

MEASUREMENT OF INTRACAMERAL CONCENTRATION OF MOXIFLOXACIN ACHIEVED AFTER TOPICAL APPLICATION OF THE DRUG

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ABSTRACT

Aim: To estimate Intracameral concentration of Moxifloxacin applied topically in different schedule. **Methods:** Measurement of moxifloxacin was done in Aqueous aspirated before cataract extraction of different patients instilling topical drug in different schedule. **Results:** Significant difference was found between drug concentration of two groups, one instilling single drop of drug 30 min before surgery (1.20 ± 0.43 $\mu\text{g/ml}$) and other, instilling 4 drops a day before and one drop on day of surgery (1.80 ± 0.34 $\mu\text{g/ml}$). **Conclusion:** Instilling moxifloxacin drops 1 day before cataract surgery results in higher concentration and is effective in endophthalmitis prevention.

Key Words: Intracameral , Endophthalmitis, Neutrophils

Introduction:

Endophthalmitis is the most dreaded and potentially vision threatening condition of the eye particularly following cataract surgery. Once bacteria are introduced into the eye, risk factors that may increase the risk of endophthalmitis include rupture of the posterior capsule, retained lens material, and surgical procedure. Published studies have demonstrated an increased risk of endophthalmitis after placement of a secondary intraocular lens, possibly due to increased surgical time or ocular manipulation. Once clinical infection occurs, damage to ocular tissues is believed to occur due to direct effects of bacterial replication as well as initiation of a fulminant cascade of inflammatory mediators. Endotoxins and other bacterial products appear to cause direct cellular injury while eliciting cytokines that attract neutrophils, which enhance the inflammatory effect. Thus, recent efforts in

controlling the damaging effects of endophthalmitis in experimental models have focused on identifying not only appropriate antibiotics for control of the infectious agent but also on anti-inflammatory agents that might disrupt the immunologic events that occur after infection.

Moxifloxacin is a novel fourth-generation fluoroquinolone with high potency against both gram positive and gram negative bacterial pathogens. It has the highest potency in its class against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Moxifloxacin has been developed as a 0.5% solution for topical, ocular use as moxifloxacin ophthalmic solution 0.5%. In addition to high potency, a desirable characteristic of topical antibiotics is the rapid migration across the cornea and extensive penetration into anterior ocular tissues. The ocular bioavailability of different antibiotics can be compared on the basis of the concentration

achieved in the tears, cornea, conjunctiva, aqueous, or vitreous humor. The concentration of the antibiotic in these tissues should be maintained for sufficient time above the minimum inhibitory concentration (MIC) for important pathogens in order to achieve effective bacterial killing. The higher the concentration above the antibiotic's MIC, the greater the protection against infection. Investigators have conducted numerous non clinical and clinical studies to measure the ocular uptake and pharmacokinetics (PK) of moxifloxacin in comparison to other topical fluoroquinolones. Prophylactic preoperative antibiotic drops are instilled in the tear film with basic aim of reducing microbial flora in the precorneal tear film prior to surgery and to allow diffusion of topically applied antibiotic into the anterior chamber with the intention of combating bacteria at that site.

After topical administration, antibiotic is assumed to penetrate by simple diffusion from the precorneal tear film, through corneal layers, to the anterior chamber. Whenever these measured aqueous humor levels exceeded common bacterial MICs, "efficacy" against the microbe was implied.

Despite the very high antibiotic concentrations delivered to tears by topical antibiotic drops, several factors mitigate against achieving meaningful antibiotic levels inside the eye after drop administration. But the topically applied drug undergo rapid washout from tear film and nasolacrimal drainage system. There is a high inter patient variability in the percentage of an administered drop that is retained in the conjunctival cul-de-sac. Only a very small volume of fluid can be added and retained in the conjunctival cul-de-sac. Because the volume of commercial drops is so much larger than this small

volume, much of an administered drop spills out of the conjunctival cul-de-sac, and is lost.

Elimination of normal conjunctival flora is dependent on drug-pathogen contact time, which is thus dependent on the drug washout time.

Drug level in the aqueous humor is dependent on factors such as drug retention time on conjunctival sac, diffusion of drug across the cornea which in turn is dependent on many factors as described later, dose of the applied drug and physical and chemical property of the ophthalmic drug solution which is liable to vary with different commercial formulations. Prevention from intraocular infection is thus dependent on the bacterial killing property of the drug which is controlled by many factors as described above.

Aim of Study

The purpose of this study is:

1. To look at the concentration of moxifloxacin in aqueous of different individuals applying topical drug in fixed dosage at fixed schedule.
2. To quantify moxifloxacin in aqueous of different individuals applying topical drug in fixed dosage at different application schedule.
3. To study the difference in concentration of moxifloxacin in aqueous when applied with same strength of different commercially available eye drops in same dosing schedule.

Material and Methods:

The study titled "Measurement of intracameral concentration of moxifloxacin achieved after topical application of the drug" was conducted in Department of Ophthalmology, Institute of Medical Sciences, Banaras Hindu University, in

collaboration with Dept of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University. 20 patients were enrolled in this study.

Inclusion criteria: All normal patients undergoing cataract surgery were eligible for this study.

Exclusion criteria: Those patients with

- Corneal opacity or scarring
- Uveitis at the time of surgery
- Lens induced glaucoma
- Traumatic cataract
- Those using topical or oral fluoroquinolones prior to surgery.

All patients were divided in four groups each containing five patients.

Group A: Patients in this group were applied with single drop of standard commercially available topical moxifloxacin half an hr before surgery.

Group B: Patients in this group were applied with one drop four times one day prior and one drop on the day of surgery of standard commercially available topical moxifloxacin.

Group C: Patients in this group were applied with single drop of local commercially available topical moxifloxacin half an hr before surgery. **Group D:** Patients in this group were applied with one drop four times a day prior and one drop on day of surgery of local commercially available topical moxifloxacin. 0.1 ml of aqueous humor was aspirated from all patients at the time of surgery.

Surgical technique of aqueous humor aspiration:

Ocular surface was cleaned with 5% povidone iodine solution and methanol was used to clean adenexae taking precaution

not to spill spirit solution on ocular surface. Two forms of anaesthesia are used based on type of surgery: topical anaesthesia using proparacain drops applied on cornea, or peribulbar anaesthesia using xylocain injection. Ocular surface was cleaned again with 5% povidone iodine solution. The eye was draped using standard eye drape. In case of SICS, sclerocorneal tunnel was made using standard technique. 1 ml syringe fitted with 30 gauge needle was inserted in the anterior chamber through the cornea from the predetermined site of side port formation just before making of side port, taking care not to touch lens capsule with needle tip which can perforate the capsule making capsulorrhexis difficult and releasing liquefied lens matter leading to contamination of clear aqueous with protein. 0.1 ml of clear aqueous was aspirated gently taking precaution not to allow anterior chamber to collapse. Accidentally collapsed anterior chamber poses difficulty in rest of surgery. The aqueous contained in same syringe is stored at -4 degree celcius before processing in the lab and patient was continued with the surgery.

Validated High Performance Liquid Chromatography (HPLC) method for the Determination of Moxifloxacin:

Moxifloxacin protocol was adopted from Ahmed A. Abdelaziz, Tarek E. Elbanna, Noha M. Gamaleldeen¹.

Chromatographic condition: The solution were analysed by waters HPLC 515 having rheodyne7725i injector fitted with 20 micro lit loop and equipped with photodiode array (PDA) 2998 detector (waters, USA) using a waters column, c18 spherisorb 5.0 micro meter ODS2 4.6mm x 250mm column.

The mobile phase consisted of 20 mM Ammonium dihydrogen ortho-phosphate

solution (pH adjusted to 2.5) and acetonitrile (50: 50) and the flow rate was 1.0 ml/min. The effluent was detected at 295 nm.

Preparation of analytical calibration: The stock solution of moxifloxacin (1.0 µg/ml) in acetonitrile is prepared. The working standard solutions (con. 10, 20, 100, 200, 400, 600, 800 and 1000 ng/ml) were prepared from primary stock solution using acetonitril as solvent. 1 ml of the each solution was filtered through 0.22 syringe nylon filter. 20 µl of above filtrate were directly injected into the HPLC. Plot the graph between concentrations on x-axis vs area under the curve on y-axis. Determine the linearity and r² value.

Preparation of bio-analytical calibration: The stock solution of moxifloxacin (1.0 µg/ml) in acetonitrile is prepared. Aliquots of 500 µl of aqueous humor were placed in eppendrof tube and spiked with increasing concentration of working standard solutions to give final moxifloxacin

Observations and Results:

The study included 20 patients. These 20 patients were divided into four groups each containing 5 patients. Mean concentration of moxifloxacin in each group are shown in the table 1.

Table 1: Mean value of Moxifloxacin in the Aqueous Humor of Patients in the respective groups.

Group	Mean Conc. of Moxifloxacin (µg/ml)
A (N = 5)	1.20 ± 0.43
B (N = 5)	1.80 ± 0.34
C (N = 5)	0.51 ± 0.34
D (N = 5)	1.71 ± 0.48
Total (N = 20)	1.31 ± 0.64

The mean concentration of moxifloxacin in aqueous of group A using single drop of standard brand was 1.20 ± 0.43 µg/ml whereas in group C using single drop of local brand was 0.51 ± 0.34µg/ml. For those patients using single drop with a day prior, mean moxifloxacin concentration in aqueous were 1.80 ± 0.34 and 1.71 ± 0.48 µg/ml respectively for standard and local brand respectively.

The table 2 compares the mean concentration of drug in group A and C. The result shows a statistically significant difference between moxifloxacin concentration between group A and C with p value < 0.01

Table- 2: Compare the mean concentration of drug in A and C groups

Group	Mean Conc. of Moxifloxacin ($\mu\text{g/ml}$)
A	1.20 \pm 0.43
C	0.51 \pm 0.34
P value = < 0.01	

The table 3 compares the mean concentration of drug in group B and D. The result shows that there is no statistically significant difference between moxifloxacin concentration between group B and D with p value = 0.094

Table- 3: Compare the mean concentration of drug in B and D groups

Group	Mean Conc. of Moxifloxacin ($\mu\text{g/ml}$)
B	1.80 \pm 0.34
D	1.71 \pm 0.48
P value = 0.094	

Discussion:

Cataract extraction is by far the most common intraocular surgery performed worldwide. It is estimated that in India alone, more than 5.1 million patients have cataract surgery annually². Postoperative endophthalmitis is a rare but dreaded complication of cataract surgery, with a reported incidence currently in the range of 0.04% to 0.41%^{3,4}. In most cases, this complication is unforeseeable, its progression is unpredictable, and the visual outcome can be devastating. With a projected steep rise in the already sizable global volume of cataract surgery, minimizing the rate of endophthalmitis and the cost of performing surgery will be extremely important.

The prospective multicenter multinational ESCRS study of endophthalmitis prophylaxis⁵ reports an

overall endophthalmitis incidence of 0.17% (29 of 16603 patients). The incidence of endophthalmitis was statistically significantly higher after suture less manual SICS than after phacoemulsification. Kalpadakis et al.⁶ found a much higher incidence of endophthalmitis after large-incision ECCE (1.13%) than after phacoemulsification (0.57%) in a socio-economically poor community. In Indian setup the incidence of endophthalmitis in patients who had phacoemulsification was only 0.02%. This is comparable to recently reported rates with phacoemulsification from the United States and Europe, which range from 0.04% to 0.07%.

The ESCRS study⁵ of endophthalmitis prophylaxis reports an incidence of 0.05% after phacoemulsification in patients receiving a combination

of topical and intracameral antibiotic prophylaxis.

Obstructed nasolacrimal passages and conjunctival microbial colonization are among the most common causes of postsurgical infection after cataract surgery in our Indian population⁷.

A majority of contaminants during, and even after, surgery can be traced to the patient's own ocular surface flora. Infection stemming from contaminated surgical instruments, tubing or the surgical environment, where occasional clusters of infection suggest a local epidemic. Surgical complications are a known risk factor for endophthalmitis, with higher endophthalmitis rates cited where complications occur. Delayed wound healing increases the risk for infection. An influx of ocular surface tears may occur postoperatively, allowing access of surface flora to the internal eye.

A recent study has shown that the spectrum of causative organisms of post-operative endophthalmitis in India is distinctly different from the Western countries⁸. This may be due to the tropical climate, lack of proper surgical protocols for cataract surgery and social, racial or economic factors.

Although no topical antibiotic have been approved by the Food and Drug Administration with a surgical prophylaxis indication, topical antibiotics have been used extensively with the intent to prevent postsurgical bacterial endophthalmitis. In recent years, the fluoroquinolone antibiotics (ciprofloxacin, levofloxacin, and ofloxacin) have found favour as the choice for topical prophylaxis perioperatively surrounding ocular surgery. Multiple studies have shown that topical ciprofloxacin, ofloxacin, and levofloxacin penetrate the ocular tissues and enter the

anterior chamber. New fluoroquinolones such as moxifloxacin and gatifloxacin have been developed to provide better activity against gram-positive bacterial infections and to provide better penetration into the ocular tissues.

It is believed that topical antibiotics help prevent bacterial postsurgical endophthalmitis by sterilizing the topical ocular surface and by penetrating the ocular tissues to provide intracameral antibiotic concentrations to eliminate bacteria that may enter the eye during surgical intervention. Others have demonstrated that antibiotics can reach elevated concentrations in the anterior chamber, and it was surmised that these concentrations were adequate to prevent post-surgical bacterial endophthalmitis. Various *in vivo* animal and human clinical studies support the fact that moxifloxacin penetrates ocular tissues better than other fluoroquinolones when instilled topically. In the single-dose animal studies, the instillation of a single drop topically of a 0.3% solution of moxifloxacin achieved maximum levels in the rabbit cornea, aqueous humor and iris-ciliary bodies of 12.5, 1.8, and 13.5 µg/ml/g, respectively. Concentrations of moxifloxacin were typically two-fold higher than the corresponding values for ofloxacin and remained two-fold higher throughout the study. Moxifloxacin concentration in the cornea was 0.25 µg/g at 48 hours, about four-fold above the MIC for methicillin-susceptible *Staphylococcus aureus*. In contrast, ofloxacin corneal concentrations were below its MIC threshold by 8 hours. The mean moxifloxacin tear film concentration at the initial 10-minute time-point was 366 µg/ml and remained at or above 1 µg/ml for up to at least 6 hours post-dose. Plasma concentrations of both drugs were low (0.01 µg/ml or less) and

declined rapidly. Moxifloxacin exhibited a better penetration profile than ofloxacin which penetrates better than ciprofloxacin.

It has been shown in animal studies that 0.5% moxifloxacin administered topically starting 1 hour (every 15 minutes, five drops) before bacterial challenge, immediately after challenge, and four times over 24 hours after bacterial challenge prevented the onset of endophthalmitis with no clinical signs of intraocular infection. The choice of antibiotic can be difficult, as there are many different aspects by which the efficacy of an antibiotic is determined. One of these aspects is bioavailability. The bioavailability of an antibiotic determines its ability to penetrate the tissues of concern and reach bacteria. To be bioavailable, a topical ophthalmic antibiotic must have a high rate of penetration and good solubility. Intravitreal concentration of 0.5% moxifloxacin 2hrly comes very close to the MIC₉₀ for *Staphylococcus epidermidis* (the most common causative organism in bacterial endophthalmitis). This concentration may be sufficient for prophylaxis, but not for treatment of active infection.

Topically administered 0.5% moxifloxacin is very well tolerated, with most adverse reactions described as mild. These most commonly include dry eye, ocular hyperemia, ocular discomfort, and ocular itching. The 0.5% moxifloxacin preparation is unique in that it is free of preservatives, specifically benzalkonium chloride. The lack of this preservative is valuable when using a collagen shield delivery device, as there is a theoretical risk of preservatives causing corneal damage after sustained drug delivery.

Vasavada et al⁹ measured moxifloxacin aqueous humour

concentrations in cataract patients. In the first group, moxifloxacin was instilled four times a day 1 day before surgery plus one drop 2 h before surgery. In second group, moxifloxacin was first instilled 2 h before surgery and then every 15 min for 1 h. Aqueous humour concentrations at the time of surgery were significantly higher in the second group (2.05 µg/ml) than in the first group (1.58 µg/ml). Mather et al¹⁰ conducted a retrospective study of 93 bacterial endophthalmitis isolates and found that the MIC level ranges for moxifloxacin was 0.06–0.19 µg/ml.

Yonca Aydin Akova et al applied the topical antibiotics four times per day for 2 days prior to surgery. Patients were further subdivided to receive additional doses of antibiotic drops as two drops 30 min apart (subgroup 1) versus four drops 10 min apart (subgroup 2) 1 h prior to the surgery. The mean concentrations of moxifloxacin in the aqueous humour were 0.72±0.40 µg/ml in the first subgroup and 1.95±1.05 µg/ml in the second subgroup.

In our study, group A and C received topical moxifloxacin 0.5% single drop 30 min before surgery of standard commercially available brand and a local brand respectively. Group B and D received topical moxifloxacin 0.5% single drop 30 min before surgery and 4 times day before the surgery of standard commercially available brand and a local brand respectively. The mean drug concentration in group A was 1.20 ± 0.43 µg/ml and in group B was 1.80 ± 0.34 µg/ml. There is statistical difference between these two groups. Mean drug concentration in group C and D are 0.51 ± 0.34 and 1.71 ± 0.48 µg/ml respectively. These two values are also statistically different.

We have multiple commercially available brands of moxifloxacin 0.5% eye

drops. Behaviour of these formulations has not been documented before. We conducted study to compare penetration of moxifloxacin in aqueous. In our study, group A and C are showing statistical difference in drug concentration which indicate that penetration of moxifloxacin in aqueous is significantly higher in some brands compared to others. However, when both the brands are applied for few days regularly, they may attain close values in aqueous. The factors responsible for such differential penetration may be related to PH or different electrolyte concentration; however details are to be studied. Close values after prolonged drug administration are the result of chemical equilibrium progressively attained in the cornea.

Conclusion:

Moxifloxacin penetrates the cornea rapidly to achieve a good concentration in aqueous when applied single drop 30 min before surgery. This concentration is significantly higher than MIC90 of most of the bacteria involved in postoperative endophthalmitis. Application of one drop 4 times a day before surgery is even more beneficial as it achieves statistically significant higher concentration. Thus it is better to start moxifloxacin eye drops a day before the surgery as a prophylactic measure. A steady application of drug for a day is facilitating the concentration of drug in aqueous compared to one or two drops prior to surgery which is unfortunately common practice in this part of the world.

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