Acute Bacterial Conjunctivitis

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Introduction

The eye is considered as one of the sensitive sensory organs. It is affected by many diseases, infection being the prominent. Ocular infections, due to bacteria are most prevalent worldwide. The other ocular infections are related to factors such as age, chronic nasolacrimal duct obstruction, contact lenses, trauma, dry eye state, surgery, and previous other ocular infections also [Yellepeddi & Palakurthi 2016; Galvis *et al*, 2014; Choudhury 2012; Iwalokun *et al*, 2011]. Ocular infections can be categorized into keratitis, conjunctivitis, blepharitis, endophthalmitis, orbital cellulitis and dacryocystitis manifestations. Bacterial conjunctivitis and keratitis are the common ocular infections and are caused bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Coagulase negative Staphylococci* [Tesfaye *et al*, 2013; Bertino, 2009].

Bacterial keratitis is an infection of the cornea due to bacteria. It accounts for approximately 90% of all microbial keratitis cases (Musa *et al*, 2010). The common bacteria causing keratitis are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Serratia* species [Lakhundi *et al*, 2018; Dakhil *et al*, 2017; Wong *et al*., 2012]. This disease is usually associated with acute pain, redness, photophobia and corneal ulceration [Stapleton *et al*, 2007]. Pseudomonas ulcers are more severe than other bacterial ulcers and are often difficult to treat. It may lead to worsening of condition than other bacterial ulcers (Sy *et al*, 2012; Green *et al*, 2008). Microbial keratitis is also common in contact lens wearers. Although there is increased use of daily disposable contact lens wear, but, the incidence of bacterial ulcers related to contact lens wear still remains high [Becmeur *et al*, 2017; Cheung *et al*, 2016; Sauer *et al*, 2016]. The approximate yearly incidence is 2 per 10,000 and most frequent causative organisms are Gram-negative organisms [Rahimi *et al*, 2015; Fleiszig, 2006; Schein *et al*, 2005; Zaidi *et al*, 2004; Cheng *et al*, 1999].

LITERATURE REVIEW

A literature study was carried out in order to understand research work carried out by other workers for improvement in ocular absorption and bioavailability

FORMULATION STRATEGIES TO IMPROVE OCULAR BIOAVAILABILITY LIMITATIONS

1 short residence time of the drug at the site of administration
2 Limitation in amount of drug administered
3 poor penetration through tissues
4 Tear secretion and reflex blinking
5 induction of tear flow because of irritation caused by the drug formulation
6 The topical administration of drugs seems to be an ideal route, but has certain limitations as given below: (Fangueiro *et al*, 2016; Cholkar *et al*, 2013)

Based on above considerations target should be

1 enhancement of the precorneal residence time by slow removal of drug from the absorption site to allow more time for absorption
2 Improvement of both paracellular and intracellular pathways

Such approach would not only reduce the dose, but also reduce frequency of instillation thereby improving patient compliance and reducing side-effects.

In general, Formulation development takes into consideration physicochemical properties of drug molecule i.e., solubility and permeability of drug.

- High solubility criteria : the drug molecule should be soluble in instilled volume (7 µl)
- High permeable criteria: the drug molecule itself has sufficient permeability to cross ocular barriers

The formulation strategy can be broadly classified as:

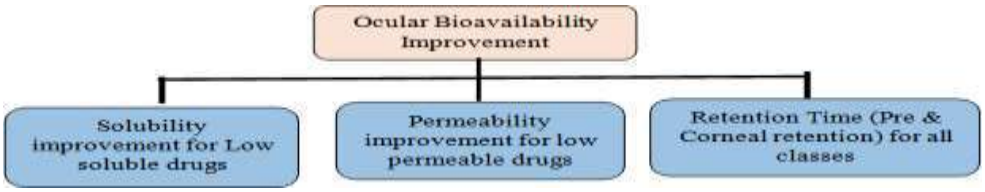

Figure: Formulation strategy approach

The above limitations can be overcome by either prodrug approach or encapsulation of drug in polymeric carriers. These are useful strategies to overcome/ bypass these barriers, and hence, the drug bioavailability at targeted ocular tissue can be improved.

Altering Formulation Properties

Bioavailability enhancement approaches has been investigated for topical ocular delivery to improving bioadhesion properties. It includes solid ophthalmic devices, liposomes, dendrimers, solid lipid nanoparticles, niosomes, contact lenses, viscous liquids, gels, suspensions, colloidal systems (nanoparticles and nanosuspension), matrix system (ocular inserts, minitablets and collagen shields) and microparticles [Rodriguez Villanueva *et al*. 2016; Yellepeddi & Palakurthi, 2016]. Table 5 highlights advantages and disadvantages of each delivery system. These micro- and nano-sized dimensions particulate systems has also been explored as an appropriate alternative to conventional options in ophthalmology. They offer the possibility to enhance delivery and transport of drugs across ocular tissues [Bravo-Osuna *et al*. 2016a].

Altering Physico-chemical properties

The physicochemical barriers include properties of the drug such as lipophilicity, solubility, molecular size and shape, and loss of drug from the ocular. The chemical approaches such as prodrugs (Xalatan™), chemical delivery systems, and soft drugs. The pro drug approach was effective in overcoming barriers to topical administration of drugs, but some demerits such as enhanced lipophilicity and lack of target specificity limit their clinical success. Such systems targets number of membrane transporters present in various ocular tissues such as the cornea, conjunctiva, and retina. Hence, transporter- targeted pro drug approach is a recent advancement in topical drug delivery.

Prevalence of Bacterial Keratitis in different parts of world

Country	Inference	References
USA	The incidence is 25,000 annually and is most prevalent in northern locations.	Estopinal *et al*, 2016
UK	Temperature correlation observed with type of bacteria. Gram-positive bacteria grew with increasing temperature. Moraxella sp. grew with decreasing temperature.	Walkden *et al*, 2018
Canada (Toronto)	Toronto: The most commong gram-negative bacteria isolated was *Pseudomonas aeruginosa* and coagulase- negative Staphylococcus respectively. Vancouver: The majority of bacterial ulcers were caused by Gram-positive bacteria. Contact lens wear was identified as the major risk factor for development of Gram-negative ulcers.	Lichtinger A *et al*, 2012 Termote *et al*, 2018
Saudi Arabia	Gram-positive bacteria make up a greater proportion of bacterial keratitis.	Al-Dhaheri *et al*, 2016
China	Fusarium species being the most common pathogens	Pan *et al*, 2016
Iran	Gram-positive bacteria is the most common causative organism for bacterial keratitis	Rahimi *et al*, 2015
India	Bacteria identified were Streptococcus pneumonia, Pseudomonas aeruginosa, and Nocardia sp.	Chidambaram *et al*, 2018
	Gram-positive organisms were coagulase-negative Staphylococci and Staphylococcus aureus (including one	Watson *et al*,

Australia	methicillin-resistant Staphylococcus aureus [MRSA]). Gram-negative organisms were Pseudomonas aeruginosa methicillin-sensitive Staphylococcus aureus (MSSA).	2018
Muscat (Oman)	Gram-positive bacteria accounted for majority was due to *Streptococcus pneumonia* and gram-negative bacteria was *Pseudomonas aeruginosa*.	Al-Ghafri & Al-Raisi, 2018

Mechanism

Tears have three layers. 1) lipid-rich layer which covers the surface of the tears and limit its evaporation - meibomian layer 2) aqueous layer which lubricates the anterior eye. It also contains multiple antibacterial proteins, peptides, 3) a mucous layer that interacts with the corneal epithelial cells. The aqueous layer contains sIgA, IgG, and IgM immunoglobulins. The IgA molecules are more prevalent than the other immunoglobulins and those that recognize bacterial adhesins can prevent bacterial attachment to cells of the corneal or conjunctival epithelium. Aqueous layer also contains antimicrobial peptides (AMP) (cathelicidin LL-37 and beta-defensins) that bind to and kill bacteria [Suzuki *et al*, 2010; Gordon *et al*, 2005].

Phospholipase A2 and antimicrobial molecules found in aqueous layer activities increases in response of the epithelial cells to the presence of the bacteria. The amount of phospholipase A2 is increased by five-fold, once infection reaches anterior portion of the eye. Antimicrobial peptides called KDAMP's can kill bacteria of variable nature including both *S. aureus* and *S. epidermidis*. Human corneal epithelial cells can produce beta-defensin 2 (hBD-2), and this has a lethal effect on S. aureus and other bacteria [Tam *et al*, 2012; Kumar *et al*, 2006; Girgis *et al*, 2003; McDermott *et al*, 2001].

SECONDARY BACTERIAL INFECTION DUE TO DISORDER

Secondary Bacterial infections are not caused directly by airborne bacteria. But, related to infection or inflammation of the lacrimal duct system, conjunctival intraepithelial neoplasm, floppy eyelid syndrome, and immunologic reaction. The conditions are as below:

Chronic Dacryocystitis

This condition is more likely limited to one eye due to obstruction of the nasolacrimal duct. It is characterized by redness of the eye, epiphora and a chronic mucopurulent discharge and, sticking of eye lashes. Also, swelling may be at medial canthal region overlying the lacrimal sac. Since, lacrimal sac is motionless, there is bacterial growth causing infection. Retrograde drainage of purulent material into the eye causes the conjunctivitis [Pinar-Sueiro *et al*, 2012; Burduk *et al*, 2008; Mills *et al*, 2007; de la Cuadra-Blanco *et al*, 2006].

The organisms isolated includes *S epidermidis, S aureus, P. aeruginosa, E. coli Streptococcus, Pseudomonas, Pneumococcus, Peptostreptococcus, Propionibacterium, Prevotella, Fusobacterium, and Staphylococci species* [Mishra *et al*, 2017; Eshraghi *et al*, 2014; Tesfaye *et al*, 2013; Mills *et al*, 2007; Bharathi *et al*, 2008; Sun *et al*, 2005; Chaudhry *et al*, 2005].

MOXIFLOXACIN: OCULAR FORMULATION DEVELOPMENT

Fluoroquinolones are powerful treatment options for preventing potentially sight- threatening bacterial infections and moxifloxacin that provides high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. [Robertson *et al*, 2005]. A literature search was carried out to study work of other researchers to improve corneal residence time using moxifloxacin as model drug.

RATIONALE

Bacterial conjunctivitis and keratitis are common ocular infections and are mainly caused bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae* and Coagulase negative *Staphylococci*. Topical antibiotic eye drops are most preferred route of administration. These drops are capable of achieving high tissue levels. However, this route has limitation of frequent dosing usually every half an hour for the first 24 – 36 hours. Among topic antibiotics, fluoroquinolones (specifically fourth generation fluoroquinolones) are treatment of choice in both bacterial keratitis & conjunctivitis. It use and effectiveness has been validated by multiple studies.

The medication frequency depends upon the severity of infection. It is usual to start half- hourly drops all through 24 h for most patients. A loading dose of a drop every 5 min for the first 30 min

is used in severe ulcers [Bacterial Keratitis: Preferred Practice Pattern, 2018.]. Hence, there has always been focus in reducing dosing frequency and increasing patient compliance, sustained release ocular formulation that provides increased pre- corneal residence time, improved permeability and intra-stromal bioavailability.

Commercial extended release formulation

Drug	Route	Indication	Disease
Moxeza (Moxifloxacin Hydrochloride)	Opthalmic	A Xanthan gum base formulation to be instilled in the affected eye(s) 2 times daily for 7 days.	Bacterial conjunctivitis
Besivance **(**Besifloxacin Hydrochloride)	Opthalmic	A Polycarbophil base formulation to be instilled in the affected eye(s) 3 times a day, four to twelve hours apart for 7 days	Bacterial conjunctivitis

ANTIBACTERIAL ACTIVITY

In order to evaluate the antibacterial activity of MOX loaded SLNs, the agar cup plate method was used and testing carried out using *S. aureus (MTCC96)*, *Ecoli* (MTCC 3850) and *B Subtilis (*MTCC 441). The method was based on inverse relation between minimum inhibitory concentration (MIC) and diameter of zone of inhibition. A layer of nutrient agar (20 ml) was allowed to solidify in petriplate the cultures of microbes in nutrient broth were transferred on solidified agar and cultures were uniformly dispersed. SLNs formulations and marketed eye drops were poured in cups. All petriplates were kept at room temperature for 2 hrs to diffuse drug into medium then incubated for 24hrs at 37˚C. Diameter of zone of inhibition was noted at various time intervals.

Pharmacodynamic evaluation of Bacterial Conjunctivitis & Keratitis in Rabbit Model

Female Rabbits (9) of 1.5 – 2 kg was used for each study. Both eyes of each experimental animal were examined before testing starts using ophthalmoscope. Animals showing eye irritation, ocular defects, or pre-existing corneal injury was rejected. Animals were divided into three groups: i.e., Group 1: Control infected and untreated, Group 2: infected and treated with commercial formulation [Moxicip], Group 3: Infected and treated with formulation OPT-07-1. The test proceure for each study was as follows:

Pharmacodynamic evaluation in Bacterial Conjuctivitis Rabbit Model

Bacterial suspension of S. aureus (≈ – 200 CFU/ml) was adjusted to contain with sterile physiological saline. 100 µl of bacterial suspension was inserted into cul-de-sac into both eyes. After 48 hrs infection was confirmed and following signs were noted for redness score: 0-5, inflammation score 0-5, tears score 0-5. After confirmation of conjunctivitis, treatment initiate for each group i.e., group 1: 50 µl saline, group 2: 50 µl marketed formulation every 8hrs, group 3: 50 µl SLN formulation every 12 hrs. The eyes were examined for signs of bacterial inflammation every day in each group. After completion of study, degree of infection was evaluated in Control group and treatment.

Pharmacodynamic study in Bacterial Keratitis in Rabbit Model

Rabbits were anesthetized with ketamine (5 mg/kg). The ocular surface then was locally anesthetized using lidocaine solution. 20 µl of bacterial suspension of **S. aureus** (≈ 1000 CFU/ml) was inserted into stroma of both eyes. After 24 hrs infection was confirmed and following signs were noted i.e., Redness Score 0-5, Opaqueness 0-5, Lacrimal secretion 0-3, Mucoidal discharge 0-4, Response to ocular stimulus (shining torch light on to the eye) 0-2 Swelling of eye lid 0-4. After confirmation of conjunctivitis, treatment initiate for each group i.e., group 1: 50 µ saline, group 2: 50 µ marketed formulation every 6 hrs, group 3: 50 µ SLN formulation every 12 hrs. The eyes were examined for signs of bacterial inflammation every day in each group. After completion of study, degree of infection was evaluated wrt control group.

RESULTS & DISCUSSION

Antibiotic therapy for ocular infection (bacterial conjunctivis and keratitis) is usually continued till 7 days. Hence, study of CQAs (entrapment efficiency & drug release) was carried out from reconstitution till use. A sample was withdrawn at 5, 10 & 15 days and analzed for entrapment efficiency & drug release. Analytical results are shown in table 44.

Table : Results of CQAs for formulation OPT-07-1 till use after redispersion

		Initial	Day 5	Day 10	Day 15
Physical appearance		Light yellow colored suspension	Light yellow colored suspension	Light yellow colored suspension	Light yellow colored suspension
Entrapment Efficiency		74.53 ± 1.9	73.18 ± 2.5	72.62 ± 3.3	68.32 ± 3.6
Drug Release	0.5 hr	19.8 ± 1.3	20.6 ± 1.5	21.3 ± 1.2	24.3 ± 1.3
	8 hr	60.6 ± 1.2	61.2 ± 1.0	61.6 ± 1.4	63.8 ± 1.1
	16 hr	99.6 ± 0.9	98.7 ± 0.7	99.2 ± 0.6	99.5 ± 0.7

Results indicated a dropping time in hold time study. However, drop in entrapment efficiency is less than 2% in 10 days. Hence, formulation can be used till 10 days.

Anti-bacterial activity

Bioassay is *in vitro* test to identify activity of formulation against desired microorganism, which is otherwise not estimated through spectroscopic technique. The formulation should maintain its free concentration above MIC in order prevent microbial growth. Similar experiments carried out by Kersala *et al*, 2016, Baig *et al*, 2016 and kalam *et al*; 2010 to check microbial activity for their respective formulations (moxifloxacinnanosuspension dispersed in gellan gum and gatifloxacin formulation dissolved in Gelrite).

Antimicrobial activity of the optimized formulation (OPT-7-1) was tested using agar diffusion method employing ‗cup plate technique'. Standardmicrobes used in this study were: *staphylococcus aureus* (MTCC 96) and *Escherichia coli* (MTCC 3850) and *bacillus subtilis (MTCC 441)*. A standard suspension of bacteria was titrated to get 10^4 - 10^5cfu per ml. A layer of nutrient agar (20 ml) seeded with the test micro organism (1 ml) was allowed to solidify in Petri dish. Cups were made on thesolidified agar layer with the help of sterile borer with 4 mm diameter. Marketedsterile formulation and optimized formulation (50 µl) solution and were poured into cups of agar plates. After allowing diffusion of solution for two hours, the agar plates wereincubated at 37°C for 24 hrs. The zone of inhibition (ZOI) was measured around eachcup and was compared with the marketed formulation. The entire operation except theincubation was carried out in an aseptic area with laminar air flow unit. Theantimicrobial activities were measured as the diameter (cm) of clear zone for growth inhibition. The tests were carried in triplicate four reading were measured from each plate. The mean of 12 observations and standard deviation is shown in table 47. The representative picture of plates for each bacterium is shown in figure 60 – 62.

Pharmacodynamic evaluation in Bacterial Conjuctivitis Rabbit Model

The pharmacodynamics model developed to study POC study is development of bacterial keratitis in rabbit eye using *Staphylococcus aureus* [MTCC 96]. This model has also been studied by other researchers for studying therapeutic effectiveness of moxifloxacin HCl and hyaluronic acid contact lenses, tobramycin sulfate in-situ gel systems, lomefloxacin HCl niosomal system and proniosomes [Maulvi *et al*, 2018; Khan *et al*, 2018; Khalil *et al*, 2017 a&b]. The evaluation criteria used was physical evaluation of signs and symptoms i.e., redness, inflammation and tear score.

Pharmacodynamic study to formulation effectiveness in Bacterial Keratitis

The pharmacodynamics model developed to study POC study is development of bacterial keratitis in rabbit eye using *Staphylococcus aureus*. [MTCC 96]. The selection is based on epidemiological data which indicate *Staphylococcus aureus*.are most common species associated with bacterial keratitis in humans. This method was first reported by Kupferman and Leibowitz [1975] where bacterial keratitis was induced using intrastromal injection to test efficacy of topical antibiotic therapy of *S. aureus* keratitis. Recently this method has been used to study efficacy of ciprofloxacin hydrochloride pre-formed gels and thermally triggered (*in situ*) gels, moxifloxacinmucoadhesive microspheres, gatifloxacinion-activated mucoadhesive hydrogel, ofloxacin loaded nano structured lipid carriers modified with chitosan oligosaccharide lactate and nanostructured lipid carriers, gelatin-capped silver nanoparticles [Luo *et al*, 2019; Bharti & Kesavan, 2017; Üstündag˘- Okur *et al*, 2015; Kesavan *et al*, 2015;

Üstündağˇ-Okur *et al*, 2014; Abdelkader& Mansour, 2013; Dandagi *et al*, 2013].
Nine rabbits were taken for this study and bacterial keratitis was induced by instilling strains of S. aureus in rabbit‘s eyes. The eyes were observed for signs and symptoms such as redness, Opaqueness, lacrimal secretion, mucoidal discharge, swelling of eyelid, and response to ocular stimuli. The scoring was carried out for above signs based on visual inspection up to 5 days. The total score (mean ± sd) for control group (untreated group), and treated with marketed formulation and Investigational hybrid formulation is formulation is provided in table 54 and figure 66 depict pictorial view of disease condition observed Overall, OPT-07-1 showed promising results in treating bacterial keratitis with reduced dosing frequency.

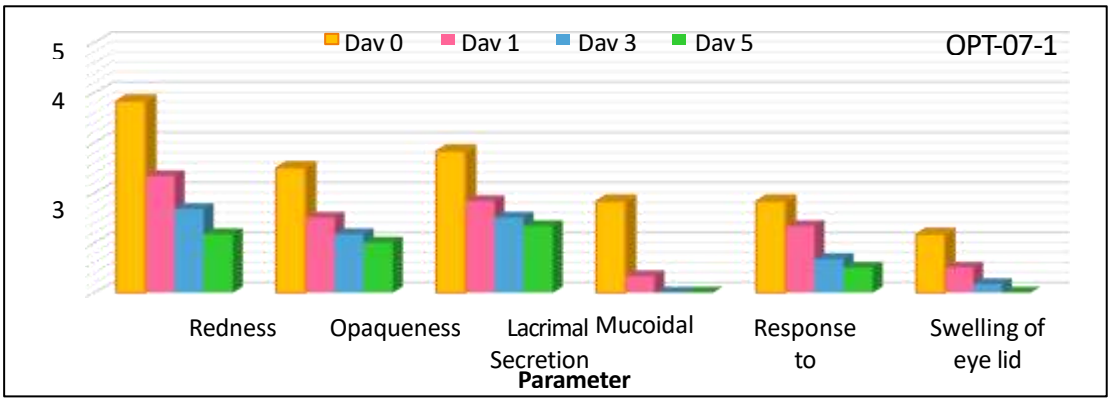

Figure : Disease severity in eyes infected with S. aureus for control group, treatment with marketed formulation and after treatment with Hybrid formulation OPT-07-1 at initial, day 1, 3 & 5th day of treatment

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