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Research Article

DEVELOPMENT OF AN OPHTHALMIC FORMULATIONS CONTAINING CIPROFLOAXACIN AND DEXAMETHASONE

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Abstract

Aim:

The use of steroids concomitantly with antibiotics is common to treat the severe and destructive inflammation, occurring during a bacterial infection of the external eye. In the present work, we have attempted to develop long acting eye drops containing ciprofloxacin and dexomethasone, a potent antibiotic and anti-inflammatory agent in the treatment of bacterial conjunctivitis.

Methods:

A clear ophthalmic solution and an ophthalmic suspension of the combination and ophthalmic solution of Ciprofloxacin alone were prepared and evaluated by various tests.

Results:

The ophthalmic solutions formulated using the Hydroxypropyl-beta-cyclodextrin (HP-beta-CD) exhibited better stability, biological activity and ocular tolerance in comparison to another ophthalmic preparation formulated without hydroxypropyl-beta-cyclodextrin (HP-beta-CD).

Conclusions:

These eye-drops were found to be non-irritating to rabbit eyes and were found to be effective in prevention of nonvascularisation in infected rabbit eyes.

Keywords: Ciprofloxacin HCL, Dexamethsone, Hydroxypropyl-beta-cyclodextrin (HP-beta-CD), Conventional

solution, Suspension, Evaluation.

Introduction

The most commonly used formulations for applying drugs topically to the eye are the conventional solution (eye drops). Although they are the simplest form of drug delivery, they suffer from certain drawbacks, such as: i) the eye drops on instillation cause reflex tearing whereby most of the instilled dose gets washed out of the eye quickly, thereby reducing its effectiveness. The eye drops have to be therefore instilled several times in a day, which often leads to patient non-compliance. ii) The eye drops on instillation cause reflex tearing due to irritation.

Various other ophthalmic drug delivery systems that are commercially available or are being evaluated include: ocular insets, aqueous polymeric gels and those containing nanoparticles or liposomes. Amongst these, the polymeric gels are the simplest to fabricate and other significant increase in contact time with the eye when instilled.

Ciprofloxacin degrades on prolonged exposure to light. Ultraviolet light causes maximum degradation with loss in antibacterial activity. Maximum stability occurs in the pH range 3-4 and degradation is accelerated at pH > 6[1, 2]. Complexation with cyclodextrins significantly reduces photodegradation of ciprofloxacin[3]. Dexamethasone is insoluble in water[4].

We decided to prepare aqueous solution containing combination of Ciprofloxacin and Dexamethasone. Attempt has been made to increase the stability of Ciprofloxacin, to solubilize Dexamethasone and to decrease eye irritation by using Hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and studied the drug release from it in vitro and vivo.

Materials

Ciprofloxacin hydrochloride, Dexamethasone, Hydroxypropyl-beta-cyclodextrin (HP-beta-CD), and Benzalkonium chloride. All other chemicals were of analytical reagent grade and water was obtained freshly from an all glass distillation still.

Methods

Preparation of the eye-drops: Two formulation were designed, viz, a clear ophthalmic solution and an ophthalmic suspension. The processes of preparation of these eye-drops were as follows

a) Preparation of 0.3%w/v ciprofloxacin ophthalmic solution: This formulation was prepared by dissolving ciprofloxacin, disodium EDTA, benzalkonium solution, sodium acetate trihydrate and sodium chloride in freshly double distilled water and adjusted the pH to 4.5 with acetic acid. The solution was sterilized by membrane filtration. Sterile amber glass vials of 10ml capacity were used for dispensing the solution. The solution was filled in the vials under laminar flow and the vials were capped with the pre-sterilized plugs.

b) Preparation of 0.3%w/v ciprofloxacin + 0.1%w/v dexamethasone ophthalmic solution:

The ciprofloxacin**,** benzalkonium chloride, EDTA & sodium chloride were dissolved in a quantity of acetate buffer solution. Separately, a quantity of buffer was heated and HP-β-cyclodextrin was dissolved in it, subsequently dexamethasone was added to it in parts after which HPMC was added & allowed to disperse. This solution was added to the ciprofloxacin hcl solution. Volume was made up & resulting solution was sterilized. This sterile solution was filled into sterile glass vials which were capped with pre-sterilized stoppers and then sealed.

c) Preparation of 0.3%w/v ciprofloxacin + 0.1%w/v dexamethasone ophthalmic suspension: To the sterile and concentrated solution of benzalkonium chloride and HPMC, sterile dexamethasone powder was added under stirring. Ciprofloxacin, EDTA and sodium chloride were dissolved in buffered solution and the solution was sterilized by autoclaving. The sterile Ciprofloxacin hcl solution was added to the sterile suspension and stirring was continued. After making up the volume, the suspension was filled in sterile glass vials. The vials were capped with pre-sterilized stoppers and sealed.

1. EVALUATION OF THE EYE-DROPS

i) Physicochemical evaluation

a) The ophthalmic solution was evaluated for the following parameters: visual appearance, clarity, pH, drug content and preservative content.

b) The ophthalmic suspension was evaluated for the following parameters: visual appearance, pH, particle size (by microscopy) and sedimentation time, ease of re-dispersion, drug content and preservative content.

Visual inspection was carried out by observing the solution against white and black background under fluorescent light. Resuspendability was as settled suspension. The container was inverted at the rate of about 8-10 times in a minute. The number of inversions required to completely resuspend the settled dexamethasone particles was noted.

Drug content was estimated by a simultaneous stability-indicating HPLC method, the chromatographic conditions followed are given below:

Column: C18, Phenomenex Bondclone, 10µm, 150× 3.9 mm i.d.

Mobile phase: 10 mM heptanesulfonic acid (pH 3.0± 0.1): methanol (50:50 v/v).

Flow rate: 1ml/min Detection: UV, at 254 nm Injection volume: 20µ L

The sample and standard solution were prepared in a 1:1 mixture of methanol: water, which contained about 150 µg/mL of ciprofloxacin and 50 µg/mL of dexamethasone. The chromatographic separation of ciprofloxacin, dexamethasone and Analog A (degradation product of ciprofloxacin) is shown fig. 1.

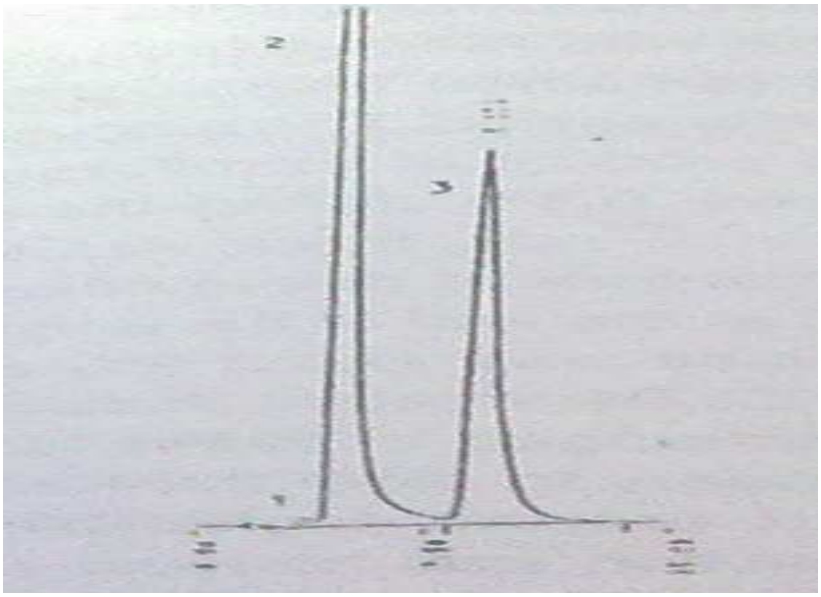

Fig. 1: Chromatogram showing sepration of

1. Ciprofloxacin Analogue – A
2. Ciprofloxacin
3. Dexmethasone

Since this method was stability-indicating, it was used to assay drugs during the accelerated stability studied also. For the accelerated stability studies the eye-drops were subjected to the following conditions: 5^{O}c (refrigeration), 25^{o}C (controlled room temperature), 37^{o}C, 45^{o}C. Benzalkonium chloride the preservative used in the formulation was assayed by the method reported by Santoni et al. The chromatographic conditions used were as follows:

Column: C18, Phenomenex Bondclone, 10µm, 150× 3.9 mm i.d.

Mobile phase: 30 mM tetrabutylammonium hydroxide (pH 2.5± 0.1):acetonitrile (30:70 v/v).

Flow rate: 1mL/min Detection: UV, at 260 nm Injection volume: 20µ L

(i) Microbiological evaluation: The prepared eye-drops subjected to test for sterility as per guidelines given in USP 23 by membrane filtration method. Antimicrobial preservative effectiveness was carried out as per guidelines given in USP 23. Five test microbes were used viz. E. coli, S. aureus, P. aeruginosa, C. albicans and A. niger.

(ii) Biological evaluation: Biological evaluation included the test for eye irritation and the test for efficacy of the formulations.

(a) Eye-irritation test: The eye-irritation potential of the prepared eye drops was assessed by the Draize test as modified by Bayard[5]. On albino rabbits. Four rabbits weighing between 2-2.5 kg of either sex used for each formulation. The eye drops were instilled in the one eye of each rabbit for 5 days as per the following schedule.

Day 1:2 drops every 30 mins for first 2 hours, then 2 drops every 4 hours (total of 8 doses per day)

Day 2-5:2 drops every 2 hours (total of 6 doses)

The contralateral eye received normal saline in the same dosing schedule. The degree of irritation was assessed and scored as propsed by Bayard[5].

(b) Efficacy study: This study was carried out in New Zealand albino rabbits of either sex, weighing between 2-2.5 kg. Six rabbits were used for each formulation. Bacterial keratitis was induced in both eyes of the rabbits using S. aureus ATCC 6538P and the ability of the formulation under test to prevent corneal vascularisation was assessed. The rabbit were anaesthetized with a mixture of ketamine (5mg/kg) and xylazine (25mg/kg). The ocular surface was locally anaesthetized using lidocaine solution. After anaesthetized had been achieved, 20µ L of an 8 hours

incubated broth containing 100 c.f.u. of S. aureus was injected into the cornea which led to the formation of corneal lab. Treatment was initiated 8 hours after the injection was induced. The right eye of all 6 animals received prepared eye drops. The left eyes of 4 rabbits received eye drops containing ciprofloxacin alone (0.3%w/v), whereas normal saline was instilled in the left eye of the remaining 2 rabbits. The dosing regimen during the treatment phase was as same as that used for the eye irritation test except in that case treatment was continued for 12 days. The rabbits were observed daily and the occurrence of prevention of corneal vascularisation at the site of injection was assessed and compared in each case.

Results and Discussion

Bacterial infections of the eye often cause severe inflammation. This inflammation can prove to the destructive to ocular tissues and may lead to partial or total impairment of vision. In order to prevent this treatment should be started not only with antibiotics but also with anti-inflammatory agents such as steroids. The use of steroids concomitantly with antibiotics greatly improves the clinical outcome of bacterial infections of the eye.

In the present study three types of eye drops were prepared viz. clear solutions and a suspension. Preparation of solutions involved a fairly simple process. The method reported by loffson et al was used lot solubilisation of the suspension of dextramethasone using cyclodextrin. On the other hand process for the preparation of the suspension was slightly cumbersome as it involved sterilization of ingredient separately and mixing them aseptically. EDTA was used as a chelating agent and Methocel as a viscosity-increasing agent. Sodium chloride was used to adjust the tonicity.

The results of the evaluation of the prepared eye-drops as follows:

(i) Physicochemical evaluation:

Table-1: **Physical evaluation test results.**

Sr. No.	Formulation	Parameter	Value
1.	Ciprofloxacin	Ciprofloxacin content	99.34±0.67 of label claim (n=6)

	solution		
		pH	4.48
		Preservative content	95.30±7.75 of label claim (n=6)
2.	Ciprofloxacin + Dexamethsone Solution	Ciprofloxacin content	98.74± 0.92% of label claim (n=6)
		Dexamethasone content	95.51± 1.41% of label claim (n=6)
		pH	4.46
		Preservative content	91.26± 1.31% of label claim (n=6)
3.	Ciprofloxacin + Dexamethsone Suspension	Ciprofloxacin content	97.09± 1.31% of label claim (n=6)
		Dexamethasone content	93.26± 0.95% of label claim (n=6)
		pH	4.48
		Preservative content	93.84± 4.90% of label claim (n=6)
		Particle size distribution of dexamethasone	5.23± 2.72µm (n=300)

The drug contents were within official limits of ± 2% for both ciprofloxacin and dexamethasone. The aim of the evaluation was closed to the expected value of 4.5. The solution was found to be clear and free from particulate matter. The suspension could be easily resuspended by inverting the container manually to about 4-5 times only, which indicated good resuspendibility. Once resuspended, the suspension about 12 hours to settle completely. It will count that the particle size distribution of dexamethasone in the suspension was same as that of the powder. The results of the accelerated stability study as follows:

(a) **Ciprofloxacin + Dexamethasone solution:** The solution was stable with respect to ciprofloxacin, as the drug content remained unchanged throughout the 6 month stability period. The percentage of Analog-A too did not increase in this duration, at any temperature. The amount of Analog-A (a degradation product of ciprofloxacin) also did not increased above 0.1% during the accelerated stability studies. The solution was however clear and no visible

changes could be seen. The pH of the formulation too remained unchanged throughout the stability period, probably due to the addition of acetate buffer in the formulation. At the end of stability studies, the preservative content, in vials stored at 45^0C dropped down to about 80% of the initial value.

(b) **Ciprofloxacin + Dexamethasone solution:** The solution was stable with respect to ciprofloxacin, as the drug content remained unchanged throughout the 6 month stability period. The amount of Analog-A (a degradation product of ciprofloxacin) also did not increased above 0.1% during the accelerated stability studies. However, there was significant decreased in the content of dexamethasone stored at 37^oC and 45^oC at the end of the 6 month stability study. The decrease was about 9% at 37^oC and about 15% at 45^oC. The content of dexamethasone was however unchanged at lower temperatures. Hence, it is necessary to store this formulation under refrigeration to prevent degradation of dexamethasone. The solution was however clear and no visible changes could be seen. The pH of the formulation too remained unchanged throughout the stability period, probably due to the addition of acetate buffer in the formulation. At the end of stability studies, the preservative content, in vials stored at 45^oC dropped down to about 80% of the initial value.

(c) **Ciprofloxacin + Dexamethasone suspension:** The suspension was physically unstable at temperature of 37^oC and 45^oC at the end of the first month of accelerated stability studies. The sedimented dexamethasone particles clumped together to form a cake which could not be redispersed; the exact cause for this behavior was not known. However at low temperatures, the sediment could be easily resuspended, with little agitation and no change in particle size was observed. Therefore, this formulation too needs to be stored under refrigeration. The content of both the drugs and the preservative remained unchanged at 25^oC and under refrigeration. The pH of the suspension too remained constant throughout the period of stability studies.

(ii) **Microbiological evaluation:** All the three eye drops complied with the test for sterility. In the test for efficacy of antimicrobial preservative, the formulations were found to be sterile at the end of first week of the 28 day test period. Hence, all the three formulations complied with the anti-microbiological preservative effectiveness test as well.

(iii) **Biological evaluation:**

(a) Eye Irritation study:

This study is of importance because the infected/inflamed eye already causes irritation and pain to the patient and if the eye-drops too cause irritation, it will result in greater discomfort. Moreover an irritating solution, when instilled into the eye results in profuse reflex tearing and hence the medication would be quickly washed out from the eye by nasolacrimal drainage, thus resulting in decreased effectiveness. It is therefore imperative that the eye drops be non-irritating to the eye.

The Bayard's system of scoring is based on reaction of individual components of the eye to the instillation of solution under test. Reflex tearing conjuctival redness etc. is mild reaction and their severity scores are therefore multiplied by a factor of 1. However, damage to the cornea or the iris discharge is more severe reaction and their severity scores are multiplied by a factor of 5-15. In our study, the only parameters that could be scored were conjuctival redness appearing immediately after instillation and reflex tearing occurring on instillation. The sum of scores of all tissues response for a marginally irritating substance is rated as 49. In our cases, the sum of scores never crossed 20, for all three formulations, which indicated that the solution were non-irritating to the eye.

(b) Efficacy study:

Efficacy studies for many drugs are carried out in animal models by experimentally inducing the required disease in suitable animals. Animal disease state models are an integral part of the ophthalmic product development process[6]. Major advances in the ability to prevent, diagnose and treat diseases in the past two decades have been possible because of animal studies[6]. Animal models of bacterial keratitis and corneal vascularisation are reported in the literature[7-10] and one such model was used for our study.

The eye treated with normal saline underwent severe neovascularisation along with formation of a corneal scar. The eyes treated with ciprofloxacin alone developed moderate degree of vascularisation. However the eyes treated with ciprofloxacin and dexamethasone solution or suspension showed total absence of vascularisation.

Conclusion

A combination of ciprofloxacin and dexamethasone was developed in the form of eye-drops, as a clear solution and a suspension. The formulations were simple and prepared relatively easily. In both the formulations, dexamethasone was found to be stable at and below temperatures of 25^{o}C. At higher temperature it showed considerable degradation. These eye-drops were found to be non-irritating to rabbit eyes and were found to be effective in prevention of nonvascularisation in infected rabbit eyes.

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References

1. Tornianen K, Tammilehto S and Ulvi V; The effect of pH, buffer type and drug concentration on the photodegration of ciprofloxacin. Int. J. Pharm. 1996, vol.132, pp 53-61.
2. Kane MP, Bailie GR, Moon DG and Siu I; Stability of ciprofloxacin injection in peritoneal dialysis solutions. Am. J. Hosp. Pharm., 1994, vol.51, pp 373-377.
3. Williams D and Ding Z-Q; Photochemical stability of the fluoroquinolone, ciprofloxacin HCl. AAPPS Annual Meeting 1994, contributed papers abstracts, Abstract No. APQ 1083.
4. Cohen EM; Dexamethasone-a monograph in Florey KP ed., Analytical Profiles of Drug Substances, ,. Academic Press NY, 1973, Vol. 2, pp 163-199.
5. Gilman M. R., in Hayes A. W. eds, ‘Skin and eye testingin animals,’ Priciples and methods of toxicology, Ravan Press, NY 1986, pp 209-212.
6. Novck G. D., Am. J. Ophthalmol. 1992, vol. 114 (3), pp 357.
7. ColleganM. C., Hobden J. A. Jill J. M. Inseler M. S. and O’callaghan R. J., Invest Ophthalmol. Vis. Sci., 1992, vol. 33 (11), pp 3017.

8. Sugar A., Cohen M. A., Bien P. A., Griffin T. J. Hetetz C. L., Mehta S., Arch. Ophthalmol., 1986, vol.104, pp 1230.
9. Tommila P., Summanen P. and Tervo T., Acla Ophthalmol, 1987, 65(suppl), 182 (89).
10. Nikooloc L. Friend J., Taylor S and Thoft R. A, Invest Ophthalmol. Vis. Sci., 1986, vol. 27 (4), pp 449.

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