STUDIES ON OCULAR FORMULATIONS OF ACECLOFENAC

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BY

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Synopsis
According to the World Health Organization (WHO), corneal diseases are major cause of vision loss and blindness, second only to cataract.

NSAIDs have proven to be a safe and effective alternative to corticosteroids in the topical management of ocular inflammations [1]. The beneficial effects of NSAIDs over corticosteroids include stabilisation of IOP, provision of analgesia and reduction in the risk of secondary infections [2]. NSAIDs comprise of several chemically heterogeneous class of drugs which possess potent cyclooxygenase inhibitory activity. However, the topical use of NSAIDs in ophthalmology is limited to relatively water soluble salicylic acid, indole acetic acid, aryl acetic acid, aryl propionic acid and enolic acid derivatives.

There are four NSAIDs currently approved by the US Food and Drug Administration for the treatment of postoperative inflammation after cataract surgery, namely ketorolac, bromfenac, nepafenac and diclofenac[3]. For ophthalmic use, diclofenac is commercially available as 0.1% w/v aqueous solution of its sodium salt. Diclofenac is applied topically in the eye for the management of pain in corneal epithelial defects following surgery or accidental trauma, treatment of postoperative ocular inflammations, chronic non-infectious inflammations, and prevention of intra-operative miosis during cataract surgery and for symptomatic relief of seasonal allergic conjunctivitis [4].

In this context, aceclofenac, 2-[[2-[2-[[2, 6-dichloro phenyl] amino] phenyl] acetyl] oxy] acetic acid, is a NSAID of the phenyl acetic acid group which is structurally related to diclofenac. It possesses good anti-inflammatory and analgesic activities, while maintaining better gastric tolerance in comparison with other NSAIDs such as indomethacin and diclofenac. Aceclofenac acts as such by inhibiting the secretion of tumor necrosis factor (TNF-α) and interleukin-1 along with preferential selective cyclooxygenase-2 (COX-2) inhibition after conversion into active metabolite [5-7].

In comparison with more sophisticated multiphase systems, aqueous ocular formulations are preferred because they are generally easier to manufacture and potentially provide better dose uniformity. Most of the commercialized eye drops are available in aqueous form, however, majority of active components are lipophilic in nature viz.-cyclosporine,
ketorolac, diclofenac and aceclofenac. It is quite difficult to formulate the aqueous solution of drugs which are lipophilic in nature.

Taking above information in view, in chapter 2, attempts were made to prepare aqueous formulations of aceclofenac which is slightly soluble in water. The different formulation factors have to be considered when drug delivery to the eye is attempted. So the purpose of this investigation was to evaluate the effect of formulation factors on in-vitro permeation of aceclofenac from aqueous drop through freshly excised goat, sheep, and buffalo corneas. The stability studies were conducted as per ICH guidelines for different aqueous formulations of aceclofenac. Aqueous isotonic ophthalmic solutions of aceclofenac of different concentrations (pH 7.2) or 0.1% (w/v) solutions of different pH or 0.1% solutions (pH 7.2) containing different preservatives were made. Permeation characteristics of drug were evaluated by putting 1 mL formulation on freshly excised cornea (0.50 cm²) fixed between donor and receptor compartments of an all-glass modified Franz diffusion cell and measuring the drug permeated in the receptor (containing 10 mL bicarbonate ringer at 37°C under stirring) by spectrophotometry at 270 nm, after 120 min. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s test. Increase in drug concentration in the formulation resulted in an increase in the quantity permeated but a decrease in percentage permeation. Increase in pH of the solution from 5.5 to 7.2 decreased drug permeation, indicating pH-dependent transport. Compared with control formulation, aceclofenac 0.1% (w/v) solution (pH 7.2) containing sodium sulphite (0.2% w/v) produced significantly ($p < 0.05$) higher permeation with all the corneas. The results suggest that aceclofenac 0.1% ophthalmic solution (pH 7.2) containing sodium sulphite (SS) (0.2% w/v) provides increased in-vitro ocular availability through goat, sheep, and buffalo corneas. During stability study, aceclofenac showed instability in aqueous environment, therefore subsequent studies were attempted to prevent the hydrolytic degradation of aceclofenac either by replacing the aqueous environment with oily vehicles or encapsulating the drug within a polymer matrix. The potential of different oily vehicles like olive, castor and sesame oil for ocular delivery have been well documented in earlier studies for improving
the delivery of poorly soluble drugs [8, 9]. In healthy subjects, pilocarpine dissolved in castor oil has shown greater degree and duration of effect on the pupil compared with an aqueous solution. Statistically significant drug effects have been noticed as long as 24 h after administration of oil based drops [10]. Aceclofenac is poorly soluble in water and gets hydrolyzed in aqueous environment. Thus, the oily vehicles could be a promising alternative to improve the drug stability. Further, the oil formulation would improve the bioavailability of aceclofenac by enhancing the residence time on ocular surface.

In chapter 3, oily formulations of aceclofenac were made in different food grade oily vehicles. For this purpose, studies were conducted to evaluate transcorneal permeation characteristics through freshly excised mammalian corneas. The stability studies were conducted as per ICH guidelines for different oily formulations of aceclofenac. The efficacy of the selected formulation was also evaluated against arachidonic acid-induced ocular inflammation in rabbits. Ophthalmic solutions of aceclofenac with or without (0.5% v/v) benzyl alcohol were formulated in different vegetable oils based on saturation solubility and permeation studies were carried out. Post permeation corneal hydration was measured to assess corneal damage. The arachidonic acid-induced rabbit ocular inflammation model [11] was used to compare the anti-inflammatory activity of prepared aceclofenac formulations. The aceclofenac ophthalmic solution in linseed oil containing benzyl alcohol exhibited maximum permeation through all corneas. The partition characteristics of drug reinforce the results of permeation studies. The optimized formulation (linseed oil containing benzyl alcohol) showed better stability profile along with higher transcorneal permeability. The optimized formulation has shown significant inhibitory effect on ocular inflammation induced by arachidonic acid ($p < 0.05$). Aceclofenac (0.12%, w/v) ophthalmic solution in linseed oil containing benzyl alcohol appears ideal on the basis of all aspects of formulation strategies (corneal permeability, stability and pharmacodynamic study).

Mostly, all ocular therapeutics has been administered to the eye as solutions. About 90% of the dose applied topically from such solutions is lost due to pre-corneal losses
(lacrimation and drainage) which lead to poor ocular availability [12]. Accordingly, there is a need for an appropriate delivery system which could increase the contact time of the drug with the eye surface and facilitate the transport of drug molecules into the eye tissue. In this role, a controlled or sustained delivery of ophthalmic drugs would be beneficial.

A number of colloidal drug delivery systems such as liposomes [13], polymeric micelles [14], nanocapsules [15] and nanoparticles [16] have been evaluated for improved ocular bioavailability. Nanoparticles, because of their submicron size are well tolerated and have the tendency to deposit in the cul-de-sac for prolonged period. Nanoparticles of several synthetic polymers, e.g. poly(alkyl cyanoacrylate) [17], poly(lactic-co-glycolic acid) [18], poly(epsilon-caprolactone) [19], as well as natural polymers such as chitosan [20] and gelatin [21] have demonstrated promising results for efficient drug delivery to the ocular tissues.

Eudragit RS and RL polymers are copolymers of poly (ethylacrylate,methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate), containing an amount of quaternary ammonium groups between 4.5-6.8% and 8.8-12% for RS and RL, respectively. Eudragit RL or RS 100 is insoluble at physiological pH and capable of limited swelling, thus appears to be a good polymeric carrier for the dispersion of drugs. The presence of quaternary ammonium group renders positive charge to the polymer by which it can interact with anionic drugs and mucin. The positive charge on the polymer may also impart mucoadhesion to the anionic cornea having isoelectric point (pI) of 3.2 and thereby increase its residence on corneal surface. Polymeric nanosuspensions prepared from Eudragit RL 100 and RS 100 have been investigated for the ocular delivery of flurbiprofen [22], cloriocromene [23], amphotericin B [24], methylprednisolone [25] and piroxicam [26].

Taking above information in view, in chapter 4, attempts were made to prepare Eudragit RL 100-based nanoparticles of aceclofenac by nanoprecipitation and evaluate the particle size, zeta potential, drug entrapment, particle morphology, in-vitro drug release and in-vivo efficacy. The arachidonic acid-induced rabbit ocular inflammation model [12] was used to compare the anti-inflammatory activity of prepared aceclofenac nanoformulations.
with aqueous solution of acceclofenac of same concentration \( (in-vivo) \) efficacy. Change in drug-polymer ratio from 1:5 to 1:20 increased the particle size and entrapment efficiency. The particles showed sustained \( in-vitro \) drug release which followed the Higuchi square-root kinetics. The results indicate that the nanoparticles release the drug by a combination of dissolution and diffusion. Based on the particle size (134.97 nm) and entrapment efficiency (95.73%), the formulation made with 1:10 drug-polymer ratio was selected for further studies. The particles were spherical with a polydispersity index of 0.186 and zeta potential of +30.5 mV. Powder X-ray diffraction and differential scanning calorimetry indicated decrease in crystallinity of drug in the nanoparticle formulation. In the \( in-vitro \) permeation study, the nanoparticle formulation showed 2-fold higher permeation of drug through excised cornea compared to an aqueous solution of drug with no signs of corneal damage. The \( in-vivo \) studies involving arachidonic acid-induced ocular inflammation in rabbits revealed significantly higher inhibition of polymorphonuclear leukocytes migration \( (p < 0.05) \) and lid closure scores by the nanoparticle formulation compared with the aqueous solution. The formulation was quite stable to ensure two year shelf life at room temperature.

**In chapter 5**, attempts were made to prepare Eudragit RS 100-based nanoparticles of acceclofenac by nanoprecipitation and evaluate the particle size, zeta potential, drug entrapment, particle morphology, \( in-vitro \) drug release profile and \( in-vivo \) efficacy. Change in drug-polymer ratio from 1:2.5 to 1:10 increased the particle size and entrapment efficiency. The particles showed sustained \( in-vitro \) drug release following the Higuchi square-root kinetics. The results indicated that the nanoparticles released the drug by combination of dissolution and diffusion. Based on the particle size (238.9 nm) and entrapment efficiency (94.53%), the formulation made with 1:10 drug-polymer ratio was selected for further studies. The particles were spherical with a polydispersity index of 0.273 and zeta potential of + 40.3 mV. Powder X-ray diffraction and Differential scanning calorimetry indicated decrease in crystallinity of drug in the nanoparticle formulation. In the \( in-vitro \) permeation study, the nanoparticle formulation showed 2-fold higher permeation of drug through excised cornea compared to an aqueous solution of
drug with no signs of corneal damage. The \textit{in-vivo} studies involving the arachidonic acid-induced ocular inflammation in rabbits revealed significantly higher inhibition of polymorphonuclear leukocytes migration ($p < 0.05$) and lid closure scores by the nanoparticle formulation compared with the aqueous solution. The formulation was quite stable to ensure two year shelf life at room temperature.

It can be concluded from the present investigation that aceclofenac (0.12\% w/v) ophthalmic solution in linseed oil containing benzyl alcohol (0.5\% v/v) appears ideal taking stability, corneal permeability and therapeutic efficacy into consideration. Similarly, Eudragit RL 100 or Eudragit RS 100- based nanoparticles of aceclofenac appear promising. However, further studies are needed to comment more in this respect.
References


