Sci Pharm. 2008; 76: 505–514 doi:10.3797/scipharm.0804-22

Effect of Formulation Parameters on Corneal Permeability of Ofloxacin

Munish AHUJA * [1], Gurmeet SINGH [1], Dipak K. MAJUMDAR [2]

[1] Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar – 125 001, Haryana

[2] Delhi Institute of Pharmaceutical Sciences and Research, (University of Delhi), Pushp Vihar, Sector-III, New Delhi – 110 017, India

Abstract

Influence of pH, buffer, preservative, tonicity and viscosity modifiers on *in vitro* transcorneal permeation of ofloxacin was studied using excised goat cornea. Permeation studies were carried out by putting 1 ml of test formulation on the cornea (0.78cm^2) mounted between the donor and receptor compartments of an all glass modified Franz diffusion cell and measuring ofloxacin concentration in the receptor (containing bicarbonate ringer under stirring at 35°C) by spectrophotometry at 288 nm, at various time intervals up to 120 min. Buffering the formulation with phosphate buffer or use of combination of methyl and propyl paraben or benzalkonium chloride or combination of benzalkonium chloride and disodium edetate as preservative provided significant increase in the apparent corneal permeability coefficient of ofloxacin. While the use of phenyl mercuric acetate as preservative or tonicity adjustment with mannitol showed a significant decrease in apparent corneal permeability of ofloxacin. Raising the pH of test formulation from 6.4 to 7.2 or addition of hydroxyl-propyl-β-cyclodextrin or use of viscosity modifier had no significant effect on apparent corneal permeability of drug.

Keywords

Ofloxacin • Corneal permeation • Formulation factors • Papp

* Corresponding author: Tel.: +91-1662-263162; Fax: +91-1662-267240.
E-mail: munishahuja17@yahoo.co.in (M. Ahuja).

Introduction

Fluoroquinolones elicit their bactericidal response by inhibiting bacterial DNA gyrase and topoisomerase IV [1]. Fluoroquinolones possess excellent bactericidal activity against most frequently occurring gram+ve and gram-ve ocular pathogens [2]. As a result fluoroquinolones have carved their own niche in the topical management of ocular infections. Ofloxacin (0.3%, w/v) ophthalmic solution is official in USP [3] and is indicated in the treatment of conjunctivitis and keratoconjunctivitis. Intraocular penetration of ofloxacin has been found to be significantly greater than that of ciprofloxacin and norfloxacin [4]. Tear film levels of ofloxacin were found to be greater than MIC90 for a wide range of clinical isolates even 4 hours after the topical instillation [5]. Ofloxacin (0.3% w/v) ointment has been successfully employed in the management of ocular chlamydial infections [6]. Earlier studies have demonstrated the enhancement of corneal penetration of ofloxacin by chitosan and its derivatives [7, 8].

Topical delivery of drugs to the ocular tissues is affected by a complex interplay of biological, physiochemical and formulation factors. Formulators usually have to design a dosage form which provides a balance between corneal penetration, ocular irritation and formulation stability. Manipulation of formulation parameters to enhance the corneal penetration is one of the approaches of increasing ocular availability [9, 10].There is very little information available on the effect of formulation parameters on the corneal permeation of ofloxacin. In the present study the effect of pH, buffer, tonicity modifier, viscosity modifier, preservative and stabilizers on the corneal permeation of ofloxacin was evaluated using freshly isolated goat cornea.

Results and Discussion

Tab.1 summarizes the effect of pH, buffer and tonicity modifiers on corneal permeation of ofloxacin. The average pH of tears is 7.2 and eyes can tolerate pH of 6.5-8.0 without much discomfort [9]. Ofloxacin yields a slightly turbid solution at

0.3% w/v concentration at pH 7.2, which becomes clear when pH is reduced to 6.4. Ofloxacin ophthalmic solution (0.3%, w/v) USP has a pH of 6.0-6.8. Increasing the pH of the formulation to physiological pH will reduce its irritation potential. So, in order to enhance the solubility of ofloxacin at pH 7.2, hydroxypropyl-β-cyclodextrin was employed. Cornea has an isoelectric point (pI) of 3.2, and above this pH, cornea is negatively charged, and thus becomes selectively permeable to cations [11]. Ofloxacin is a racemate with pKa_1 of 5.5 (for the carboxyl group), pKa_2 of 8.0(for the piperazinyl group) and pI of 6.75 [12]. Thus, increasing the pH of ofloxacin solution from 6.4 to 7.2 will increase the unionized fraction of ofloxacin, which should increase its corneal permeation. But the unionized drug concentration exceeds the solubility of drug in water at pH 7.2; as a result it gives a cloudy solution. Inclusion of cyclodextrin improved the solubility of drug at pH 7.2. Cyclodextrins have earlier been reported to enhance [13] and dimnish [14] the corneal permeation of drugs. However, the results of present study show that there was no significant effect of addition of hydroxypropyl-β-cyclodextrin on the corneal permeation of ofloxacin.

Tab. 1. Effect of pH, buffer and tonicity modifier on corneal permeation of ofloxacin

pH	**Buffer**	**Tonicity Modifier**	**Papp* (cm/sec x 10^6)**	**% Corneal hydration***
6.4	Unbuffered	Sodium chloride	0.73±0.25	81.20±1.05
6.4	Citrate	Sodium chloride	0.72±0.07	80.27±0.20
6.4	Phosphate	Sodium chloride	1.31±0.30[b]	81.04±1.05
6.4[a]	Phosphate	Sodium chloride	1.22±0.10	81.61±0.08
7.2[a]	Phosphate	Sodium chloride	1.50±0.07	81.19±0.10
6.4	Phosphate	Glucose	0.96±0.04	81.23±0.11
6.4	Phosphate	Mannitol	0.64±0.16[†]	81.06±1.00

* Values represent mean ± SD (n=3), [a] Formulations contained hydroxypropyl-β-cyclodextrin, [b] Significant difference compared to unbuffered formulation, [†] Significant difference compared to formulation containing sodium chloride as tonicity modifier.

On comparing the corneal permeation of ofloxacin from different buffered vehicles, it was observed that there was a significant increase (P<0.05) in corneal permeation of ofloxacin from isotonic phosphate buffer compared with unbuffered vehicle, while citrate buffer had no effect on ofloxacin permeation.

The results also reveal that tonicity adjustment with sodium chloride provided maximum permeation of ofloxacin followed by glucose and mannitol. There was a significant decrease (P<0.05) in corneal permeation when mannitol was employed.

Tab. 2. presents the data for corneal permeation of ofloxacin from ophthalmic solution preserved with different preservatives. The results show that there was a significant increase (P<0.05) in corneal permeation of ofloxacin when combination of methylparaben (MP) and propylparaben (PP), benzalkonium chloride (BAC) or combination of BAC and disodium edetate (EDTA) were employed, while the use of phenylmercuric acetate (PMA) was associated with a significant decrease (P<0.05) in permeation. Benzyl alcohol (BA), thiomersal (TM) and phenylmercuric nitrate (PMN) showed no significant change in corneal permeation of ofloxacin compared with control formulation containing no preservative.

Tab. 2. Effect of preservatives on corneal permeation of ofloxacin

Preservative	**Papp* (cm/sec x10^6)**	**% Corneal hydration***
Control	1.31±0.30	81.04±1.05
MP/PP	5.94±0.77†	80.01±0.80
BAC	2.19±0.47†	80.69±1.72
BAC/EDTA	2.22±0.66†	81.10±0.48
BA	1.26±0.49	79.01±0.93
THM	1.13±0.23	80.37±1.08
PMA	0.42±0.17†	80.01±0.80
PMN	1.31±0.17	80.38±1.04
SMS	1.25±0.35	81.03±1.04
EDTA	1.12±0.10	80.15±0.48
* Values represent mean ± SD (n=3), † Significant difference compared to control formulation containing no preservatives		

Combination of MP and PP have been found to increase the corneal transport of insulin [15] and diclofenac [16]. BAC, a cationic surfactant has been reported to increase the corneal permeation of drugs by emulsification and disruption of corneal epithelium [17]. Combination of BAC and EDTA has been observed to increase the corneal permeation of fluoroquinolones like moxifloxacin [18] and gatifloxacin [19].

Ofloxacin undergoes photochemical degradation [20], so sodium metabisulphite (SMS), an antioxidant with small preservative action, and EDTA, a chelating agent were also evaluated for their effect on permeation. SMS has been found to enhance corneal transport of diclofenac [16]. EDTA [21] also has been found to increase the corneal permeation of drugs. However, in the present study, no significant change in corneal permeation of ofloxacin was observed with the use of SMS or EDTA.

Table 3 shows the effect of viscosity modifier on corneal permeation of ofloxacin. Viscosity modifiers are used in eye drops to prolong the precorneal residence of drugs. The results show that there was no significant change in the corneal permeation of ofloxacin from formulations containing viscosity modifiers compared with control formulation containing no viscosity modifier.

Tab. 3. Effect of viscosity modifiers on corneal permeation of ofloxacin

Viscosity modifier	**Papp* (cm/sec x10^6)**	**% Corneal hydration***	**Viscosity (mPa.s)**
None	1.31±0.30	81.04±1.05	0.87
MC	1.15±0.04	81.07±0.17	3.37
HPMC	1.11±0.79	82.02±0.20	1.34
PVA	1.21±0.66	79.13±1.49	3.57
* Values represent mean ± SD (n=3)			

It is expected that during *in vivo* use polyvinyl alcohol (PVA) and methyl cellulose (MC) having higher viscosity will provide maximum precorneal residence.

PVA containing drops are however easy to filter than products containing cellulose derivatives, when the drops are sterilized by filtration. Cellulose derivatives precipitate from solution on heating and thus need to be redispersed by shaking after autoclaving and cooling while PVA does not present any such problem. Thus, PVA appears to be an ideal viscosity modifier compared with the cellulose derivatives.

From the results of the present study, it can be concluded that formulation of ofloxacin (0.3%w/v) ophthalmic solution in phosphate buffer made isotonic with sodium chloride containing combination of methyl- and propylparaben or benzalkonium chloride and EDTA or benzalkonium chloride favors corneal permeation of ofloxacin.

Experimental

Materials

Ofloxacin (purity 99.2%) and hydroxypropyl-β-cyclodextrin were obtained as a gift sample from Ranbaxy Research Laboratory (Gurgaon, India). All other chemicals purchased were of analytical grade and were used as received. Fresh whole eyeballs of goat were obtained from local butcher shop (Hisar, India).

Preparation of test formulations

(a) *Ofloxacin ophthalmic solutions of different pH* - Ofloxacin (0.3% w/v) solutions in isotonic phosphate buffer (0.0667M) of pH 6.4 or pH 6.4 (containing 2 mmol hydroxypropyl-β-cyclodextrin) or pH 7.2 (containing 2 mmol hydroxypropyl-β-cyclodextrin) were prepared.

(b) *Ofloxacin ophthalmic solutions in different buffers* - Ofloxacin 0.3% (w/v, pH 6.4) solution in water or phosphate buffer (0.0667M) or citrate buffer (0.0667M) as a vehicle was prepared and made isotonic by adding sodium chloride.

(c) *Ofloxacin ophthalmic solutions containing different tonicity modifier* - Ofloxacin 0.3% (w/v) solution in phosphate buffer (pH 7.4, 0.0667M) made isotonic with either of sodium chloride or mannitol or glucose, was prepared.

(d) *Ofloxacin ophthalmic solutions containing antimicrobial preservative* - Ofloxacin 0.3% (w/v) solution in isotonic phosphate buffer (0.0667 M, pH 6.4) containing either BAC (0.01%, w/v) or combination of BAC (0.01%, w/v) and EDTA (0.01%, w/v) or phenylmercuric acetate PMA (0.001%, w/v) or PMN (0.001%, w/v) or TM (0.005%,w/v) or BA (0.5%, v/v) or combination of MP (0.02%, w/v) and PP (0.01%, w/v) or SMS (0.3%, w/v) or EDTA (0.1%, w/v) was made.

(e) *Ofloxacin ophthalmic solutions containing viscosity modifiers* - Ofloxacin 0.3% (w/v) solution in isotonic phosphate buffer (pH 6.4), containing either MC (0.25%, w/v) or HPMC (0.25%, w/v) or PVA (1.4%, w/v) was made.

Viscosity Measurement - Viscosity of ofloxacin 0.3% (w/v) ophthalmic solution containing viscosity modifiers was measured using an Ostwald viscometer

In vitro corneal permeation studies - Whole eye ball of goat was transported from the local butcher shop to the laboratory in cold (4 ^{o}C) normal saline within one hour of slaughtering of the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from protein. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of an all glass modified Franz diffusion cell [22] in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.78 cm^2. The receptor compartment was filled with 11 ml of freshly prepared bicarbonate ringer (pH 7.4). One milliliter of test formulation was placed on the cornea. Evaporation of the test formulation was prevented by sealing the opening of the donor compartment with a glass cover slip, while the receptor fluid was maintained at 35^{o} C with constant stirring, using a Teflon-coated magnetic stir bead. One ml sample was withdrawn from the receptor compartment at various time intervals up to 120 min and withdrawn samples were replaced with equal volume of bicarbonate ringer. The samples were analyzed for ofloxacin by measuring absorbance at 288 nm in a spectrophotometer (Cary 5000, Varian, Australia). At the end of the experiment, each cornea, freed from sclera, was weighed, soaked in

1 ml methanol, dried overnight at 90 ^{o}C and reweighed. From the difference in weights corneal hydration was calculated.

Calculation of apparent permeability coefficient-Apparent permeability coefficient was calculated using the following equation-

$$Papp = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{(A \cdot Co \cdot 60)}$$

Where, ΔQ/Δt (μg/min) is the flux across the corneal tissue. A is the area of diffusion (cm^2), Co (μg/cm^3) is the initial concentration of drug in donor compartment, and 60 is taken as the factor to convert minute into second. The flux across the cornea was obtained from the slope of the regression line obtained from the linear part of the curve between the amount permeated (Q) Vs time (t) plot.

Acknowledgement

Authors are grateful to Ranbaxy Research Laboratories (Gurgaon, India) for the gift samples of ofloxacin and hydroxypropyl-β-cyclodextrin.

References

[1] Hardman JG, Limbird LE.
In: Goodman & Gilman's The Pharmacological Basis of Therapeutics.
New York: McGraw-Hill, 2001.

[2] Adenis JP, Colin J, Verin P, Saint-Blancat P, Malet F.
Ciprofolxacin ophthalmic solution versus rifamycin ophthalmic solution for the treatment of conjunctivitis and blepharitis.
Eur J Ophthalmol. 1995; 5: 82–87.

[3] The United States Pharmacopoeia.
United States Pharmacopoeial Convention, Inc, Rockville, 2005: 1413–1414.

[4] Donnenfield ED, Schrier A, Perry HD, Aulicino T, Gombert ME, Snyder R.
Penetration of topically applied ciprofloxacin, norfloxacin, and ofloxacin into the aqueous humor.
Ophthalmology. 1994; 101: 902–905.

[5] Borman L, Tang-liu D, Kann J, Nista J, Lin ET, Frank J.
Ofloxacin in human serum, urine and tear film following topical application.
Cornea. 1992; 11: 226–230.

[6] Aoki K, Moroboski ST.
Clinical effect of ofloxacin ointment for chlamydial conjunctivitis.
Jap J Clin Ophthalmol.1986; 40: 985–988.

[7] Di Colo G, Zambito Y, Burgalassi S, Nardini I, Saettono MF.
Effect of chitosan and of N-carboxymethylchitosan on intraocular penetration of topically applied ofloxacin.
Int J Pharm. 2004; 273: 37–44.
[doi:10.1016/j.ijpharm.2003.12.018]

[8] Di Colo G, Burgalassi S, Zambito Y, Monti D, Chetoni P.
Effects of different N-trimethyl chitosans on in vitro/in vivo ofloxacin transcorneal permeation.
J Pharm Sci. 2004; 93: 2851–2862.
[doi:10.1002/jps.20197]

[9] Smolen VF, Bull L, editors.
The Control of Drug Bioavailability from Ophthalmic Dosage Forms.
In: Bioavailability Control by Drug Delivery System Design.Volume 3.

[10] Malhotra M, Majumdar DK.
Permeation through cornea.
Indian J Exp Biol. 2001; 39: 11–24.

[11] Rojanasakul Y, Robinson JR.
Transport mechanisms of the cornea: characterization of barrier permselectivity.
Int J Pharm. 1989; 55: 237–246.
[doi:10.1016/0378-5173(89)90047-1]

[12] Kawazu K, Midori Y, Shiono H, Ota A.
Characterisation of the carrier-mediated transport of levofloxacin, a fluoroquinolone antimicrobial agent, in rabbit cornea.
J Pharm Pharmacol. 1999; 51: 797–801.
[doi:10.1211/0022357991773168]

[13] Usayapant A, Karara AH, Narukar MM.
Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate.
Pharm Res. 1981;12: 1495–1499.

[14] Davies NM, Wavy G, Tucker IG.
Evaluation of hydrocortisone/hydroxypropyl - β-cyclodextrin solution for ocular drug delivery.
Int J Pharm. 1997; 56: 201–209.
[doi:10.1016/S0378-5173(97)00199-3]

[15] Sasaki H, Tei C, Yamamura K, Nishida K, Namammura J.
Effect of preservatives on systematic delivery of Insulin by ocular instillation in rabbits.
J Pharm Pharmacol. 1994; 46: 871–875.

[16] Ahuja M, Dhake AS, Majumdar DK.
Effect of formulation factors on *in vitro* permeation of diclofenac from experimental and marketed aqueous eye drops through excised goat cornea.
Yakugaku Zasshi. 2006; 126: 1369–1375.
[doi:10.1248/yakushi.126.1369]

[17] Fu RC, Lidgate DM.
In vitro rabbit corneal permeability study of ketorolac tromethamine, a non steroidal anti-inflammatory agent.
Drug Dev Ind Pharm. 1986; 12: 2403–2430.

[18] Pawar PK, Majumdar DK.
Effect of formulation parameters on *in vitro* permeation of moxifloxacin from aqueous drops through excised goat, sheep and buffalo corneas.
AAPS PharmSciTech. 2006; 7: E12–E17.
[doi:10.1208/pt070113]

[19] Rathore MS, Majumdar DK.
Effect of formulation factors on in vitro transcorneal permeation of gatifloxacin from aqueous drops.
AAPS PharmSciTech. 2006; 7: E89–E94.
[doi:10.1208/pt070357]

[20] Tiefenbacher EM, Haen E, Pryzbilla B, Kurz H.
Photodegradation of some quinolones used as antimicrobial therapeutics.
J Pharm Sci.1994; 83: 463–467.
[doi:10.1002/jps.2600830403]

[21] Grass GM, Wood RW, Robinson JR.
Effects of calcium chelating agents on corneal permeability.
Invest Ophthalmol Vis Sci. 1985; 26: 110–113.

[22] Malhotra M, Majumdar DK.
Effect of preservative, antioxidant and viscolizing agents on in vitro transcorneal permeation of ketorolac tromethamine.
Indian J Exp Biol. 2002; 40: 555–559.

Received April 20th, 2008
Accepted (after revision) July 3rd, 2008
Available online at www.scipharm.at August 9th, 2008