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International Journal of Pharmaceutics 290 (2005) 155–159
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Note

Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system

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Received 2 June 2004; received in revised form 20 October 2004; accepted 29 October 2004
Available online 5 January 2005

Abstract

In the present study chitosan (REVTMbio1) or Carbopol (REVTMbio2 and 3) coated niosomal timolol maleate (0.25%) formulations were prepared by reverse phase evaporation (REV) and compared to timolol solution (TMS; 0.25%) in terms of in vitro release and IOP lowering pharmacodynamic effect. The in vitro release phase of timolol (91% release in 2 h) was extended significantly by its incorporation into niosomes and further by the polymer coating (40–43% release upto 10 h). The developed formulations were evaluated for their pharmacodynamics in albino rabbits, by measuring intraocular pressure (IOP) using a non-contact pneumatonometer, and were compared to a marketed in situ gel forming solution of timolol (Timolet GFS, 0.5%; Sun Pharma). REVTMbio1 formulation showed a more sustained effect of upto 8 h (vis a vis 6 h for carbopol-coated niosomes). TMS in comparison showed effect for only 2 h though the peak effect was slightly more (14%). Lowering of IOP in the contralateral eye (20–40% as compared to 100% in case of TMS), considerably reduces with REV and REVbio formulations indicating lesser systemic side effects. Moreover, the results of REVTMbio1formulation containing 0.25% of timolol maleate compared well with the 0.5% marketed gel formulation, indicating our formulation to be significantly better considering that similar effect is obtained at half the concentration. The later becomes especially important in context to the cardiovascular side effects associated with ocular timolol maleate therapy.

Keywords: Timolol maleate; Niosomes; Bioadhesion; Glaucoma; Intraocular pressure; Corneal permeability

Timolol maleate (TM) is one of the drugs of choice for treatment of open angle glaucoma (Uusitalo et al., 1999). Since excessive loss of drug through nasolacrimal drainage can cause respiratory and cardiovascular side effects (Everitt and Avorn, 1990; Wolfhagen et al., 1998), it is important to minimize the systemic absorption and enhance ocular bioavailability of TM. This problem can be addressed by use of suitable carrier systems. Niosomal vesicular system is one of the potential approaches, which can be suitably used (Kaur et al., 2004; Saettone et al., 1996; Vyas et al., 1998). Even though a controlled release can be expected with

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doi:10.1016/j.ijpharm.2004.10.026

a vesicular system, an increase in precorneal retention (to delay the washout) would make such a system more effective. This will also reduce the amount of drug and the dose frequency necessary for therapeutic effect (Rosenlund, 1996).

Aim of the present study was to develop a suitable niosomal preparation of TM with an optimal ocular pharmacodynamics extended over a prolonged period, and a limited systemic absorption and side effects. The niosomes (REVTM) were coated with three different bioadhesives and their role was evaluated in terms of an improvement in release and prolongation of the pharmacodynamics.

The niosomes were prepared by different methods (using a span 60:cholesterol ratio of 1:1) e.g. hydration, ether evaporation and reverse phase evaporation, reported in the literature (Azmin et al., 1985; Ballie et al., 1985; Szoka and Papahadjopoulos, 1978). Unentrapped drug was removed by ultracentrifugation and the niosomal sediment was resuspended in an equivalent volume of phosphate-buffered saline (PBS). Since in the final formulation (REVTM, selected for the study), approximately 25% (24.3%) of the drug was entrapped, so, even though we started with a 1% TM solution, strength of the final formulation was approximately 0.25% (w/v). An aqueous solution of timolol maleate (TMS) containing an equivalent amount (0.25%, w/v) was thus taken as a control for the developed niosomal formulations.

Primary objectives of achieving effective ocular delivery are prolonged retention and enhanced penetration. Controlled ocular delivery can be attained through different strategies that include the use of bioadhesive polymers, penetration enhancers and the advanced design of micro- and nanoparticulate delivery systems (Zimmer and Kreuter, 1995; Kaur and Smitha, 2002). Use of chitosan as a bioadhesive in development of a new generation of ocular drug delivery systems holds a great promise (Alonso and Sanchez, 2003). It shows penetration enhancing properties and an excellent ocular tolerance in addition to a considerable increase in the corneal residence time (Schipper et al., 1997; Koch et al., 1998; Dodane et al., 1999; Felt et al., 1999). Carbopols is another important class of ocular bioadhesives. Use of Carbopol-coated liposomes has been reported to increase both the residence time and the bioavailability of the entrapped drug (Ayers et al., 1996; Davies et al., 1992; Durrani et al., 1992; Koleng and Mc Ginity, 2001).

Based on this premise the reverse phase evaporation vesicles (REVs) were coated with 0.5% chitosan (REVTMbio1), 0.05% Carbopol 934P (REVTMbio2) and 0.05% Carbopol 974P (REVTMbio3) by incubating pre-formed vesicles at 37 °C in the respective polymer solution for 5 min. The polymer solution was not removed, thus allowing the vesicles to remain dispersed in the polymer solution. This was done considering the fact that solutions of these polymeric mucoadhesives show viscoelastic behaviour. Chitosan and Cabopol solutions have been well-characterized in terms of their pseudoplastic and viscoelastic behaviour (Mucha, 1997; Park and Robinson, 1987). Furthermore, a synergism between rheological behaviour and mucoadhesion of chitosan has also been described (Caramella et al., 1999).

The entrapment efficiency of niosomes prepared by each of the methods was determined by ultracentrifuging the niosomal dispersions at 40,000 × *g* for 30 min. The clear supernatant was analyzed for timolol maleate spectrophotometrically and gave the amount of unentrapped drug. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated.

$$\text{Percentage entrapment} = \frac{\text{entrapped drug (mg)}}{\text{total drug added (mg)}} \times 100$$

In vitro release pattern of niosomal preparations was studied and compared with a 0.25% timolol maleate aqueous solution (TMS) at pH 7.4 using sigma dialysis tubing (Sigma, USA). Niosomal preparation/TMS was taken in the dialysis bag and the bag was placed in a beaker containing 100 ml simulated tear fluid (STF), pH 7.4 (O'Brien and Edelhauser, 1977). The beaker was placed over a magnetic stirrer and the temperature was maintained at 37 ± 1 °C. Two milliliters of samples were withdrawn periodically and were replaced by equal volume of fresh STF. Sink conditions were maintained throughout the experiment. The withdrawn samples were analyzed for drug content spectrophotometrically.

Adult male normotensive rabbits weighing 1.5–2.0 kg were used for the in vivo pharmacodynamic studies as described elsewhere (Aggarwal et al., 2004). The IOP was measured in both the eyes immediately prior to giving the drug ($IOP_{zero\ time}$), and at regular