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COMMUNICATIONS

Topically effective ocular hypotensive acetazolamide and ethoxyzolamide formulations in rabbits

T. LOFTSSON, H. FRIÐRIKSDÓTTIR, E. STEFÁNSSON, S. THÓRISDÓTTIR, Ö. GUÐMUNDSSON, T. SIGTHÓRSSON, *Departments of Pharmacy and Ophthalmology, Faculty of Medicine, University of Iceland, Reykjavik, Iceland*

Abstract—The effect of topically active 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) eye-drop formulations containing solutions of acetazolamide, ethoxyzolamide or timolol on the intra-ocular pressure (IOP) was investigated in normotensive conscious rabbits. Both acetazolamide and ethoxyzolamide were active but their IOP-lowering effect was less than that of timolol. The IOP-lowering effects of acetazolamide and ethoxyzolamide and that of timolol appeared to be to some extent additive. Combination of acetazolamide and timolol or ethoxyzolamide and timolol in one HP-β-CyD formulation resulted in a significant increase in the duration of activity compared with HP-β-CyD formulations containing only acetazolamide, ethoxyzolamide or timolol. Also, it was possible to increase the IOP-lowering effect of acetazolamide by formulating the drug as a suspension in an aqueous HP-β-CyD vehicle.

Orally administered carbonic anhydrase inhibitors, such as acetazolamide and ethoxyzolamide, are very effective in lowering the intraocular pressure (IOP) in glaucoma patients, but, due to their side-effects, patient compliance is often poor. It is generally recognized that topical carbonic anhydrase-inhibitor formulations, possessing similar efficacy to the oral formulations would be a significant advancement in the treatment of glaucoma. The water-insolubility of acetazolamide and ethoxyzolamide limits their ocular bioavailability, but formulations that increase the drug contact time with the eye cornea, e.g. contact lenses and gels, have been shown to be topically active (Friedman et al 1985; Tous & Nasser 1992).

2-Hydroxypropyl-β-cyclodextrin (HP-β-CyD) is a non-toxic β-cyclodextrin derivative which is capable of forming water-soluble inclusion complexes of many water-insoluble drugs (Pitha et al 1986; Loftsson et al 1991a). Under normal conditions the large and very hydrophilic HP-β-CyD molecules do not penetrate biological membranes but act as penetration enhancers by assuring constant high concentration of dissolved drug at the membrane surface (Szejtli 1988; Loftsson et al 1991b). Thus, it is believed that HP-β-CyD improves ocular bioavailability of drugs by keeping the water-insoluble drug molecules in solution and delivers them to the surface of the corneal barrier where they partition into the eye. Previously we have shown that HP-β-CyD forms water-soluble inclusion complexes with both acetazolamide and ethoxyzolamide, and that through the complexation, topically active drug solutions could be obtained (Loftsson et al 1994). The purpose of this present investigation was to try to improve the IOP-lowering effect even further by formulation of acetazolamide in a suspension or by combination with timolol maleate.

Materials and methods

Materials. Acetazolamide was obtained from Agrar (Rome, Italy), ethoxyzolamide from Sigma Chemical Co. (St Louis, MO, USA), timolol maleate from Industrie Chimiche Italiane S.P.A. (Milan, Italy), HP-β-CyD of molar substitution 0·6 from Wacker-Chemie (Munich, Germany) and hypromethylcellulose from Mecobenzon (Copenhagen, Denmark). Alcaine was obtained from Alcon Laboratories (Fort Worth, TX, USA). All other chemicals used were of pharmaceutical or special analytical grade.

Preparation of eye-drop solutions. Acetazolamide (1 or 2 g) or ethoxyzolamide (0·3 g) and timolol maleate (0·68 g) were dissolved in 100 mL of a vehicle consisting of HP-β-CyD (12·5% (ethoxyzolamide), 16% (acetazolamide) or 20% (preparations containing timolol maleate)), hydropromellulose (0·1%), benzalkonium chloride (0·01%), sodium edetate (0·05%) and sodium chloride (0·46, 0·36 and 0·12% for HP-β-CyD concentrations of 12·5, 16 and 20%, respectively), all weight-volume percents, in water. The solutions were sterilized in an autoclave (120°C for 20 min). The isotonicity was monitored with an automatic osmometer from Knauer (Germany) and the pH of the final solutions (or 2% acetazolamide suspension) was about 5.

IOP studies. English brown rabbits (0·5–1·5 years old) of either sex were used and kept in individual cages with free access to food and water. A Digital Mices one pneumatonometer (Bio-Rad, USA) was used to determine the IOP in the unrestricted, conscious rabbits. Before each measurement, one drop of Alcaine (containing 5 mg proxymetacaine mL^{-1}) solution was administered to the eye as local anaesthetic. A single 50-μL drop was administered to one eye at time 0 and the IOP measured at various time intervals after the administration. For each drug tested, no animal was used more than twice with a resting period between experiments of at least three days. The results are expressed as the difference in mmHg from the pH-treatment value ($22{\cdot}79 \pm 0{\cdot}34$ mmHg). Each value represents the mean of ten experiments ± s.e.m.

Results and discussion

The stability constants for the 1:1 drug-HP-β-CyD complexes were previously determined from phase-solubility diagrams to be 85 and 685 M^{-1} for acetazolamide and ethoxyzolamide, respectively (Loftsson et al 1994). Addition of the water-soluble timolol salt to the aqueous 20% (w/v) HP-β-CyD solution resulted in only 4–5% reduction in the complexation. In normotensive, conscious, English brown rabbits both acetazolamide and ethoxyzolamide had significant IOP-lowering effects (Table 1). The maximum decline after topical administration of one drop (50 μL) of 1% acetazolamide solution or one drop of 0·3% ethoxyzolamide solution was 2·6 mmHg, the maximum effect was 1–2·5 h after the administration and the duration of activity was almost 4 h. The duration was increased up to about 8 h when acetazolamide was administered in the form of a 2%

Correspondence: T. Loftsson, Department of Pharmacy, PO Box 7210, University of Iceland, IS-127 Reykjavik, Iceland.

Table 1. Effect of one drop of aqueous HP-β-CyD solution containing timolol maleate equivalent to 0·5% (w/v) timolol, 1% (w/v) acetazolamide, 2% (w/v) acetazolamide in suspension, 1% acetazolamide and timolol maleate equivalent to 0·5% (w/v) timolol, 0·3% (w/v) ethoxyzolamide, or 0·3% (w/v) ethoxyzolamide and timolol maleate equivalent to 0·5% (w/v) timolol on the intraocular pressure (IOP) of normotensive, conscious, English brown rabbits. Results are expressed as the difference in mmHg from the treatment value (22·79 ± 0·34 mmHg). Each value represents the mean of ten experiments ± s.e.m.

Time (h)	Timolol maleate (0·5% timolol)	1% Acetazolamide	2% Acetazolamide suspension	1% Acetazolamide and 0·5% timolol	0·3% Ethoxyzolamide	0·3% Ethoxyzolamide and 0·5% timolol
0	0		0	0	0	0
0·5	−2·02 ± 0·84[e]	−0·45 ± 0·49[c]	−1·3 ± 0·43[c]	−2·7 ± 0·97[c]	−0·30 ± 0·99[a]	−3·06 ± 0·49[e]
1	−3·94 ± 0·73[e]	−2·11 ± 0·57[e]	−2·52 ± 0·58[e]	−3·00 ± 0·73[e]	−2·23 ± 0·87[e]	−4·51 ± 0·85[e]
1·5	−4·36 ± 0·87[e]	−1·97 ± 0·76[e]	−3·00 ± 0·55[e]	−3·65 ± 1·37[e]	−2·63 ± 0·70[e]	−5·20 ± 0·77[e]
2	−5·17 ± 0·82[e]	−2·39 ± 0·76[e]	−3·08 ± 0·77[e]	−4·70 ± 1·55[e]	−2·40 ± 0·54[e]	−5·25 ± 0·80[e]
2·5	−2·90 ± 1·05[e]	−2·64 ± 0·95[e]		−3·78 ± 0·72[e]	−0·98 ± 0·57[e]	−5·61 ± 0·72[e]
3	−2·12 ± 0·76[e]	−1·69 ± 0·66[e]	−2·78 ± 0·61[e]	−2·29 ± 0·74[e]	−0·51 ± 0·77[b]	−3·01 ± 0·95[e]
3·5	−1·95 ± 0·92[e]	−1·70 ± 0·87[e]		−1·99 ± 0·68[e]	+0·00 ± 0·98	−3·09 ± 1·13[e]
4	−1·50 ± 1·01[e]	+0·10 ± 0·44[a]	−2·64 ± 0·48[e]	−1·33 ± 0·92[e]		−3·19 ± 0·64[e]
4·5	+0·62 ± 0·56			−1·32 ± 0·86[e]		−1·03 ± 0·64[e]
5			−2·01 ± 0·56[e]	−1·10 ± 0·94[d]		−1·70 ± 0·40[e]
6			−1·71 ± 0·74[e]			
8			−0·02 ± 1·35			

[a] Significantly reduced from baseline by paired t-test ($P < 0·1$). [b] Significantly reduced from baseline by paired t-test ($P < 0·05$). [c] Significantly reduced from baseline by paired t-test ($P < 0·01$). [d] Significantly reduced from baseline by paired t-test ($P < 0·005$). [e] Significantly reduced from baseline by paired t-test ($P < 0·001$).

suspension ($P < 0·001$ by paired t-test). In the suspension, about 50% of the drug was in solution and about 50% in a solid form resulting in a sustained drug release. The maximum decline after administration of one drop of the suspension was about 3 mmHg. Timolol had a much larger IOP-lowering effect than the two carbonic anhydrase inhibitors. The maximum decline after topical administration of one drop of 0·5% timolol in the form of timolol maleate solution was about 5 mmHg, but the duration of activity was less than 4·5 h.

In combination formulations, the IOP-lowering effects of the carbonic anhydrase inhibitors and timolol appeared to be to some extent additive. Thus, the IOP decline after topical administration of one drop of an aqueous HP-β-CyD formulation containing both 1% acetazolamide and 0·5% timolol was significantly larger than the one obtained after a HP-β-CyD formulation containing only 1% acetazolamide ($P < 0·001$). Similar results were obtained when a formulation containing both 0·3% ethoxyzolamide and 0·5% timolol was compared with a formulation containing only 0·3% ethoxyzolamide ($P < 0·001$). For a formulation containing both 0·3% ethoxyzolamide and 0·5% timolol, the maximum decline was 5·6 mmHg at 2·5 h after the drug administration and this represents a reduction of 25% from the mean baseline value of 22·79 mmHg in normotensive rabbits. The duration of activity was significantly increased compared with the activity of the single agent formulations, being for both combination formulations more than 5 h (the last recorded time point).

In conclusion, both acetazolamide and ethoxyzolamide are topically active IOP-lowering agents when formulated in aqueous HP-β-CyD eye-drop formulations but they are less active than timolol. It is possible to increase their effect either by formulating the drugs as suspensions or by combination with a topically active β-blocker such as timolol.

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