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Note

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

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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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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

intervals ($IOP_{time,t}$) following the treatment. Each animal was given a washout of three days after every treatment.

Change in IOP (ΔIOP) for each eye is expressed as follows: $\Delta IOP = IOP_{zero\ time} - IOP_{time,t}$; ΔIOP is reported as the mean ($\pm$ S.E.M.) for $n = 6$. Reverse phase method gave the best results in terms of higher entrapment (33 and 50% higher than niosomes prepared by ethanol evaporation and film hydration methods, respectively), and hence was used for further studies. The vesicles obtained from this method upon evaluation with optical microscope revealed large unilamellar vesicles (LUVs) and this explains the reason for its higher entrapment efficiency. Timolol maleate is a water soluble drug and LUVs have a large internal aqueous space relative to the amount of lipid contained in the bilayer. The former can encapsulate high percent of water soluble material within the vesicle.

The colloidal suspensions prepared above had low viscosity (0.8 cps at shear rate of 200), considering that this may not allow sufficient retention of the dosage form in the eye upon instillation, the vesicles were coated with bioadhesive polymers. In vitro release studies of the prepared vesicles show that TMS is released within 1.5 h whereas an extended release (>10 h) was observed in case of vesicle formulations (Fig. 1).

The pharmacodynamic evaluation is presented as the change in IOP (ΔIOP) versus time data (Fig. 2). Instillation of TMS showed a significant IOP lowering effect at 1 and 1.5 h. In case of REVTM (uncoated niosomes) the onset of action started within 1 h and peak effect was obtained at 2 h. Whereas, in case of chitosan-coated vesicles (REVTMbio1) the peak effect was observed in 3 h and it was maintained for upto 8 h. Carbopol-coated formulations (REVTMbio2 and REVTMbio3) also showed a peak effect at 3 h and a significant effect was observed for upto 6 h. Moreover, a more efficient control of IOP was observed for REVTMbio1 at times $\geq$4 h. The low effectiveness of Carbopol(s) compared to chitosan can be explained on the basis that we are using Carbopol at pH 7.4. The pK_a of Carbopol polymer is 6.0 ± 0.5 and above this point the carboxylic acid groups are ionized to a great extent thus reducing H-bonding. It has been reported that pH of the carbopol solution should be kept at/below 6.0 for a significant effect (Ch'ng et al., 1985; Davies et al., 1992; Park and Robinson, 1985). On the other hand chitosan is a polycationic biopolymer obtained by alkaline deacetylation of chitin. Due to its positive charge at neutral pH, an ionic interaction with the nega-

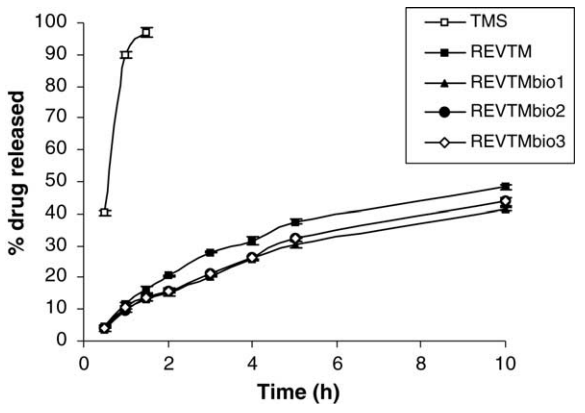

Fig. 1. A plot of percent drug release vs. time, comparing release rate of different formulations of timolol maleate. Note: the apparent permeability coefficient values for all formulations (except for REVTMbio2 and REVTMbio3) are significantly different ($p < 0.05$).

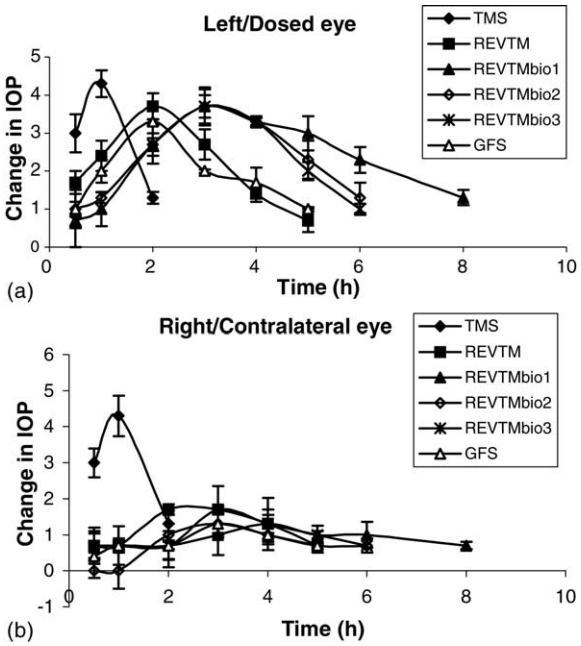

Fig. 2. Change in IOP vs. time for different formulations in (a) dosed eye and (b) contralateral eye. Note: Control IOP was 10 ± 0.7 mmHg ($n = 6$); no significant difference in baseline IOP was observed between both the eyes. All values for all formulations, at each time, are significantly different ($p < 0.05$), except for REVTMbio2, REVTMbio3.

tive charges of sialic acid residues of the mucus occurs (He et al., 1998; Lehr et al., 1993).

However, no significant difference was observed between vesicles coated with Carbopol 934P and 974P. Both the polymer solutions have approximately similar viscosity at 0.05% (w/v) and both can be suitably used in topical preparations (Koleng and Mc Ginity, 2001).

An interesting observation of the study was that upon administration of TMS a corresponding decrease in IOP was also observed in the contralateral eye (extent of lowering being the same; Fig. 2b). The lowering of IOP in the control eye is possibly due to the systemic absorption of TM upon topical instillation into the dosed eye. Thus, extent of reduction in IOP of the control eye can be taken as an indirect measure of the extent of systemic absorption. Fig. 2b indicates a significantly lower fall in IOP of the control eye in case of the developed niosomal (REV) preparations in contrast to that observed with TMS.

IOP measurements with a marketed formulation of TMS (Timolet® GFS, Sun Pharmaceuticals India limited, Mumbai), showed a peak effect at 2 h (compared to 3 h with vesicular formulation), but the effect was not sustained sufficiently and diminished after 5 h. On the other hand chitosan coated vesicles (REVTMbio1) showed an effect which was sustained for upto 8 h (ΔIOP ranging from 3.7 to 2.4 from 2 to 6 h; while for Timolet® GFS ΔIOP was 3.4 at 2 h and reduced to 1.7 at 4 h). Peak effect obtained with REVTM was 3.7 at 2 h while the same peak effect at 3 h was obtained for all the REVTMbio formulations. Moreover, Timolet® GFS is recommended as an once a day (o.d.) formulation and since the duration of effect of the vesicular formulation (REVTMbio1) is greater than that observed with GFS, we can safely propose the vesicular formulations developed by us to be even more effective o.d. formulations (Fig. 2a). Further, it maybe noted that the ΔIOP in the contralateral eye for different vesicular formulations showed a similar pattern, which was comparable to the marketed formulation (Fig. 2b).

Thus, it may be concluded that the chitosan-coated REVTMbio1 formulation was significantly better. Lowering of IOP in the contralateral eye (20–40% as compared to 100% in case of solution), considerably reduces with REV and REVbio formulations in comparison to TMS. Moreover, the REVbio formulations containing 0.25% of timolol maleate compared well with the 0.5% marketed gel formulation, Timolet® GFS, indicating our formulations to be significantly better, considering that, similar effect is obtained at half the concentration. The later becomes especially important in context of the cardiovascular side effects associated with ocular timolol maleate therapy.

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