Comparison of Aqueous Humour Concentration after Single High Dose Versus Multiple Administration of Topical Moxifloxacin in Rabbits

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For the prevention of postoperative ocular infections prophylactic topical antibiotics are routinely used. Studies evaluating comparative difference between single dose versus multiple dose administration on aqueous humour concentration of moxifloxacin are lacking. This study compared the aqueous humour concentration of moxifloxacin following its topical administration in rabbit eyes with two dose regimens. Twelve albino rabbits were divided into two groups. In group-1, two drops were administered thrice (total six drops) at 2 min intervals, in both the eyes; in group-2, two drops of moxifloxacin were administered three times a day for three days and also two h before aqueous humour collection i.e. on fourth day. Mean aqueous humour concentrations were calculated and compared using Student's '*t*' test and $P<0.05$ was considered significant. Moxifloxacin concentration in aqueous humour in group-1 was 23.79 µg/ml and in group-2 was 42.08 µg/ml. Both dosing regimens produced substantially higher aqueous concentrations than the known minimum inhibitory concentration for most bacteria. Moxifloxacin concentration in aqueous humour with multiple instillations is significantly higher than single instillation ($P<0.05$), which is adequate to cover ciprofloxacin-resistant gram-negative bacteria. Repeated topical moxifloxacin administration achieved significantly higher aqueous humour concentrations than single administration.

Key words: Aqueous humour, MIC_{90}, moxifloxacin, rabbiteye

Fluoroquinolones were introduced in the 1980s, with good antibacterial coverage, tolerability, high penetration in ocular tissue and delayed development of resistance. Fluoroquinolones are also used topically for ocular infections[1,2]. Moxifloxacin, a 4th generation fluoroquinolone, has broad-spectrum of coverage for both Gram-positive and Gram-negative bacteria. It acts by inhibiting DNA-gyrase enzymes which is necessary for bacterial DNA separation[3,4]. For the prevention of postoperative ocular infections prophylactic topical antibiotics are routinely used. Before selecting an antibiotic, its pharmacokinetic and pharmacodynamic properties should be considered. An agent with high intraocular tissue penetration, lower minimum inhibitory concentration (MIC) and broader spectrum should be chosen for higher efficacy[5]. Action of fluoroquinolones is concentration dependant[6]. *In vitro* MIC_{90} of moxifloxacin against common ocular isolates is 0.06 µg/ml for *Staphylococcus aureus* (methicillin-sensitive) to 6 µg/ml for fluoroquinolone resistant strains; 0.125 µg/ml to 3 µg/ml for coagulase-negative *Staphylococci*; 0.13 µg/ml for *Staphylococcus epidermidis*; 0.19 µg/ml for *Streptococcus pneumonia*; 0.12 to 0.75 µg/ml for *Pseudomonas aeroginosa*; 0.12 to 2 µg/ml for *Haemophilus influenzae*[7,8]. Moxifloxacin formulation is preservative free and has better penetration over other fluoroquinolones. However, head to head studies evaluating comparative difference between single high dose versus multiple dose administration on its aqueous humour concentration are lacking. In the present study moxifloxacin levels were estimated after single time- high dose and multiple times instillation in the aqueous humourin rabbit eyes.

Albino rabbits (2-3 kg) were housed in standard laboratory conditions with diet and water available *ad libitum*. The study was approved by IAEC (Institutional Animal Experimentation Ethics Committee) and care of the animals was as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals),

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India guidelines for laboratory animal facilities. Moxifloxacin 0.5% ophthalmic solution was purchased from the market. All other reagents used in the experiments were of the highest grade available.

Twelve rabbits were divided into two groups and moxifloxacin administered topically. In group-1, two drops were administered thrice (total six drops) at 2 min intervals, in both the eyes. In group-2, moxifloxacin two drops three times a day for three days and 2 h before sample collection i.e. on fourth day, were administered in both the eyes. Rabbits were fasted 12 h prior to sample collection, but water was given *ad libitum*. Animals were pre-treated with atropine (0.1 mg/kg s.c.) and anesthetized with xylazine + ketamine (5 mg/kg + 50 mg/kg i.m.). Aqueous fluid was aspirated from the anterior chamber under aseptic conditions, using air cannula needle attached to a tuberculin syringe 2 h after the last instillation. The aspirate was immediately stored at −70º until estimation.

Moxifloxacin was dissolved in acetonitrile to make a working stock solution of 1.0 µg/ml. Aqueous humour (50 µl) was spiked with increasing known concentration of working stock solution to make moxifloxacin concentration of 20, 40, 80, 160, 320, 650, 1250, 2500 ng/ml. Standards were processed according to the sample preparation and analyzed by high-performance liquid chromatography (HPLC) as described below.

Aqueous humour was allowed to thaw before the start of the experiment. Fifty microlitres of rabbit aqueous humour was pipetted out and poured into a tube and 100 µl of acetonitrile was added. The samples were vortex mixed for 2 min and left for 30 min. Then this solution was filtered with a syringe filter 0.22 µm pore size. Of this filtered sample, 10 µl was directly injected into the HPLC.

Waters Acquity HPLC system was used to perform HPLC analysis, which is equipped with a binary solvent manager, an auto sampler, column manager composed of a pre-column heater and a photo-diode array detector. A volume of 5 µl was injected into the Waters Acquity BEH C18 (250×5 mm, 45 µm) HPLC column kept at 40° and the chromatographic separation was performed by gradient elution. A mixture of acetonitrile and 0.1% trifluoroacetic acid in water was used as mobile phase, with a flow rate of 1 ml/min. Chromatogram was performed at a wavelength of 296 nm and total run time for estimation was 20 min. Data handling, data acquisition and instrument control was done with the help of Empower Software v1.0®.

Mean aqueous humour concentrations were calculated and compared using Student's *'t'* test and $P<0.05$ was considered significant. fig. 1(a) shows the chromatogram of blank aqueous humour samples. fig. 1(b) shows the chromatogram of aqueous humour spiked with moxifloxacin (400 ng/ml). Mean concentration of moxifloxacin following single-time high dose instillation group is 23.79±3.78 µg/ml. Mean concentration of moxifloxacin following multiple instillations is 42.08±9.27 µg/ml. Mean concentration of moxifloxacin in aqueous humour in multiple doses group is significantly higher compared to single-time instillation group ($P<0.05$). Moxifloxacin concentration in aqueous humour of 24 eyes from 12 rabbits in both the groups is well above the MIC values for common eye pathogens.

Antibiotics are administered preoperatively to prevent postoperative infection. Prophylactic flouroquinolones are widely used for the prevention of conjunctivitis and also for the treatment of ocular infections post surgery. Risk of keratitis is increased after ocular surgery. It has been shown that penetration of topical antibiotics is better in aqueous humour than systemic antibiotics[5]. Topical antibiotics can prevent blebs formation and reduce the incidence of keratitis in postoperative period. Topical use of antibiotics is associated with fewer side effects and there is reduced risk of resistance to antimicrobial drugs.

In the present study, mean concentration of moxifloxacin in aqueous humour, after single dose administration group is 23.79±3.78 µg/ml which is remarkably close to an earlier study by Lindstrom *et al.* which showed concentration of moxifloxacin in aqueous humour after single dose administration as 24.1 µg/ml[5]. Further, mean concentration of moxifloxacin in aqueous humour after multiple doses administrations group was 42.08±9.27 µg/ml in our study.

Sugioka *et al.* compared the intraocular penetration levels of moxifloxacin, gatifloxacin and levofloxacin in rabbit's conjunctiva, cornea and aqueous humour

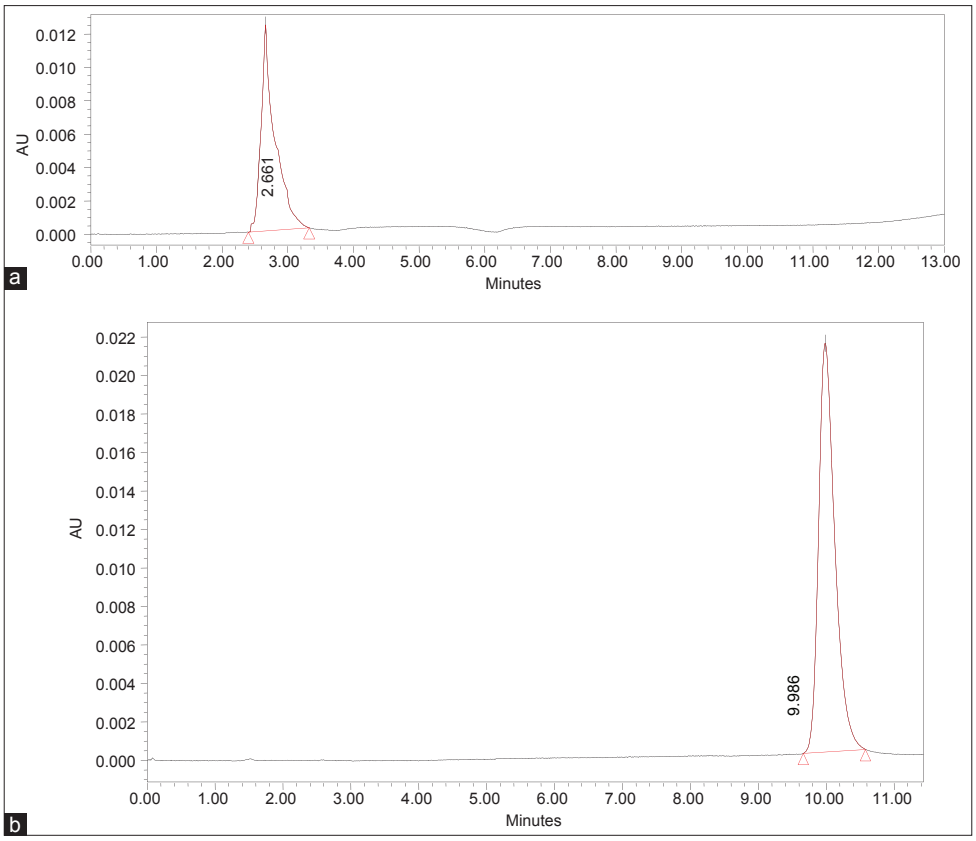

Fig. 1: Representative HPLC chromatograms.
Representative HPLC chromatograms of (a) blank aqueous humor and (b) moxifloxacin spiked aqueous humor at a concentration of 400 ng/ml.

after sequential topical instillation[9]. Moxifloxacin levels were significantly higher in all the tissues than gatifloxacin and levofloxacin. The aqueous humour demonstrated mean concentration of moxifloxacin as 2.677 µg/ml. Our study demonstrated considerable difference in the aqueous humour levels of moxifloxacin as compared to the above study possibly due to a different dosing protocol. In a study by Levine *et al.*[10], moxifloxacin 0.5 % drops were instilled topically in the rabbits'eye every 15 min for 4 h. The mean concentration of moxifloxacin in the aqueous (n=9) was 11.057 mg/ml (range 7.66 to 18.87 mg/ml), showing a greater accumulation on increasing the dose. The superior penetration and accumulation of moxifloxacin in aqueous may be attributed to its high degree of lipohpilicity, greater solubility at neutral pH and higher concentration in the commercial formualtion.

In an experimental study, Yagci *et al.* found that concentration of moxifloxacin in aqueous and vitreous humour topical administration in inflamed as well as normal eyes were significantly higher than other fluoroquinolones[11]. Concentration of moxifloxacin in aqueous humour in normal and inflamed eyes was 4.92 and 43.33 µg/ml, respectively. Thus, concentration in inflamed eyes was around 10 fold higher than normal eyes. This may be due to the loosening of tight junctions in ocular tissues during inflammation. Our results demonstrate that with multiple administrations, moxifloxacin accumulates in the aqueous humour and levels can be as high as shown in inflammatory conditions of the eye.

In various other animal and human studies moxifloxacin concentration in ocular tissues is significantly higher than other topical antibiotics but a study done by Stewart *et al.* shows that concentration of topical azithromycin 1% is higher than moxifloxacin 0.5% in conjunctiva and cornea[12]. However, in this study also concentration of moxifloxacin in aqueous humour was higher i.e. 0.77 µg/ml as compared to 0.053 µg/ml of azithromycin.

Above studies show that moxifloxacin levels in aqueous humour with topical moxifloxacin 0.5%

with different dose regimens were high enough for therapeutic effect, only in one study by Stewart *et al.* where level was subtherapeutic[13]. Levels of moxifloxacin in aqueous humour in these studies were 0.77 µg/ml to 43.33 µg/ml. In the present study, level in single high dose instillation group is 23.96 and 41.99 µg/ml in multiple instillations group. Present study shows that moxifloxacin reaches well above MIC levels in aqueous humour after topical administration. We found that the concentration difference in both the groups is clinically and statistically significant. Moxifloxacin levels after multiple instillations are significantly higher than after single instillation (P<0.05).

The choice of antibiotic depends on its spectrum of activity and penetration in ocular tissue to prevent intraocular infection. Moxifloxacin is a newer fourth generation flouroquinolones with wider spectrum of action and better penetration in aqueous humour and it is free of preservative. Many studies have shown that moxifloxacin reaches well above MIC_{90} of various pathogens in aqueous humour[11].

In the present study, the moxifloxacin level in both the regimens exceeded MIC for most of the bacteria. Level in multiple dose regimens is significantly higher than single instillation. On the basis of our data we recommend multiple instillation of moxifloxacin 0.5% topically two drops three times a day for 3 days and 2 h prior to ocular surgery to prevent anterior segment infection. In case it is not possible to follow multiple dosing regimens, a single high dose i.e. 2 drops repeated thrice at 2 min intervals is also suitable as it also exceeded MIC for most of the bacteria except ciprofloxacin-resistant gram-negative bacteria.

Moxifloxacin concentration in aqueous humour achieved therapeutic levels in the noninflamed eyes in rabbits after topical administration for most pathogenic bacteria. Moxifloxacin concentration in aqueous humour with multiple instillations is significantly higher than single instillation. Therefore, we recommend use of multiple instillations of moxifloxacin 0.5% ophthalmic solution three drops 3 times a day for 3 days and 2 h prior to surgery.

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