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Formulation and evaluation of ophthalmic preparations of acetazolamide

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Abstract

The orally administered acetazolamide has a limited use in glaucoma due to the systemic side effects associated with its use. It has been reported to show little effect on the intraocular pressure (IOP) of human and rabbit eyes upon topical application, probably owing to its poor bioavailability and instability at pH > 5.0. In order to enhance the bioavailability of the drug, contact time between the drug molecules and the ocular surface was increased using high viscosity, water soluble polymers (PVA, HPMC), and by incorporating acetazolamide into an in situ-forming ophthalmic drug delivery system. Moreover, a penetration enhancer (EDTA) was also used in these formulations to increase the extent of absorption of the drug. Acetazolamide at a concentration of 10% was used and the formulations (eyedrop suspensions) were evaluated for their in vitro release pattern. The effect of these formulations on the IOP in normotensive conscious rabbits was also investigated. These formulations were found to be therapeutically effective with a peak effect at 2 h. A fall in IOP of up to 46.4% was observed with repeated administration of one of the formulation containing PVA, EDTA and Tween 80 (MK-5). Results indicated that a topical effect of acetazolamide can be observed if the formulation, (a) contains a suitable polymer—to increase the residence time; (b) a penetration enhancer—as acetazolamide has a low permeability coefficient i.e. 4.1×10^{-6} cm/s [Duffel, M.W., Ing. I.S., Segarra, T.M., Dixson, J.A., Barfknecht, C.F., Schoenwald, R.D., 1986. J. Med. Chem. 29, 1488–1494]; and (c) pH of the formulation is maintained at the point of maximum stability (pH ≤ 5.0).

Keywords: Acetazolamide; Intraocular pressure; Topical preparation; Polymers; Penetration enhancer

1. Introduction

Acetazolamide (a carbonic anhydrase inhibitor, CAI) is used orally for the reduction of intraocular pressure (IOP) in patients suffering from glaucoma. It is used to relieve the acute symptoms of open-angle glaucoma, prolong the onset of blindness in persons with advanced glaucoma and reduce IOP preoperatively by reducing the aqueous humour formation. To obtain the desired lowering in IOP, large oral doses of acetazolamide are

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used, and this usually leads to systemic side effects, the most common of which are diuresis and metabolic acidosis. To reduce or abolish these systemic side effects, a number of scientists sought to develop an effective topical CAI. However, in studies performed in the 1950s acetazolamide administered topically, subconjunctivally, intravitreally, or by iontophoresis failed to reduce IOP in experimental animals (Green and Leopold, 1955; Foss 1955; Friedenwald, 1955). The other commercially available CAIs, like methazolamide, ethoxzolamide and dichlorphenamide were equally ineffective when administered topically. As these four drugs failed to reduce IOP despite different physicochemical properties, many scientists concluded that it would be impossible to develop an effective topical CAI. Thus, research on this subject was largely abandoned for more than 20 years.

Over the past few years, however, there has been a renewed interest in topical CAIs. It now appears that the therapeutic approach of CAIs has been limited by inadequate penetration of the drug moiety to reach the active sites in the ciliary epithelium (Maren and Conroy, 1993). To improve penetration and therapeutic effectiveness, many of these drugs have been administered after structural modifications (Stein et al., 1983; DeSantis et al., 1986; Surgue et al., 1990; Balfour and Wilde, 1997), in high concentration (Flach and Peterson, 1981), in multiple doses (Flach et al., 1984; Lewis et al., 1984), in gel vehicle (Putnam et al., 1987; Rozier et al., 1989; Tous and Abd-El Nasser, 1992), as water soluble salts (Lotti et al., 1984), with a high water content lenses (Friedman et al., 1985), in liposomal forms (El-Gazayerly and Hikal, 1997) and as aqueous solutions containing cyclodextrins (Loftsson et al., 1994a Loftsson et al., 1994b; Fridriksdottir et al., 1997).

Amongst the available CAIs, acetazolamide is still systemically the most effective drug in the treatment of glaucoma. Maus et al. (1997) found that 2% dorzolamide hydrochloride (newer CAI), when applied topically, was not as effective as systemically administered acetazolamide. Dorzolamide is already being marketed as topical eyedrops, under the trade name of Trusopt® by Merck and Co., for the treatment of glaucoma. Several attempts have been made to formulate acetazolamide in a topical preparation, e.g. (a) topical administration of 10% acetazolamide in water loaded pigmented rabbits (Flach and Peterson, 1981); (b) using high concentration (10%) of topical acetazolamide in combination with systemically administered acetazolamide (Flach et al., 1984); (c) topically active surfactant gel preparation (Tous and Abd-El Nasser, 1992) of acetazolamide; (d) contact lenses containing acetazolamide (Friedman et al., 1985); and (e) topically applied aqueous 1% acetazolamide solution using 2-hydroxypropyl-β-cyclodextrin (Loftsson et al., 1994a). Recently, El-Gazayerly and Hikal (1997) have successfully prepared acetazolamide liposomes as an ocular delivery system.

Various water soluble viscosity increasing polymers and permeation enhancers have been investigated and have been found to enhance corneal drug absorption. This is achieved through their ability to increase the residence time at the corneal and conjunctival surfaces and increase transcorneal flux of hydrophilic drugs through loosening of the tight junctions of the corneal epithelium, respectively. Hydrogel polymers like HPMC and PVA have been reported to improve solubility of various drugs (Loftsson et al., 1996), in addition to increasing its residence time. Topical bioavailability of ophthalmic drugs have been reported to improve by enhancing the corneal permeability with appropriate substances (EDTA, BAC) known as penetration enhancers or absorption promoters (Lee, 1990, 1993a,b; Ashton et al., 1991; Liaw and Robinson, 1993; Saettone et al., 1986).

The aim of this work was to formulate polymeric suspensions of acetazolamide to be applied topically and to evaluate the in vitro and in vivo performance of these formulations.

2. Materials and methods

2.1. Materials

Acetazolamide (supplied by Shallaks Pharma-

ceuticals, New Delhi, as a free gift), polyvinyl alcohol (PVA, M wt. App. 125 000; S.D. Fine Chem.), hydroxypropyl methylcellulose (HPMC, Methocel K4M; Cobrcon Asia, Bombay), sodium alginate (S.D. Fine Chem.), disodium ethylenediaminetetraacetic acid (EDTA disodium, Chem. Div. Glaxo, Bombay), benzalkonium chloride (BAC, Loba Chemie, Bombay), cellophane membrane (a cellulose membrane film, av. cut off 12 000 D obtained as a free gift from UCB Cellophane, UK).

2.2. Preparation of polymeric suspensions

A high concentration of acetazolamide was used since the drug has a very low aqueous solubility (0.7 mg/ml) and also a lower penetration coefficient. So we proposed, that, if a large concentration of drug (say 10%) was made available at the corneal surface, in a suspended form, along with a viscosity imparting agent which would increase the residence time of the drug, then more drug would pass through the cornea and inhibit the carbonic anhydrase. It may be added here, that an inhibition of at least 99% or even more (Brechue, 1994) of the carbonic anhydrase activity is essential to show the IOP decreasing response. Furthermore, there is evidence that topical acetazolamide at a 10% concentration has the ability to lessen the increase in IOP after water loading in pigmented rabbits (Flach and Peterson, 1981; Flach et al., 1984) and acetazolamide at a 5–7% in sodium carboxymethyl cellulose gel showed a significant reduction in IOP of both normotensive rabbits and humans, but lower concentrations were ineffective (Tous and Abd-El Nasser, 1992). Formulations MK-1 to MK-9 (Table 1) were prepared as follows:

MK-1: Acetazolamide was suspended in a solution of Tween 80 (1%) in triple distilled water and was stirred for 3 h.

MK-2: Acetazolamide was suspended in a solution of Tween 80 and EDTA disodium in triple distilled water and was stirred for 3 h.

MK-3: Acetazolamide was suspended with the help of Tween 80 in the polymer solution and the volume was made up with triple distilled water. The resulting formulation was then stirred for 3 h.

MK-4, MK-5, MK-6, MK-7, MK-8: Acetazolamide was dispersed in the solution of Tween 80 and permeation enhancer. This was then poured into the polymer solution. The volume was made up with triple distilled water and the mixture was stirred for 3 h.

MK-9: Sodium acetazolamide solution was prepared by dissolving acetazolamide in 0.75 M NaOH.

Table 1
Composition of formulations

Formulation	Polymer (%)	Permeation enhancer (%)	Acetazolamide (%)	Tween 80 (%)[b]	pH
MK-1	–	–	10.0	1.0	5.00
MK-2	–	EDTA disodium (0.5)	10.0	1.0	4.30
MK-3	PVA (2.0)	–	10.0	1.0	4.90
MK-4	PVA (2.0)	EDTA disodium (0.5)	5.0	1.0	4.85
MK-5	PVA (2.0)	EDTA disodium (0.5)	10.0	1.0	4.95
MK-6	HPMC (1.0)	EDTA disodium (0.5)+BAC (0.02)[a]	10.0	1.0	4.90
MK-7	HPMC (1.0)	BAC (0.02)[a]	10.0	1.0	4.85
MK-8	Alginate sodium (1.0)	EDTA disodium (0.5)	10.0	1.0	4.80
MK-9	–	–	10.0	–	9.60

[a] BAC is incorporated in the formulation containing HPMC as a preservative. In addition, it is also reported to enhance permeation of drugs.

[b] Tween 80 (1.0%) was used in all the preparations except MK-9 as the suspending agent. Concentrations of Tween 80, BAC, PVA, and HPMC used are well within the specified limits for use in the ocular preparations and have been reported to be well tolerated by the cornea.

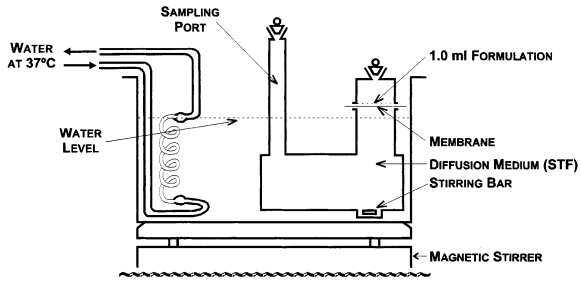

Fig. 1. Diffusion cell assembly.

2.3. Drug release studies

For the in vitro release studies of acetazolamide, membrane diffusion technique (Cohen et al., 1997) was used. The studies were conducted within a cell, maintained at a constant temperature (37 ± 0.5°C), under mixing conditions using a magnetic stirrer. The cell is a 135 ml two limbed reservoir (Fig. 1) immersed in a constant temperature waterbath. One limb of the cell was covered with the pre-treated cellophane membrane (rinsed in acetone and soaked for 24 h in the diffusion medium). The formulation (1 ml) to be studied was placed on the membrane. The diffusion medium used was freshly prepared simulated tear fluid (STF), equilibrated at 37 ± 0.5°C. STF was prepared using NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2 \cdot 2H_2O$ 0.008 g and water up to 100.0 g (Rozier et al., 1989). The pH of the solution was maintained between 7.2 and 7.4 by passing CO_2. Aliquots (4.0 ml) of the medium were withdrawn periodically from the sampling port and were replaced with an equal quantity of fresh STF to maintain a constant volume. Sink conditions were maintained throughout the study. Samples were analysed spectrophotometrically at 265 nm. The solubility of acetazolamide in STF was 1.9 mg/ml at 37°C.

2.4. Particle size determination

The particle size of all the formulations was determined using light microscope. Hundred particles (in different fields) were observed per sample for their size distribution after vortexing the formulation for 1 min.

2.5. In vivo studies

Adult male normotensive (18.1 ± 1.6 mm Hg) rabbits weighing 1.5–2.0 kg were used. The rabbits were provided with food and water ad libitum in a temperature-controlled room (18–24°C). They were exposed to 12 h light:12 h dark cycles. IOP was measured using a Schiötz tonometer, by the same operator, using the same tonometer after instilling a drop of sensorcaine (a local anaesthetic, 0.5%). All the measurements were made three times, at each interval, and a mean of these was taken. All measurement periods began during the same hour on each day. The animals used were accustomed to the experimental procedure. The only restraint was the hands of the investigator lightly laid on the back and shoulders of the rabbits. Rabbits that showed a consistent difference in IOP between the eyes during the baseline measurements or any signs of eye irritation were excluded from the study. Formulations were instilled topically into the upper quadrant of the eye and the eye was manually blinked three times; one eye received 50 μl of the suspension, and the contralateral eye served as the control. IOP was measured immediately, prior to giving the drug and at 15, 30, 60, 120 and 180 min following the treatment. Each formulation was tested on a group of at least six healthy male rabbits. Each animal was given a washout period of 6 days after every treatment.

Repeated administration: After measuring the baseline IOP, 50 μl of MK-5 was instilled into the right eye for five times at an interval of 5 min. Then IOP was measured at 15, 30, 60, 120 and 180 min from the time of instillation of the last drop. The change in IOP (ΔIOP) was determined according to the following equation:

$$\Delta\text{IOP} = \text{IOP}_{\text{Dosed eye}} - \text{IOP}_{\text{Control eye}}$$

2.6. Data analysis

A one-way analysis of variance followed by Dunnet's t-test was used to analyse the data at each time point. Significance was considered at $P < 0.05$. Results are expressed as mean ± S.E.M.

3. Results and discussion

3.1. Particle size determination

The formulations upon evaluation with optical microscope revealed that 50–60% of the particles fall in the range of less than 10 μm. There were some particles (8–10%) even bigger than 50 μm. The percentage of such particles was fairly low and the formulations were found to be nonirritating to the eye. Furthermore, the bigger particles are better retained in the cul-de-sac, thus increasing the bioavailability of the drug. They are also reported to give a slow release effect (Hecht, 1995).

3.2. pH

The eye tolerates a fairly wide range of pH in a solution at the time of its instillation (Riegelman and Sorby, 1966). As acetazolamide is reported to be unstable beyond pH 5.0, thus we did not compromise the stability of the drug and formulated various preparations without using any buffer. All the formulations had a pH between 4.3 and 5.0, the pH of maximum stability (Lloyd and Martin, 1996). A failure to formulate an effective topical preparation of acetazolamide till date, could probably be due to the use of its sodium salt, which although highly soluble, has an alkaline pH ($\cong 9$) and at this pH the drug degrades rapidly (Parasrampuria, 1993).

3.3. Eye irritation

The potential ocular irritancy and/or damaging effects of the formulations under test were evaluated by observing them for any redness, inflammation, or increased tear production. Each formulation was tested on three rabbits, the treatment was performed by a single instillation (50 μl) of the solution (or suspensions) under test into the conjunctival sac of one eye. Both eyes of the rabbits under test were examined for any signs of irritation before treatment, and 30 min, 1, 2 and 3 h after instillation. It was observed that with the exception of formulation MK-9 none showed any redness, inflammation or increased tear production. MK-9 was a solution of acetazolamide in sodium hydroxide (pH 9.6) that led to an increased tear production. The reason for this could be the hypertonicity of the solution. Stein et al. (1983) have reported that a 10% solution of acetazolamide in NaOH has a tonicity of around 1000 mosm per l.

3.4. In vitro diffusion studies

In vitro release experiments for all the formulations showed a linear relationship between the percent release versus time. In vitro release (corresponding $T_{25\%}$ (h) values are shown in Table 2) for the formulations containing PVA were found to be faster ($T_{25\%} = 9.2 \pm 0.2$) in comparison to those containing HPMC ($T_{25\%} = 11.7 \pm 0.3$). Sodium alginate was found to have the slowest release ($T_{25\%} = 18.8$), probably due to the tendency of alginates to undergo gelation when in contact with STF, thus entrapping the drug molecules within the polymeric matrix and hindering the release (Cohen et al., 1997). In vitro release, however, was slow in all the formulations, 15–20% of the drug being released to the diffusion medium in 6 h. The difference in release rate could be due to the difference in the viscosity or the difference in the solubility of acetazolamide in different vehicles.

3.5. Biological studies—IOP determination

The change in IOP versus time data of various formulations is tabulated in Table 3. Administration of 50 μl of a 10% suspension of acetazolamide (MK-1 using Tween 80 as a dispersing

Table 2
Time taken for the release of 25% of the drug ($T_{25\%}$) into the STF in in vitro diffusion studies for various formulations

Formulation	MK-1	MK-2	MK-3	MK-4	MK-5	MK-6	MK-7	MK-8
$T_{25\%}$ (h)	9.2	9.0	9.2	9.4	9.1	12.1	11.4	18.8

Table 3
Effect of topically administered acetazolamide on the IOP in normotensive rabbits

Treatment	ΔIOP ± S.E.M. (mmHg) before treatment	ΔIOP ± S.E.M. (mmHg) at various time intervals after drug administration				
		15 min	30 min	60 min	120 min	180 min
Control	0 ± 0.4	−0.1 ± 0.9	+0.0 ± 0.4	−0.0 ± 0.6	−0.8 ± 0.6	+0.6 ± 0.7
MK-1	−0.6 ± 0.7	−0.8 ± 0.6	−0.0 ± 0.4	−0.3 ± 0.5	−0.3 ± 0.5	−0.0 ± 0.6
MK-2	0 ± 0.6	−2.0 ± 0.8	−4.1 ± 0.6	−2.8 ± 0.1	−2.3 ± 0.6	−0.7 ± 0.3
MK-3	0 ± 0.9	−1.7 ± 0.9	−2.3 ± 0.4	−2.7 ± 0.5	−3.6 ± 0.6	−3.0 ± 0.4
MK-4	0 ± 0.8	−0.8 ± 0.4	−1.6 ± 0.4	−3.8 ± 0.3	−4.8 ± 0.5	−1.1 ± 0.8
MK-5	0 ± 0.4	−3.3 ± 0.6	−3.6 ± 0.8	−4.2 ± 0.4	−5.8 ± 0.5	−5.0 ± 0.6
MK-5*	−0.0 ± 0.6	−2.6 ± 0.8	−4.0 ± 0.6	−4.4 ± 0.5	−5.0 ± 0.4	−4.9 ± 0.6
MK-5*	−0.6 ± 0.8	−6.4 ± 0.9	−7.0 ± 0.9	−8.1 ± 0.9	−7.9 ± 0.9	−3.5 ± 0.5
MK-6	−0.3 ± 0.3	−1.7 ± 0.4	−2.6 ± 0.4	−3.3 ± 0.8	−4.7 ± 0.3	−3.1 ± 0.7
MK-6*	0 ± 0.4	−1.6 ± 0.4	−3.1 ± 0.3	−3.9 ± 0.6	−3.8 ± 0.4	−3.3 ± 0.8
MK-7	−0.6 ± 0.8	−0.1 ± 0.9	−2.6 ± 0.7	−3.7 ± 0.4	−3.9 ± 0.6	−2.0 ± 0.7
MK-8	−0.3 ± 0.8	−0.4 ± 0.3	−3.6 ± 0.3	−3.9 ± 0.4	−4.7 ± 0.7	−3.6 ± 0.8
MK-8*	−0.1 ± 0.6	−3.1 ± 0.3	−4.0 ± 0.7	−4.1 ± 0.4	−4.5 ± 0.6	−3.3 ± 0.6
MK-9	−0.3 ± 0.3	−0.2 ± 0.6	−1.3 ± 0.8	−1.7 ± 1.1	−2.2 ± 0.8	−1.1 ± 0.4

* For each treatment group and at all time intervals $P < 0.05$ except for MK-1 and MK-9.

agent) had no significant effect on the IOP of a normotensive rabbit (Fig. 2). The formulation containing the permeation enhancer EDTA (MK-2) indicated a marked decrease of up to 4.1 ± 0.6 mmHg at 30 min, thus showing a rapid and a higher response, indicative of a faster and a better absorption of the drug. EDTA, a calcium chelator mainly active on the tight junctions is reported to produce ultra structural changes in corneal epithelium, resulting in a water influx and a decrease in the overall lipophilic characteristics. The strategy of decreasing the lipophilicity of the corneal epithelium by addition of permeation enhancers has already been developed and is achieving success. The formulation MK-3 containing 2% PVA in addition, showed a significantly higher response with respect to MK-1, thus indicating that an increase in viscosity, can lead to an increased contact time, slower elimination and, hence, a better transcorneal penetration of the drug into the anterior chamber.

Instillation of a 50 μl freshly prepared solution of acetazolamide in NaOH–MK-9 (pH 9.6) also led to a small but significant effect starting from 30 min onwards with a peak effect of 2.2 mmHg (reduction in IOP) after 2 h of instillation. Earlier workers have reported negative results with the solution of acetazolamide either because they have tried lower concentrations or due to the fast degradation of the drug at a pH > 8.0, if the solution was not freshly prepared (Parasrampuria, 1993). Moreover, since acetazolamide is in its anionic form at pH 9.6, it is possible that the small IOP lowering effect of the aqueous 10% acetazolamide solution is due to the inability of the anionic form to penetrate into the eye. Furthermore, this solution does not contain any viscosity-increasing polymer and the pH 9.6 solution

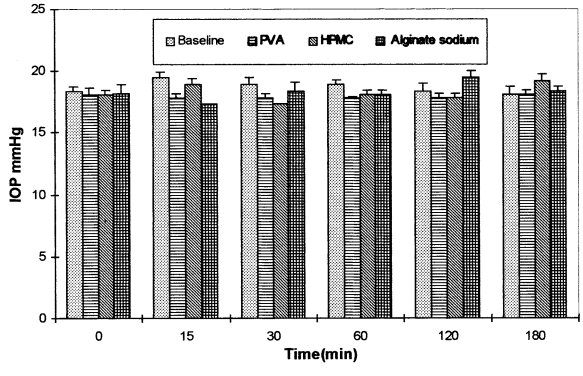

Fig. 2. Effect of different vehicles at various time intervals on IOP of normotensive rabbits. Values are mean ± S.E.M., $n = 6$; $P > 0.05$ for all formulations at all time intervals.

Table 4
Summary of activity parameters[a] of various formulations of acetazolamide in normotensive rabbits

Formulation	T_{max} (min)	$\Delta IOP_{max} \pm$ S.E.M. (mmHg)
MK-1	15	0.8 ± 0.6
MK-2	30	4.1 ± 0.6
MK-3	120	3.6 ± 0.6
MK-4	120	4.8 ± 0.5
MK-5	120	5.8 ± 0.5
MK-5*	60	8.1 ± 0.9
MK-6	120	4.7 ± 0.3
MK-7	120	3.9 ± 0.6
MK-8	120	4.7 ± 0.7
MK-9	120	2.2 ± 0.8

[a] ΔIOP_{max}, maximum reduction in IOP; T_{max}, time required to reach the peak effect.

may rapidly be washed from the surface of the eye (due to the increased tear production) thus producing a relatively small IOP lowering effect.

Three polymers were selected for the study—PVA, HPMC and sodium alginate, as these water-soluble polymers are reported to improve the solubility of drugs (Loftsson et al., 1996), in addition to increasing the viscosity and hence the contact time. In case of the preparations containing HPMC, BAC was added for its preservative action. At the concentration used (0.02%), it is also reported (Saettone et al., 1986) to increase the hydration of the cornea with the effect being more marked for hydrophilic drugs.

These polymers (MK-5, MK-6, MK-8, Table 3) indicated a significant and a prolonged (with respect to MK-2, T_{max} shift from 30 min to 2h) therapeutic effect (lowering of IOP i.e. ΔIOP) in the following order. PVA > HPMC = sodium alginate. Alginate sodium showed a slightly delayed response starting at 30 min in comparison to PVA and HPMC (15 min). The strongest promoting effect was observed with PVA. This could be due to the fact that PVA increase the thickness of the corneal tear film (Zaki et al., 1986). We tried to correlate the fraction of drug in solution (using different vehicles) with the IOP lowering effect, but as such no correlation was observed (results not shown). There was an increase, in solubility, from 0.7 mg/ml in the case of only Tween 80 as the solvent of up to two-to-three times with MK-6. Moreover, the fraction of drug present in MK-5 formulation was found to be only 0.8 mg/ml even though physiologically speaking this is the most effective formulation. Thus suggesting the superiority of PVA over the other polymers, may be because of its adhesive properties. Benedetto et al. (1975) have reported that PVA can drag with it an aqueous layer 10–20 μm, provided there is sufficient water available on the eye. They also observed a good correlation between its surface spreading characteristics and its ability to drag water. Ludwig and Van-Ooteghem (1988) have reported that the nature of polymer is highly significant in addition to its viscous effects.

Responses with MK-5*, MK-6*, and MK-8* treatments indicate the results of bilateral studies, where the vehicle was instilled into the control eye and the corresponding formulation in the experimental eye. There was no significant difference in the results when compared with those from the earlier studies, i.e. no vehicle being instilled into the contralateral eye (MK-5, MK-6, and MK-8; Table 3).

Since BAC and EDTA disodium both are permeation enhancers so an alternative formulation of HPMC was prepared in which only BAC (0.02%) was added (MK-7). It showed a ΔIOP versus time profile almost similar to MK-6 but, the ΔIOP_{max} was reduced from 4.7 for MK-6 to 3.9 for MK-7 (Table 4). This indicated a small enhancing effect of EDTA on BAC containing formulations.

As the results with MK-5 were quite promising, we concentrated further studies on this formulation and tried a similar formulation, but with a lower concentration of acetazolamide (5%)–MK-4. Although the reduction in IOP by MK-4 was less than that with MK-5, yet it was sufficiently high (Table 4). In fact, the ΔIOP_{max} of this formulation, containing only 5% acetazolamide, was comparable with formulations MK-6 (with HPMC) and MK-8 (with sodium alginate) containing 10% acetazolamide. All these findings suggest that the transport process (as the effect was increased in the presence of permeation enhancers and polymers) and the extent of inhibition of carbonic anhydrase (lower response with 5% acetazolamide–MK-4) are the rate limiting steps.

Several workers (Maren et al., 1983; Stein et al., 1983) have reported that an increase in the frequency of CAI delivery, results in a sufficient corneal drug penetration to inhibit carbonic anhydrase. Thus, MK-5 was instilled (50 μl at an interval of 5 min) for five times into the right eye of normotensive rabbits, keeping the other eye as control. This method of administration was found to give best results with up to 46.4% reduction in IOP at 60 min in comparison to 31.4, 26.0 and 25.7% for MK-5, MK-6, and MK-8 respectively, at 120 min.

The results were also evaluated in terms of activity parameters, such as ΔIOP_{max}, maximum reduction in IOP; and T_{max}, time required to reach the peak effect. Table 4 indicates the activity parameters for all the formulations. MK-5 showed a three-fold increase in effect with respect to MK-9, while MK-1 showed no effect. There was a small but significant increase in ΔIOP_{max} (from 4.9 ± 0.5 to 5.8 ± 0.5 mmHg) when the concentration of acetazolamide was increased from 5 to 10% in formulations with PVA. Upon repeated administration of MK-5 (i.e. MK-5•) ΔIOP_{max} increased 1.4 times that of single administration (MK-5) and four times that with MK-9. Sodium alginate and HPMC formulations showed almost similar results of 4.7 ± 0.7 and 4.7 ± 0.3 mmHg, respectively.

An experiment was also performed where only the vehicles were instilled into the treated eye, keeping the other eye as control. No significant change in IOP was found between the control and the treated eye, indicating the absence of any vehicle effects (Fig. 2). Moreover, the contralateral eyes in all the experiments showed no significant drop in the IOP. This observation ruled out chances of any systemic effect due to the absorption of the drug from the eye into the blood circulation, and confirmed that the observed fall in IOP was entirely due to the local action.

4. Conclusions

From the results obtained above, we can say that the transport process and the extent of inhibition of carbonic anhydrase are the limiting steps. It may also be inferred that a topical acetazolamide formulation can be successful if it includes a suitable viscolizer to increase the corneal residence; a penetration enhancer to help in the transport across the cornea and; the pH is maintained towards the acidic side (< 5) so as to prevent the degradation of acetazolamide. Although attempts are being made to enhance the solubility characteristics of acetazolamide either by structural modifications or by the use of cyclodextrins, etc., but we wonder if the addition of a high viscosity polymer and a penetration enhancer to a suspension of acetazolamide may prove to be topically effective. It is not possible to guess whether this subtle but significant IOP reducing effect of topically applied acetazolamide on the IOP of rabbits has any clinical significance for humans or not. Moreover, if the study is extended to glaucomatous rabbits and also to normotensive humans and humans suffering from glaucoma, we may get promising results. Some workers have reported better results with viscous vehicles in the human eye, in comparison to those obtained with rabbits (Saettone et al., 1986). However, this local effect reported by us supports the recommendations of others that acetazolamide warrants a further, more elaborate study.

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