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Review

Acetazolamide: future perspective in topical glaucoma therapeutics

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Abstract

Through this review it is contemplated that acetazolamide (ACZ), an age-old treatment for glaucoma with a myriad of side effects and inadequate topical effectiveness, may be formulated into a topically effective agent by utilizing various newer formulation approaches of ocular drug delivery. Even though it has a poor solubility and penetration power, various studies mentioned in the review indicate that it is possible to successfully formulate topically effective ACZ by using: (i) high concentration of the drug, (ii) surfactant gel preparations of ACZ, (iii) ACZ loaded into liposomes, (iv) cyclodextrins to increase the solubility and hence bioavailability of ACZ, and (v) viscolyzers and other polymers either alone or in combination with cyclodextrins. With the advent of newer topical carbonic anhydrase inhibitors (CAIs) like dorzolamide and brinzolamide, a localized effect with fewer side effects is expected. But whenever absorbed systemically, a similar range of adverse effects (attributable to sulphonamides) may occur upon use. Furthermore, oral ACZ is reported to be more physiologically effective than 2% dorzolamide hydrochloride administered topically, even though in isolated tissues dorzolamide appears to be the most active as it shows the lowest IC_{50} values for CA-II and CA-IV [M.F. Surgue, J. Ocular Pharmacol. Ther. 12 (1996) 363–376]. Hence, there exists considerable scope for the development of more/equally effective and inexpensive topically effective formulations of ACZ. The use of various formulation technologies discussed in this review can provide a fresh impetus to research in this area.

Keywords: Acetazolamide; Carbonic anhydrase inhibitors; Glaucoma; Intraocular pressure

1. Introduction

Glaucoma, the leading cause of irreversible blindness in the world, is estimated to be encountered by 67 million people. It generally occurs in people over 40 years of age but may also occur in younger persons including children (Sommer et al., 1991). Glaucoma is the term used for a group of ophthalmic disorders characterized by an increase in intraocular pressure (IOP), which results in a damage to the optic disc and visual field disturbances. IOP increases through an imbalance between the production and drainage of aqueous humor. Agents used to treat glaucoma are de-

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signed to decrease IOP. These drugs limit aqueous humor production in the ciliary body and/or enhance aqueous outflow through the trabecular meshwork or the uveoscleral pathway. Various classes of drugs used in the long-term management of glaucoma include β-adrenergic blockers, miotics, α-adrenergic agonists, carbonic anhydrase inhibitors (CAIs), prostaglandin analogues and hyperosmotics (Everitt and Avorn, 1990; Collingnon-Brach, 1994; Infield and O'Shea, 1998; Titcomb, 1999). In this review, we will focus on the topical use of acetazolamide (ACZ), for use in the long-term management of glaucoma.

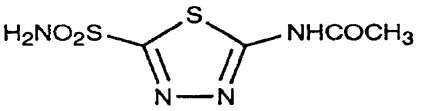
2. Carbonic anhydrase inhibitors

Carbonic anhydrase (CA), an enzyme present in the eye, reversibly catalyzes the reaction of H_2O and CO_2 to form carbonic acid and subsequently the bicarbonate ion as shown in the following equation:

$$H_2O + CO_2 \underset{\text{Zn containing enzyme}}{\overset{\text{CA}}{\rightleftharpoons}} H^+ + HCO_3^- . \qquad (1)$$

The HCO_3^- ion is responsible for the movement of Na^+ into the eye. Water follows Na^+ to form the aqueous humor. CA inhibition by agents such as ACZ, methazolamide and dichlorphenamide, decreases HCO_3^- concentration and therefore the flow of Na^+ and H_2O into the posterior chamber, thus, resulting in a decreased production of aqueous humor and hence a lowering of IOP (Maren, 1967, 1987).

ACZ (Fig. 1) and other oral CAIs have been an integral part of anti-glaucoma therapy for more than over 40 years (Becker, 1955; Centofanti et al., 1997). They are amongst the most powerful agents



$C_4H_6N_4O_3S_2$ Mol. Wt. 222.24

Acetazolamide is *N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)-acetamide.

Fig. 1. Structure of ACZ.

to lower IOP (Pfeiffer, 1997). However, due to their extraocular side effects (Table 1), they have become less popular of late (Epstein and Grant, 1977; Goodfield et al., 1982; Gamm, 1984).

CAIs, however, offer distinct advantages over:

i) Cholinergic agonists: as they are free of any effects on the pupil and accommodation, and have almost no central nervous system side effects.
ii) β-Blockers: which pose a potential risk especially for elderly patients with compromised cardiac/pulmonary function (Diggory and Franks, 1994).
iii) Prostaglandin analogues: whose full range of clinical and systemic side effects is not yet known. Some unusual effects of these agents include, an increase in eyelash growth and an irreversible darkening of iris (Khaw and Cordiero, 2000). Some post-marketing surveillance studies have also indicated a hypertensive effect in a few patients (Peak and Sutton, 1998).

CAIs are particularly useful in the management of glaucoma resistant to other anti-glaucoma therapies, and in the control of acute glaucoma attack. Moreover, these agents inhibit the production of aqueous humor without interfering with its outflow (Friedland and Maren, 1984). CAIs have also been shown to improve intracranial blood flow (hence increase ocular perfusion) and visual fields (Flammer and Drance, 1983).

To overcome the systemic side effects of CAIs, several workers tried to administer these agents topically. However, topical formulations of CAIs were initially unsuccessful due to the poor ocular bioavailability of these drug agents, which can be largely attributed to a poor penetration coefficient (Duffel et al., 1986) and a poor biphasic solubility of most of CAIs. After topical administration of an ophthalmic drug solution, it is first mixed with the lacrimal fluid and is thus diluted. Induced lacrimation causes spillage of instilled solution, thereby reducing the contact time of the drug with the ocular tissues. The existence of a well-defined corneal structure further restricts the passage of drug molecules into the inner eye. These are the

Table 1
Extraocular side effects of CAIs

General	CNS	Pulmonary	GIT	Renal	Hematologic	Others
Anorexia	Depression	Respiratory decompensation in patients with obstructive pulmonary disease	Cramps	Nephrolithiasis	Acute leukopenia	Metabolic acidosis
Weight loss	Loss of libido		Diarrhea	Renal failure	Agranulocytosis	Stevens–Johnsons syndrome
Fatigue	Erectile dysfunction		Epigastric burning		Aplastic anemia	
Malaise			Metallic taste		Hemolytic anemia	
Paresthesias of toes and fingers			Nausea		Neutropenia	
			Vomiting		Pancytopenia	
					Thrombocytopenia	

general problems faced in ocular therapeutics and are largely responsible for a lesser success of topical ocular agents. But the prospect of overcoming the systemic effects of a drug and also achieving an ocular effect at a much lower dose is very attractive and the researchers have always sought to develop topically effective agents. Development of topically effective CAIs has continued for a period of over 40 years and extensive work on structural modification of existing CAIs resulted in the development of two topically effective CAIs, dorzolamide and brinzolamide (Manners, 1993). These are the first topical CAIs to be approved by the US-FDA for the treatment of primary open angle glaucoma (POAG). These agents although administered topically are absorbed systemically. Thus, same type of adverse reactions that are attributable to sulfonamides may occur with topical administration of these agents. Furthermore, the effectiveness and safety of these CAIs still needs to be supported by exhaustive clinical studies. Thus, the need of the hour is to develop a topically effective CAI devoid of its systemic side effects, and therefore, there exists a considerable scope for the development of formulations in such a way that the otherwise orally effective drug molecule shows a topical effect rather than indulging in chemical search for a new drug molecule. Latter being a tedious process requiring time, money and manpower, and thus causing wastage of resources.

Amongst the available CAIs, ACZ is still the most highly prescribed and systemically the most effective drug for the treatment of POAG. It is the obvious choice as an anti-glaucoma agent over the other CAIs because:

i) dorzolamide reduces IOP by up to 23% as monotherapy and additional 15% in combination with timolol, whereas oral ACZ reduces IOP by about 30% (Becker, 1954; Strahlman et al., 1995);
ii) dorzolamide inhibits aqueous flow by 17% while ACZ inhibits aqueous flow by 30% (Maus et al., 1997);
iii) commercially available 2% dorzolamide solution had almost comparable IOP lowering effect as 1% ACZ eye drop solution (Loftsson et al., 1996);
iv) ocular burning and stinging was reported in 19 and 12% patients, respectively, with 2–3 times daily application of 2% dorzolamide (Strahlman et al., 1995);
v) 1% brinzolamide three times daily reduced IOP by only 20.3% (Silver, 1998);
vi) methazolamide has smaller effect on IOP in comparison with ACZ (Dahlen et al., 1978);
vii) ethoxzolamide has very high lipid solubility and corneal permeability and yet, topical application does not lower IOP (Maren et al., 1983).

The long-term clinical experience with ACZ coupled with its well-established safety and low cost (with respect to either dorzolamide and brinzolamide) has led us to explore the possibility of development of an effective topical formulation of ACZ.

2.1. Background

Mann and Keilin in 1940 observed that metabolic acidosis was produced as a side effect when sulphanilamide was administered as a chemotherapeutic agent. This observation led to a detailed in vitro and in vivo evaluation of this drug, which demonstrated that sulphanilamide is an inhibitor of CA. Schwartz in 1949 was able to show that the diuretic effect of sulphanilamide was caused by the inhibition of CA in the kidneys. Subsequently, a number of compounds were synthesized for their high CA inhibition activity so as to act as diuretics. One of these compounds ACZ has been extensively studied.

CA is present in renal tubular cells, gastric mucosa, pancreas, ciliary body of eye, iris, cornea, retina, brain and the erythrocytes. In 1954, American Cynamid Company introduced this powerful inhibitor of CA, ACZ (marketed by Lederle) as the first oral diuretic. In the same year, it was introduced in ophthalmology as it was found to lower IOP by reducing the aqueous humor production in the ciliary body (Becker, 1954). Other investigators also reported the efficacy of ACZ in

glaucoma patients, upon oral administration (Gloster and Perkins, 1955).

2.2. Mechanism of action and oral use

CA was first found to be present in the anterior uvea of the rabbit eye and subsequent studies confirmed its presence in human ciliary processes (Wistrand, 1951; Lutjon-Drecoll et al., 1983). Four isoforms of CA have been identified in human tissues. They are designated as CA-I, CA-II, CA-III and CA-IV. Out of these, CA-II and CA-IV have been found in the human ciliary processes, cornea, iris and the retina.

CA catalyses the conversion of CO_2 to HCO_3^- ions. As explained earlier, formation of HCO_3^- helps in the production of IOP. CA-IV plays an important role in the formation of HCO_3^- and aqueous humor secretion whereas CA-II is responsible for the cellular equilibrium of CO_2 and H_2O. For effective lowering of IOP, 99.99% inhibition of CA-II and 98% inhibition of CA-IV is required (Brechue, 1994).

ACZ is the most commonly used oral CAI and is available as Diamox tablets 125 and 250 mg; extended release capsules 250 and 500 mg (Diamox sequels) and a sterile ACZ sodium (ACZ-Na) powder for injection 500 mg, all marketed by Lederle Laboratories, USA. The maximum reduction in IOP is obtained with 250 mg tablets four times daily or 500 mg S.R. capsules twice daily. The reduction in IOP is up to 20–30% and is dependent on the initial IOP. It is a second line drug to be used in non-regulated glaucoma, post-surgically and in post-laser therapy. The chemical usefulness of ACZ is, however, limited by a variety of side effects. As a result, its oral use has become unpopular and a number of scientists have sought to replace oral CAIs with topical CAIs to reduce the systemic side effects.

3. Early studies to evaluate topical effectiveness

A number of approaches were adapted to develop an effective topical formulation of ACZ. Gloster and Perkins (1955) employed various routes of administration for ACZ. They administered 5% solution of ACZ-Na by sub-conjunctival and also by intraocular injection. Both these routes of administration, however, failed to exhibit any reduction in IOP. Similar findings were reported by Grant and Trotter (1954). This fact that local administration of ACZ to the eye did not reduce IOP led to a conclusion that reduction of IOP following application of ACZ to the cornea was impossible. Research on this topic was therefore abandoned for more than two decades.

However, in the 1980s there was a renewed interest in CAIs due to the introduction of a number of sulfonamide derivatives, which had varied effects on IOP in solution/suspension forms, e.g. L-645, 151; L-650, 719 and L-651, 465 (Fig. 2). Later, novel structure CAIs which were water-soluble, e.g. L-654, 230; MK-417 and MK-927 (Fig. 3) were also developed (as discussed in Section 5).

4. Reasons for poor effectiveness

It has been observed that the inability of a topically administered CAI to reduce IOP may be the result of its limited ocular penetration, causing an insufficient amount of the drug to reach the ciliary body (Maren et al., 1983). It may be noted that 99.99% inhibition of CA is required to decrease the aqueous flow. Other potentially important factors, which are found to monitor the physiological effectiveness and bioavailability of CAIs include the stability of the compound, activity against CA, activity of metabolites against CA, binding to pigment and protein in the eye and time of residence at active sites in the ciliary epithelium. Most of the workers involved in the study of topical effectiveness of ACZ used ACZ-Na solutions (as solubility of ACZ is very low, of the order of 0.7 mg/ml) having a pH of >7.0. But it has been reported that ACZ is highly unstable at an alkaline pH (Parasrampuria and Das Gupta, 1989). This could be another reason for the absence of physiological response upon topical administration of ACZ solution (in the form of Na salt) in case the solution was not freshly prepared. Moreover, at an alkaline pH, ACZ occurs in the ionized form, which may further limit its transport through the lipophilic epithelium of the cornea.

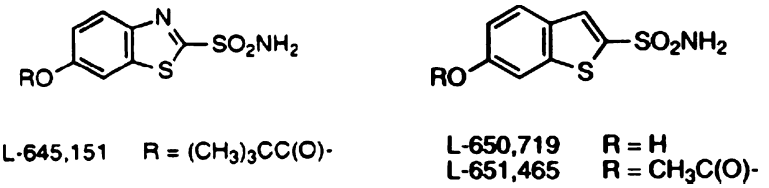


Fig. 2. Ethoxzolamide and its structurally modified derivatives.

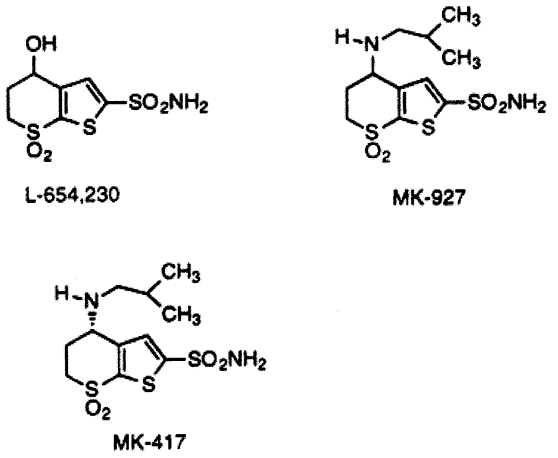


Fig. 3. Structurally modified water-soluble CAIs.

Thus, the main focus in the CAI research was to enhance the bioavailability of these agents by improving their corneal penetration by altering the physicochemical properties/structure of these agents. Another aspect, which according to us is simpler and more versatile, is to explore for a suitable penetration enhancer. Further, the residence time of the formulations of these drugs can be increased by using viscolyzers to increase the viscosity of the solution (Kaur and Kanwar, in press) or by incorporation in a gel and other suitable delivery systems (e.g. liposomes, niosomes, etc.). A more novel approach would be to incorporate a suitable mucoadhesive into such formulations and achieve an increase in the bioavailability of these agents (Kaur and Smitha, 2002).

5. Structural modifications of CAIs

The quest for a topically effective ocular hypotensive CAI resulted in an intensive research programme at various chemical laboratories and culminated in the discovery of various topically effective ocular hypotensives, most of which, however, had to be discarded after pharmacological testing. Ethoxzolamide, with its high lipophilicity and with multiple sites available in its structural formula was a suitable candidate for molecular modifications. The initial studies were therefore focused on the modification of the structure of ethoxzolamide, resulting in the synthesis of benzothiazole derivatives L-645, 151; L-650, 719 and L-651, 465 (Fig. 2).

All of the above were discarded either due to allergic reactions or due to their inefficacy in humans (Graham et al., 1989; Durand-Cavagna et al., 1996). Then, strategies were evolved to develop a topically effective water-soluble CAI by the introduction of functional groups capable of increasing water solubility which led to the discovery of L-654,230, MK-927 and its S-enantiomer, MK-417 (Fig. 3). Both MK-927 and MK-417 were assessed as safe and effectively lowered IOP in humans (Lippa et al., 1988, 1991).

Further structure–activity relationship studies resulted in the development of MK-507, later named as dorzolamide, with improved spectrum of activity and increased water solubility (Ponticello et al., 1998). This was the first topically effective CAI to be approved by US-FDA in 1995. Subsequently, brinzolamide was also approved for topical use in the treatment of glaucoma and was reported to produce less ocular discomfort than dorzolamide (Silver, 1998) (Fig. 4).

6. ‘Me too’ aspect of ACZ

Inspite of the advent of newer CAIs, the interest in ACZ was not lost and it still remained in clinical use especially for the treatment of acute glaucoma

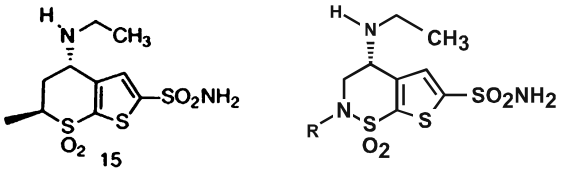


Fig. 4. Newer CAIs.

attacks, and post-laser and post-surgical control of IOP. Oral ACZ is reported to be more effective than 2% dorzolamide hydrochloride administered topically (Maus et al., 1997). The major reasons for its continued use could be:

i) It is more specific and reduces IOP by inhibiting CA. The inhibition of enzyme leads to a direct decrease in aqueous humor production and hence IOP. It does not alter the outflow of aqueous humor (Friedland and Maren, 1984).
ii) It is a cheap alternative since it is an already established drug for glaucoma therapy. Cost is especially important, since most of the patients suffering from glaucoma are aged and cannot afford expensive drugs. Moreover, long-term management of glaucoma is required and this further makes the cost an important factor.
iii) It is safer with respect to the β-blockers like timolol (which are commonly employed for the control of glaucoma) in patients with compromised cardiac/pulmonary status. In contrast to the vasoconstrictive effects of β-blockers, which may reduce the perfusion pressure of the optic disk, ACZ improves ocular perfusion (Flammer and Drance, 1983).
iv) Community use of newer agents like latanoprost has emerged with newer side effects, e.g. hypertension, which were not apparent during the clinical trials (Peak and Sutton, 1998).

Thus, while the intensive research work resulted in the discovery of new topical CAI molecules, simultaneously, formulation scientists sought to develop a topical formula for ACZ by adapting a number of approaches to enhance its ocular bioavailability.

Stein et al. (1983) attempted to improve penetration of ACZ by increasing the drug concentration and frequency of drug administration. They observed that topically administered ACZ (3–5 drops of 10% solution) did not effectively decrease IOP in normotensive rabbits but prevented ocular hypertension induced by oral water loading. They concluded that increasing the concentration and frequency of ACZ delivery resulted in a sufficient corneal drug penetration.

Flach et al. (1984) reported a lowering of IOP with 10% ACZ in water-loaded pigmented rabbits (60 mg distilled water/kg body weight intraperitonially). Change in IOP for the contralateral eye however did not rule out a systemic effect of the drug. These investigators further reported a significant increase in ocular hypotensive response when 10% topical ACZ was administered after a simultaneous IV dose of 25 mg of the drug/kg body weight. The latter studies indicated an additive effect of topical ACZ.

A statistically significant lowering of IOP was observed by using high water content soft contact lenses soaked in ACZ. Following 2.5 h application of +37 D lenses soaked in 5% ACZ, significant reduction in IOP was observed up to 5 h. Similar results were obtained for lenses soaked in 2.5% ACZ. However, no IOP reduction was observed with 1% ACZ solution. Variables such as soaking duration, wearing duration and lens thickness were also evaluated. A systemic effect was ruled out not only by lack of contralateral decrease in IOP but also by the examination of arterial blood, which revealed similar pH, bicarbonate, P_{CO_2} and base excess values both before and after the application of a 5% ACZ-soaked lens. Moreover, bilateral anterior chamber paracentesis following unilateral wearing of contact lenses soaked in 5% ACZ showed a significant reduction in the mean pH, bicarbonate level and base excess in treated eyes when compared with the contralateral untreated eyes (Friedman et al., 1985).

All the above-mentioned approaches to lower IOP have used 2.5–10% ACZ solutions which is much beyond the aqueous solubility of free acid (0.7 mg/ml). These scientists have probably used

the more soluble sodium salt of ACZ, which gives a solution of pH ≅ 9.0. ACZ has been reported to degrade at a very fast rate in an alkaline pH ≥ 8.0; the pH of maximum stability being 4–5 (Parasrampuria and Das Gupta, 1989; Khamis et al., 1993). Although none of the scientists working on the development of topical ACZ formulations have highlighted the effect of pH on stability and degradation of ACZ, it may be concluded that the results obtained during the above-mentioned studies may be deceptive and misleading in case the solutions were not freshly prepared. It may, however, be added here that even better results are expected if it can be ensured that drug degradation does not occur during preparation and storage.

In the early 1990s, formulation scientists focused on the development of a topical formulation of ACZ, using hydroxypropyl-β-cyclodextrins (HPβCDs) to enhance the aqueous solubility of ACZ. It has been reported that cyclodextrins are capable of forming water-soluble complexes with many water-insoluble drugs (Pitha et al., 1986; Loftsson et al., 1991a). The large and very hydrophilic HPβCD molecules do not penetrate the biological membranes and thus act as true carriers and penetration enhancers by assuring constant high concentration of dissolved drug at the membrane surface (Szejtli, 1988; Loftsson et al., 1991b). Topically active drug solutions (1%) were formulated by forming water-soluble inclusion complexes of HPβCD (16%) with ACZ (Loftsson et al., 1994). The IOP lowering effect of these formulations was reported to be improved further by:

i) use of water-soluble polymers (HPMC) which act by increasing the stability constant of drug–cyclodextrin complexes,
ii) formulating ACZ at a higher concentration (2%) in the form of a suspension, and
iii) combining ACZ with timolol maleate.

The 1% solution of ACZ was prepared, using 16% HPβCD, 0.5% EDTA, 0.1% HPMC, and 0.01% BAC. In a separate experiment, an addition of 0.25% PVP was found to double the aqueous solubility of ACZ (Loftsson et al., 1996).

A gel suspension of ACZ and methazolamide was formulated in an attempt to facilitate absorption, by greater adherence to the surface of the eye. No significant reduction in IOP was observed in ocular hypertensive individuals with either of the gel suspensions (Manners et al., 1993). A surfactant gel preparation was, however, reported to be topically effective (Tous and Nasser, 1992).

A liposomal preparation of ACZ for topical administration was formulated using the reverse-phase evaporation technique. Liposomes were prepared using varying concentrations of lipid components. Positively charged ACZ liposomes produced sustained reduction in IOP for a period of up to 4 h after instillation. This was attributed to the fact that positively charged liposomes have a higher binding affinity for the corneal surface than the neutral or negatively charged vesicles. As the drug molecules rely on passive diffusion to cross the corneal barrier after being released from the vesicles, the longer the contact time at the corneal surface, the more is the amount of drug released (El-Gazayerly and Hikal, 1997).

In addition to the corneal barrier (due to its trilamellate structure), eyelid movements and normal drainage of lacrimal fluid reduce the contact time between the topically administered preparation and the conjunctiva and corneal epithelium, thereby reducing the ocular bioavailability significantly. Keeping this in mind, it may be concluded that bioavailability of ocular drugs can be increased by:

i) Administration of high concentration of drugs; which would increase the amount of drug reaching the eye, producing a greater pharmacological effect; although the fraction of dose which enters the eye remains the same and hence the effective bioavailability is not improved.
ii) Making use of viscosity enhancers such as semi-synthetic derivatives of cellulose like PVP, PVA, etc. in the formulation to increase the residence time.
iii) Increasing the transcorneal passage of the drugs by using absorption enhancers like EDTA, saponins, bile salts and acids, BAC, etc.

iv) Making use of mucoadhesive properties of polymers like chitosan, CMC-Na, polyacrylic acids, carragenan, polycarbophil, hyaluronic acid, etc. to increase the contact time of the drugs with the corneal surface and hence the accumulation of the drug into the ocular tissue.

In an attempt to increase the contact time between the drug molecules and the ocular surface thereby enhancing ocular bioavailability of ACZ, we formulated ACZ (10%) suspensions using viscolyzers like PVA, HPMC and penetration enhancer, EDTA (Kaur et al., 2000). Significant reduction in IOP was observed in normotensive rabbits by incorporating 2% PVA and 0.5% EDTA into suspensions of ACZ (10%). There was no change in IOP in the contralateral eye, which ruled out a systemic effect of the drug. Later on, we successfully formulated topically effective 0.5% ACZ solutions in our laboratory, using HPβCD (10%) and 0.05% PVP (R. Smitha, M. Pharmacy Thesis, UIPS, Panjab University, Chandigarh, India, unpublished work). The two major problems which hinder the topical effectiveness of ACZ are its poor aqueous solubility (0.7 mg/ml) and a low permeability coefficient of 4.1×10^{-6} cm/s (Duffel et al., 1986). Considering these two parameters we tried to improve upon the low solubility of ACZ by the combined use of cyclodextrin, water-soluble polymers like PVP and a cosolvent like PEG 400. ACZ has high solubility of the order of 87.81 mg/ml in PEG. Further, we tried to increase the permeability of the drug by incorporating permeation enhancers like EDTA and sodium deoxycholate into the formulations. Using these agents in varying combinations, nine formulations were prepared (Table 2).

These formulations were then evaluated in terms of their in vitro permeability and physiological effectiveness in terms of lowering IOP of normotensive rabbits. The influence of the various components of the formulations on the in vitro permeability of ACZ through pig cornea was investigated using a glass diffusion cell. All the formulations showed linear permeation plots (Fig. 5) with correlation coefficients (r) in the range 0.9803–0.9997. The apparent corneal permeability coefficient (P_{app}) of these formulations was determined according to the following equation (Schoenwald and Huang, 1983):

$$P_{app} = \frac{\Delta Q}{\Delta t\ 60A\ \mathrm{Co}}\ (\mathrm{cm/s})\ , \qquad (2)$$

where $\Delta Q/\Delta t$ is the steady-state slope of the linear portion of the plots of the amount of drug in the receiving chamber (Q) vs. time (t). A is the exposed corneal surface area (1.327 cm^2), Co the initial concentration of drug in the donor cell and 60 represents the conversion of minutes to seconds. The in vitro permeation data of various formulations is shown in Table 3.

The results from in vitro experiments showed that the flux as well as the apparent permeability coefficient of formulation 2 (not containing 0.05% PVP) was significantly lower than formulation 1. P_{app} of formulation 4 was found to be significantly higher (1.25 times) than that of 1. Similarly, the P_{app} of formulation 7 was greater than that of 6. This is probably due to the presence of permeation enhancer, EDTA in these formulations. EDTA is reported to loosen the tight junctions of the corneal epithelium, thus facilitating the paracellular transport of the drug molecules (Hochman and Artrusson, 1994). P_{app} of formulations 8 and 9 were significantly lower than 1 even though the flux was similar. This could be so because of the higher initial concentration of ACZ (2%) in these formulations with respect to 0.5% for other formulations. Not much change in P_{app} was, however, observed when bile salt was used as the permeation enhancer (formulation 5).

The physiological effectiveness of these formulations was determined in terms of their IOP lowering effect in normotensive rabbits using Schiötz tonometer. The change in IOP (ΔIOP) vs. time data of various formulations is shown in Fig. 6. All the formulations were compared in terms of their activity parameters (onset time, peak effect time and duration of action/effective time period) as shown in Table 4. Instillation of 50 μl of formulation 1 showed a significant IOP lowering effect at 1.5 and 2.0 h. In the case of formulation 2, where PEG (30%) was also incorporated along with CD into the formulation, the onset of action

Table 2
Composition of formulations

Ingredients	Formulations								
	1	2	3	4	5	6	7	8	9
ACZ (%, w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2	2
HPβCD (%, w/v)	10	10	10	10	10	10	10	10	10
PVP (%, w/v)	0.05	–	0.05	0.05	0.05	0.05	0.05	0.05	0.05
PEG (%, v/v)	–	30	30	–	–	–	–	–	–
Sodium deoxycholate (%, w/v)	–	–	–	–	0.05	–	–	–	–
EDTA disodium (%, w/v)	–	–	–	0.5	–	–	0.5	–	–
Chitosan[a] (%, w/v)	–	–	–	–	–	0.5	0.5	–	0.5
Tween 80 (%, w/v)	–	–	–	–	–	–	–	1	–

[a] 3% Chitosan was prepared in 1% dilute acetic acid as a stock solution and appropriate quantities were added to each formulation to get a final concentration of 0.5%.

was shifted to 1 h (from 1.5 h) and the peak effect also increased (from 17.3% in case of formulation 1 to 23.0% in case of formulation 2). Formulation 3, containing 0.05% PVP in addition showed a sustained effect by increasing the duration of action to up to 2.5 h.

Formulation 4 containing EDTA (0.5%) as the penetration enhancer in addition to cyclodextrin and PVP showed a faster onset of action (0.5 h), a comparable peak effect and a longer duration of action maintained up to 2.5 h. The incorporation of sodium deoxycholate (0.05%) into formulation 5 produced very promising results, even though the P_{app} in the in vitro studies was not much different from formulation 1. Both a faster onset as well as a higher physiological response (25.2% reduction in IOP) were observed (Fig. 6).

Chitosan was evaluated as the bioadhesive polymer in this study. It was selected because of its polycationic nature, pseudo-plastic property, biodegradability, nontoxicity, biocompatibility and easy availability (Felt et al., 1998, 1999; Singla and Chawla, 2001). Formulation 6 showed a significant reduction in IOP, and the onset of

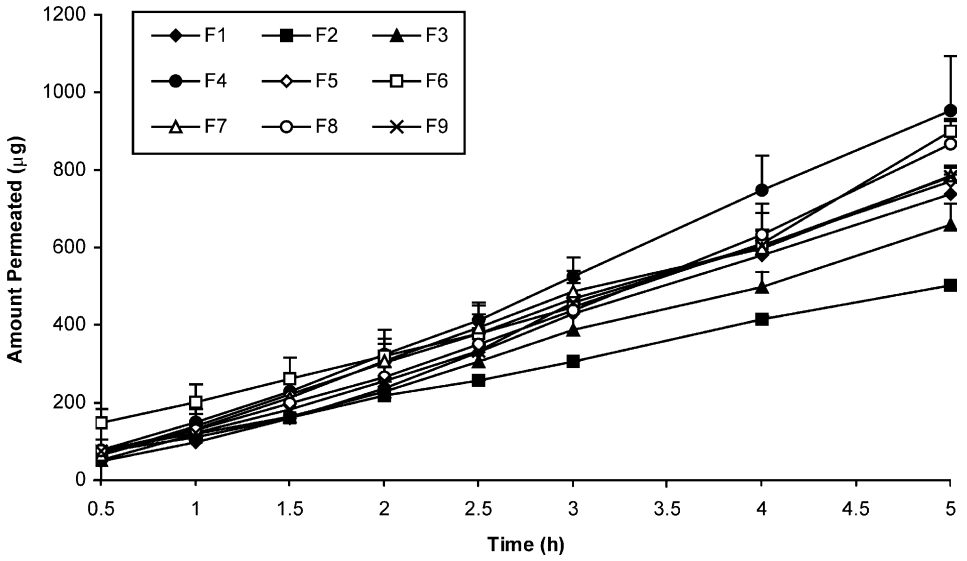


Fig. 5. Cumulative amount of drug permeated through porcine cornea vs. time (min) data of formulations 1–9.

Table 3
Comparison of various formulations in terms of % permeation, steady-state flux and apparent permeability coefficient P_{app} from in vitro permeation studies using pig cornea

Formulations	% Permeation	Steady-state flux (μg/min)	Apparent permeability coefficient, P_{app} (cm/s) ($\times 10^{-6}$)
1	29.50±2.01	2.641±0.166	6.634±0.418
2	20.09±0.16	1.620±0.013*	4.069±0.032*
3	26.35±2.20	2.237±0.173	5.621±0.435
4	38.14±5.62	3.302±0.383	8.295±0.963*
5	30.84±1.59	2.613±0.053	6.563±0.134
6	36.00±1.06	2.289±0.359	5.749±0.902
7	31.42±0.78	2.668±0.063	6.701±0.157
8	8.67±0.65	2.923±0.248	1.836±0.155*
9	7.83±0.16	2.699±0.084	1.695±0.052*

All results are expressed as mean±S.E.M. ($n=3$).
* Statistically significant difference from the corresponding value of formulation 1, $P<0.05$.

action was observed at 1 h and it lasted up to 3 h. A further addition of EDTA (formulation 7) caused a faster onset of action but contrary to expectations, the duration of action was not increased. Formulations 8 and 9 were suspensions with a higher concentration of drug (2%). Because of the tendency of particles to be retained in the cul-de-sac, the contact time and duration of action of suspension exceed that of a solution. Formulation 8 showed very promising results as indicated by its maximum IOP lowering effect (Fig. 6) and the duration of action was also prolonged up to 3 h, starting from 0.5 h (Table 4). However, formulation 9 (containing chitosan) where no dispersing agent (Tween 80) was added, was not comparable. Although IOP lowering action was retained (20% reduction in IOP), the effect subsided within 2.5 h. This discrepancy in the physiological response is contrary to our expectations and it may be due to the absence of Tween 80

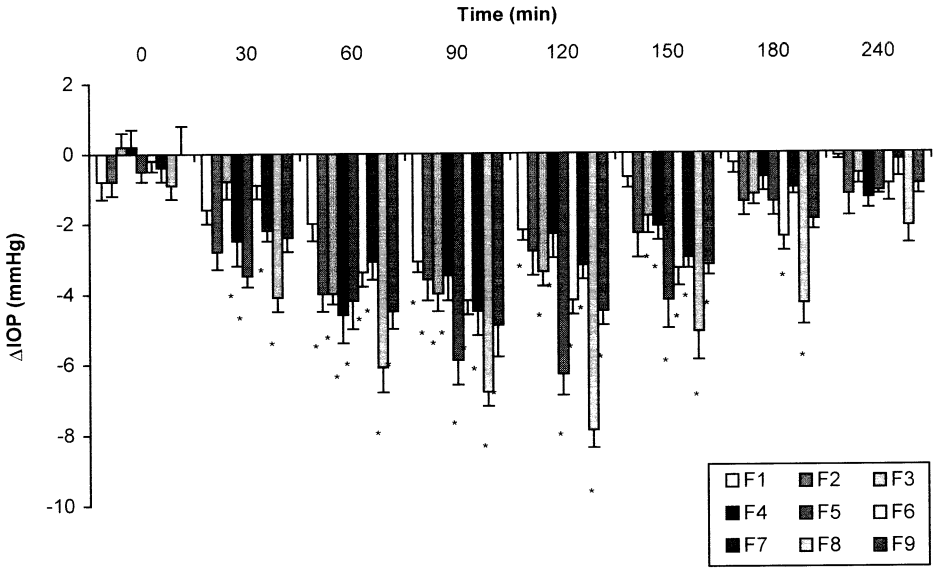


Fig. 6. Comparison of formulations 1–9 at different time intervals. Values are mean±S.E.M. ($n=6$). * Significantly different from the corresponding formulation 1 value ($P<0.05$).

Table 4
Summary of activity parameters of various formulations of ACZ

Formulations	Onset time (h)	Peak effect time (h)	Effective time period (h)
1	1.5	1.5	1.5–2
2	1	1	1–1.5
3	1	1	1–2.5
4	0.5	1	0.5–2.5
5	0.5	2	0.5–2.5
6	1	1.5, 2	1–3
7	0.5	1.5	0.5–2.5
8	0.5	2	0.5–3
9	0.5	1.5	0.5–2.5

which it seems in some way improves the bioavailability of ACZ. Formulations 1–9 have been compared in Fig. 6 to give the reader an overall view of the effect of various formulations on reduction in IOP.

The results obtained above from our study further reinforce the fact that topical ACZ can be successfully formulated by maintaining the pH of the formulation near the pH of maximum stability, i.e. 4.0; increasing the solubility of the drug using cyclodextrins (with or without copolymers) and/or co-solvents; and improving the ocular bioavailability of the formulations by (i) improving the transport characteristics of the drug across the cornea using suitable penetration enhancers and (ii) increasing the precorneal residence time by incorporating mucoadhesive polymers.

7. Conclusions and future directions

Development of topical formulation of ACZ may lead to total abolition of the systemic side effects observed upon oral and IV administration of ACZ. Attempts in this direction have been significant and noteworthy despite the initial failures. ACZ is a poorly water- and lipid-soluble drug. Its poor lipid solubility limits its transit through the corneal epithelium and endothelium whereas poor aqueous solubility prevents the transit through the hydrophilic stroma. Use of cyclodextrins (CDs) to increase the aqueous solubility of the drug without affecting lipophilicity is a successful approach to formulate a topically effective solution of ACZ. Further, CDs may also be used in combination with other polymers or co-solvents. Careful study of the approaches already adapted can help in developing effective preparations that are commercially viable and cost-effective. Future research in this field should focus on:

i) Optimization of formulation vehicles for prolonged drug retention in cul-de-sac by:
   - addition of water-soluble, natural, synthetic or semi-synthetic viscolyzers;
   - use of drug carrier systems like liposomes, nanoparticles, microspheres, etc. which would retain the drug in the cul-de-sac for a longer period of time thus giving a sustained action;
   - use of bioadhesive polymers for improving ocular absorption (Kaur and Smitha, 2002), and
   - use of drug delivery systems which provide controlled and continuous ocular delivery.

ii) Improving drug penetration through the cornea by the use of absorption promoters/penetration enhancers (Kaur and Smitha, 2002).

iii) Use of drug suspensions.

It may also be pointed out that the development of successful topical ACZ formulation not only depends upon the appropriate choice of a suitable viscolyzer or a penetration enhancer but also on maintaining its stability. It is important to maintain the pH of the solution towards the acidic side (pH 4) so as to prevent the degradation of ACZ.

Shelf life of ACZ solution should be determined by conducting accelerated and real-time studies. Elaborate physiological studies in glaucomatous animal models and humans need to be conducted to ensure a promising and an effective formulation.

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