) HPMC. Based on these results, the former TR vehicle appears as the best candidate for ophthalmic administration. Its HP-β-CD content (4% (w/v)), although relatively high, did not apparently induce toxicity or hypertonicity problems.

The improved in vivo behaviour of the CD formulations are presumably due to their physiological pH, resulting in a reduced irritant effect, although an effect of HP-β-CD on corneal permeability cannot be dismissed a priori. Further studies are under way to clarify this point.

Acknowledgements

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