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Monocarboxylate transporter mediated uptake of moxifloxacin on human retinal pigmented epithelium cells

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Abstract

Objectives—This work was aim to determine *in vitro* interaction of moxifloxacin with monocarboxylate transporter (MCT) using a human retinal pigment epithelium cells (ARPE-19).

Methods—*In vitro* moxifloxacin uptakes were performed at 37°C across ARPE-19 cells. Concentration-dependent uptake of moxifloxacin was performed to delineate moxifloxacin kinetics with MCT. Effects of MCT substrates, MCT inhibitors, pH and metabolic inhibitors on moxifloxacin uptake were conducted to delineate mechanism of moxifloxacin influx via MCT.

Key findings—Moxifloxacin uptake was found to exhibit saturable kinetics ($K_m = 1.56 \pm 0.32 \mu\text{M}$ and $V_{\text{max}} = 0.58 \pm 0.16 \mu\text{M}/\text{min}/\text{mg}$ protein). Higher uptake of moxifloxacin was observed at acidic pH. MCT substrates such as salicylic acid, ofloxacin and L-lactic acid significantly inhibited the uptake of moxifloxacin. Furthermore, moxifloxacin uptake was significantly reduced in the presence of metabolic and MCT inhibitors. Overall, this study demonstrated an interaction of moxifloxacin with Na^+ and H^+ -coupled transporter, most likely MCT1.

Conclusions—Apart from the lipophilicity, we anticipate that lowest vitreal half-life of intravitreal moxifloxacin compared with other fluoroquinolones may be due to its interaction with MCT. This information might be crucial in clinical settings and can be further explored to improve vitreous half-life and therapeutic efficacy of moxifloxacin.

Keywords

human retinal pigment epithelium cells; monocarboxylate transporter; moxifloxacin

Introduction

Localized drug delivery is an important alternative to systemic therapy owing to its fewer side effects upon chronic drug exposure. Intravitreal injection is the most common localized

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Declaration

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

drug delivery route for treating retinal ailments such as age-related macular degeneration, diabetic retinopathy, retinoblastoma and endophthalmitis.^[1,2]

Postoperative endophthalmitis remains as a most devastating complication of cataract surgery possessing significant vision threat. Moxifloxacin (Figure 1), a fourth-generation fluoroquinolone, has shown a potent and rapid bactericidal activity against postoperative endophthalmitis pathogens. Reports have suggested safe and effective intracameral delivery of moxifloxacin in an animal model of endophthalmitis.^[3] Furthermore, efficacies intravitreal delivery of moxifloxacin for bacterial endophthalmitis has been reported clinically.^[3,4] However, lowest vitreous half-life of moxifloxacin (1.76 h) among other fluoroquinolones (ofloxacin→5.6 h and ciprofloxacin→2.2 h) used for the treatment of bacterial endophthalmitis has been reported in the literature.^[4–6] Typical order of fluoroquinolones lipophilicity (moxifloxacin > ciprofloxacin > ofloxacin) explains the faster vitreal elimination of highly lipophilic moxifloxacin.^[7] Vitreal elimination of molecules largely depends on its physicochemical properties such as hydrophilicity, lipophilicity (log P), molecule size and diffusion across vitreous fluid. Moreover, substrate specificity of drug molecules for transporters expressed on retinal pigmented epithelium (RPE) or retinal blood capillaries may also have an effect on its vitreous half-life.^[7]

Monocarboxylates such as lactate, acetate, pyruvate and ketone bodies are known to be transported via H⁺-coupled, Na⁺-coupled or anionic-exchange carrier-mediated process in a variety of cells and tissues.^[8] Typically, active transport of lactate, pyruvate and ketone bodies (an energy source for retina) across eukaryotic cell plasma membrane occurs via a family of proton-coupled monocarboxylate transporters (MCTs). These MCTs are also essential for the transport of numerous other metabolically significant monocarboxylates such as branched-chain oxo-acids derived from leucine, valine and isoleucine, and various ketone bodies such as acetoacetate, β -hydroxybutyrate and acetate.^[9,10] Generally, MCT substrates are represented as a weak monovalent organic acids carrying carboxyl group attached to a relatively short side chain.^[11] Various subtypes of MCTs such as MCT-1, 2, 3 and 4 are reportedly expressed on the retinal pigment epithelium.^[12,13] Hosoya *et al.* have reported the pH, temperature and concentration-dependent transport of [¹⁴C] L-lactic acid (MCT substrate) across TR-iBRB2, a prototypical *in vitro* inner blood retinal barrier cells. The quantitative real-time PCR study has revealed messenger RNA (mRNA) expression of both MCT1 and MCT2 on TR-iBRB2 cells with higher MCT1 (~33-fold mRNA) expression relative to MCT2.^[14] Furthermore, Philp *et al.* have characterized the *in vivo* expression and cellular distribution of MCTs on RPE of human donor eye by immunofluorescence analysis. This analysis revealed an expression of MCT1 on the apical membrane of RPE, whereas localization of MCT3 was observed on the basolateral membrane. However, an expression of MCT4 was demonstrated in the neural retina and was found absent in the RPE.^[8] Majumdar *et al.* have characterized the functional activity of MCT1 on the human RPE cell line and have demonstrated ARPE-19 as a model cell line to screen intravitreal drug targeting MCT1.^[15] Interestingly, few reports have shown an evidence for the existence of Na⁺-independent (H⁺-coupled MCT) and Na⁺-dependent (sodium-coupled MCT, SMCT) mechanisms for mono-carboxylate transport in the retinal cells.^[16–18] Martin *et al.* have analysed the expression of SMCT1 and SMCT2 in the retinal cell lines (RGC-5, rMC-1 and

ARPE-19) and primary cultures of retinal Müller cells and ganglion cells. These studies have shown that SMCT1 is expressed predominantly in the retinal neurons and only on the basolateral side of RPE, whereas SMCT2 is expressed exclusively in the Müller cells.^[19]

Various reports suggest that fluoroquinolones such as ciprofloxacin, levofloxacin and ofloxacin are known substrates of MCT.^[20,21] This may be due to the presence of carboxyl group in the chemical structure of fluoroquinolones, which act as a MCT substrate recognition site. Recently, functional and molecular localization of MCT has been reported on the apical membrane of retinal pigment epithelium cells (ARPE-19).^[15] Therefore, ARPE-19 can be used as a model retinal cell line to screen substrate specificity of potential drug candidates for MCT expressed on the RPE.^[15] Here, we aim to evaluate *in vitro* interaction of moxifloxacin with MCT using ARPE-19 cells. L-lactic acid, which has been widely used as a substrate for carrier-mediated monocarboxylate transport processes in various tissues/organs, was chosen as the model substrate along with other agents such as salicylic acid and ofloxacin.^[17,22–25] Concentration-dependent uptake of moxifloxacin was performed to delineate moxifloxacin kinetics with MCT. In addition, pH dependency and competitive uptake inhibition studies were also performed to delineate the mechanism of moxifloxacin influx via MCT.

Material and Methods

Materials

A retinal pigment epithelial (ARPE-19) cell line was purchased from American Type Culture Collections (ATCC; Manassas, Virginia, USA). Fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Lawrenceville, Georgia, USA), and Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient Mixture (D-MEM/F-12) was obtained from Gibco (Invitrogen, Grand Island, New York, USA). Streptomycin, penicillin, sodium bicarbonate, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), p-chloromercuribenzoic acid (pCMB), p-chloromercuribenzenesulfonate (pCMBS), quercetin, D, L-dithiothreitol (DTT), 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS), 2,4-dinitrophenol (DNP), ouabain, and sodium azide were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). AR-C155858 was procured from Medchem Express (Princeton, New Jersey, USA). Culture flasks (75 cm² growth area) and 12-well (3.8 cm² growth area per well) culture plates were obtained from Corning Costar Corp (Cambridge, Massachusetts, USA). [¹⁴C] L-lactic acid (specific activity: 131 mCi/mmol) was procured from Perkin-Elmer, Inc. (Boston, Massachusetts, USA). Levofloxacin and Moxifloxacin were acquired from TCI America (Sunnyvale, California, USA) and Hubei Phoebe West Company Technology Co., Ltd (Mainland, China), respectively. Bradford protein assay reagent was purchased from Bio-Rad Laboratories (Hercules, California, USA). All the solvents were of HPLC grade and obtained from Fisher Scientific (St. Louis, Missouri, USA).

Cell culture

ARPE-19 cells (passages 20–30) were cultured in D-MEM/F-12 (1 : 1) and incubated at 37°C with 5% CO₂ and 90% relative humidity. The growth media was supplemented with

10% heat-inactivated FBS, 15 mM HEPES, 29 mM sodium bicarbonate, penicillin (100 U/ml) and streptomycin (100 µg/ml). For uptake experiments, cells were seeded at a density of 3×10^6 cells per 12-well tissue culture plates. The medium was changed every alternate day, and cells were allowed to grow for 26–28 days before conducting experiments.

Stock solution and sample preparation

Different stocks were prepared for the calibration standards of moxifloxacin and other compounds. Moxifloxacin stock solutions were prepared in DMSO and diluted with the uptake buffer (for experiments) or mobile phase (for analysis). Stock solutions of various substrates and inhibitors such as quercetin, pCMBA, pCMBS, AR-C155858, DNP, DTT, DIDS, ouabain and sodium azide were also prepared in DMSO. Final concentration of DMSO in the uptake medium was adjusted below 2% to avoid any damage to the ARPE-19 cells. For liquid chromatography–mass spectrometry (LC-MS) analysis, samples were thawed at 37 °C and 20 µl (10 µg/ml) of levofloxacin (internal standard (IS)) was added and vortexed for 30 s. Nine hundred microlitres of cold ethyl acetate (protein precipitating and extraction solvent) was added to the 200 µl of sample. Samples were vortexed and centrifuged at 12 000 rpm for 5 min. The supernatants were subjected to vacuum drying at 40 °C for 2 h. Finally, the vacuum-dried samples were reconstituted with 200 µl of mobile phase for analysis.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) conditions

All experiments were performed on API 3200 triple quadrupole linear QTrap spectrometer (Applied Biosystem/MDS Sciex, Foster City, California, USA) equipped with Agilent LC pump and an autosampler. Following sample preparation, 10 µl of the clear upper layer was injected on a Luna XTerra® C18 analytical column (50 × 2.1 mm, 5 µm) for chromatographic separation. The flow rate of mobile phase (0.1% trifluoroacetic acid in distilled de-ionized water and acetonitrile (50 : 50)) was adjusted to 0.25 ml/min. The MDS Sciex API 3200 Triple Quadrupole linear QTrap mass spectrometry (Applied Biosystems/MDS Sciex) system interfaced by turbo ion spray with positive ion source in multiple reaction monitoring mode was used for the detection of positive ions (electrospray ionization). Levofloxacin was used as an IS for analysis. With a scan width of 0.5 m/z and injection rate of 20 µl/min, following transitions were measured: 402.2→384.2 for moxifloxacin and 362.2→344.2 for levofloxacin. Ion spray voltage, dwell time and capillary temperature were set at 5200 V, 400 ms and 350 °C, respectively.

Concentration dependence uptake of moxifloxacin on ARPE-19 cells

In vitro uptake of moxifloxacin was performed according to earlier published protocol with minor modifications.^[26] Briefly, 28–30 days post-seeding of ARPE-19 cells, the medium was removed, and cells were washed twice with Dulbecco's phosphate buffered saline (DPBS, pH 7.4) containing 130 mM NaCl, 2.5 mM KCl, 7.5 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgSO₄ and 5 mM glucose. Different concentrations (0.5–140 µM) of moxifloxacin solutions were prepared in DPBS (pH 7.4). *In vitro* uptake was initiated by incubating the cells with 1 ml of moxifloxacin solution (0.5–140 µM) for a predetermined time at 37 °C (for total uptake) and at 4 °C (for non-saturable kinetics).

Because previously published report revealed linear *in vitro* uptake of L-lactic acid (well-known MCT substrate) for at least 60 s,^[15] therefore, we conducted moxifloxacin uptake at a similar incubation period. Following incubation, the drug solution was removed, and cells were washed twice with ice-cold stop solution (0.52 g/l HEPES and 15.64 g/l KCl). Cells were lysed by rapid freeze–thaw cycle (three times), and moxifloxacin was extracted following cold ethyl acetate extraction. Samples were further analysed using the LC-MS/MS method as described previously. All the experiments were performed in quadruplicate, and cellular accumulation was normalized using protein count measured by Bio-Rad protein assay.

pH dependency and competitive inhibition studies

The uptake experiment was performed as described previously. pH dependency of *in vitro* moxifloxacin uptake was determined using different pH buffer solutions (pH 5.0–7.4). An interaction of moxifloxacin was also evaluated using known MCT substrates such as salicylic acid (10 mM), ofloxacin (1 mM) and L-lactic acid (5 and 10 mM). Moxifloxacin (10 μ M) and MCT substrates were simultaneously incubated for 60 s for *in vitro* uptake on ARPE-19 cells. Furthermore, effect of MCT inhibitors (Quercetin, pCMBA and pCMBS), metabolic inhibitors (ouabain and sodium azide), protonophores (DNP), anion transporter inhibitors (DIDS) and sulfhydryl-group modifying agents (N-Ethylmaleimide (NEM) and Dithiothreitol (DTT)) on moxifloxacin uptake were determined by pre-incubating ARPE-19 cells for 10 min with above inhibitors and DPBS (pH 7.4) buffer (control). Finally, an interaction of moxifloxacin with MCT1 was evaluated using MCT1-specific inhibitor (AR-C155858, 0.1 and 10 μ M). *In vitro* uptake of moxifloxacin (10 μ M) was conducted for 60 s on ARPE-19 cells pre-incubated with AR-C155858 solutions for 60 min at room temperature (22–25 °C) and later samples were analysed using LC-MS/MS, as described previously.

Data treatment

Uptake data was fitted to the classical Michaelis–Menten equation represented in equation 1,

$$V = \frac{V_{\max} \times C}{K_m + C} \quad (1)$$

where V is the total rate of uptake, $V_{\max}$ is the maximum uptake rate for the carrier-mediated process, K_m (Michaelis–Menten constant) is the concentration at half maximum velocity, and C is the substrate concentration. Data were fitted to equation 1 with the aid of a nonlinear least squares regression analysis program (Kaleida Graph V3.09, Synergy Software).

Statistical analysis

All experiments were conducted in quadruplicate and results are expressed as mean $\pm$ standard deviation (SD). Michaelis–Menten parameters (K_m and $V_{\max}$) are expressed as mean $\pm$ SD. Statistical analysis between two groups were determined using Student's t test. A difference between mean values was considered statistically significant at $P < 0.05$. An

unpaired, two-tailed Student's *t*-test was used to determine the significance of difference between two group means. While comparing more than two group means, one-way analysis of variance was used, and difference between mean values was considered statistically significant at $P < 0.05$.

Results

LC-MS/MS method moxifloxacin detection

The LC-MS method of moxifloxacin was successfully developed using levofloxacin as an IS (Figure 2). The method was developed over the linear ($r^2 > 0.99$) concentration range (30–1000 ng/ml) for moxifloxacin and levofloxacin. Lower limit of quantification was found to be 5.0 and 7.8 ng/ml for moxifloxacin and levofloxacin, respectively. The analytical method was validated with respect to precision (% relative SD < 5.0), linearity ($r^2 > 0.998$), reproducibility (% relative SD < 2.0) and selectivity (absence of interfering peaks).

Concentration-dependent uptake of moxifloxacin

Concentration dependency was determined by incubating ARPE-19 cells with various concentrations of moxifloxacin (0.5–140 μM). The concentration of moxifloxacin was determined by LC-MS/MS analysis following cold ethyl acetate extraction. Figure 3 demonstrates an involvement of a saturable carrier-mediated process, wherein major and minor components represents total uptake at 37°C and saturable component (i.e. total uptake at 37 °C –non-saturable component at 4 °C), respectively. The obtained data are fitted in to equation 1, and kinetic parameters (K_m and $V_{\max}$) were determined. K_m and $V_{\max}$ of moxifloxacin uptake on ARPE-19 cells were found to be $1.56 \pm 0.32 \mu\text{M}$ and $0.58 \pm 0.16 \mu\text{M}/\text{min}/\text{mg}$ protein, respectively.

Effect of MCT substrates on moxifloxacin uptake

Moxifloxacin uptake was analysed in the presence of various MCT substrates on ARPE-19 cells. Significant ($P < 0.05$) reduction of moxifloxacin uptake (Figure 4) was observed in the presence of salicylic acid (~72%), ofloxacin (~56%) and L-lactic acid (~52% and ~64%).

Effect of MCT inhibitors on moxifloxacin uptake

Effect of MCT inhibitors such as quercetin (100 and 200 μM), pCMBA (200 and 500 μM) and pCMBS (1, 10, 50 and 100 μM) on moxifloxacin (10 μM) uptake was studied on ARPE-19 cells. Significant ($P < 0.05$) reduction of moxifloxacin uptake was observed in response to MCT inhibitors. Moxifloxacin uptake was reduced to ~70%, ~40% and ~25–65% in the presence of quercetin, pCMBA and pCMBS, respectively (Figure 5). Furthermore, AR-C155858 (MCT1 specific inhibitor) demonstrated ~58% and ~64% inhibition of moxifloxacin uptake at 0.1 and 10 μM concentration, respectively (Figure 5). Effect of sulphhydryl-modifying agents (NEM and DTT) on the uptake of moxifloxacin (10 μM) was also determined on ARPE-19 cells. Cellular uptake of moxifloxacin was reduced to ~62% and ~51% by NEM (500 μM) and DTT (500 μM), respectively (Figure 6).

Effect of protonophore and organic anion transporter inhibitor on moxifloxacin uptake

Effect of protonophore (DNP) on moxifloxacin (10 μ M) uptake was studied on ARPE-19 cells. Cellular accumulation of moxifloxacin was reduced to ~40% by DNP (500 μ M), whereas no significant change in moxifloxacin uptake was observed by DIDS (organic anion transporter inhibitor) across ARPE-19 cells (Figure 6).

Effect of pH and metabolic inhibitors on moxifloxacin uptake

Uptake of moxifloxacin (10 μ M) was performed as a function of different buffer pH compositions. Cellular accumulation of moxifloxacin was observed ~2.3- and ~1.4-fold higher at pH 5.0 relative to pH 7.4 and pH 6.0, respectively (Figure 7). Furthermore, effect of metabolic inhibitors such as ouabain and sodium azide on moxifloxacin uptake was also determined on ARPE-19 cells. Moxifloxacin uptake was reduced to 40% and 50% by sodium azide (1 and 10 mM) and 50% by ouabain (1 mM) (Figure 8).

Discussion

Clinical endophthalmitis may affect the elimination half-life of a drug depending on its physiochemical properties (e.g. lipophilicity, size and structure) and elimination route (e.g. posterior route through passive or active transport across retina and anterior route through aqueous humour). Zwitterions such as ciprofloxacin and other fluoroquinolones are eliminated by posterior route and display shorter elimination half-lives relative to cationic compounds, which are primarily eliminated by passive transport through anterior chamber and aqueous humour.^[4] An *in vitro* minimum inhibitory concentration to inhibit 90% pathogens value of moxifloxacin for bacteria commonly encountered in postoperative and post-traumatic endophthalmitis are lowest (0.25 to 2.5 μ g/ml) relative to other fluoroquinolones, making it promising candidate for treating ocular infections.^[27–29]

MCTs 1–4 have been identified on RPE and are known to transport range of endogenous and exogenous compounds including lactate, butyrate, pyruvate, (gamma-Hydroxybutyric acid), pravastatin, simvastatin and carindacillin.^[30] The specificity and affinity of various MCT isoforms differ towards its inhibitors as well as substrates. For example, MCT1 and MCT2 isoforms demonstrates very similar substrate specificities; however, they differ with respect to substrate affinity.^[31] In addition, MCT1 and MCT2 can be further distinguished by their sensitivity towards inhibitors because MCT2 activity may not inhibited by known MCT1 inhibitor (p-chloromercuribenzenesulfonic acid).^[32] In contrast with MCT2, MCT4 demonstrates lower affinity for monocarboxylates and is relatively sensitive towards p-chloromercuribenzenesulfonic acid-mediated inhibition.^[30] Majumdar *et al.* have demonstrated the presence of MCT1 and its role in cellular accumulation of L-lactic acid (MCT substrate) on human retinal cells (ARPE-19).^[15] Furthermore, Kadam *et al.* have demonstrated the presence of MCT and transport (retina to sclera) of phenylacetic acid (MCT substrate) on human RPE that suggest the possible role of MCT in vitreal elimination of its substrates.^[33] Along with H⁺-dependent MCT transporter, Martin *et al.* have recently identified two isoforms of Na⁺-coupled MCTs (SMCTs): high-affinity SMCT1 and low-affinity SMCT2.^[19] SMCT isoforms have shown to mediate active transport of monocarboxylates such as lactate and ketone bodies across retina. The expression of

SMCT1 is widely reported on epithelial cell lining of small intestine, colon and renal proximal tubule, and have been indicated that SMCT1 transporter may influence bioavailability and renal re-absorption of monocarboxylate drugs.^[34,35]

Moxifloxacin displays a low vitreous half-life compared to other fluoroquinolones indicating possibilities of its reorganization and translocation by active transport system present on the retina, which leads to rapid drug elimination from vitreous. Carrier-mediated (H^+) transport mechanism of monocarboxylic acid via MCT has been reported on retinal pigment epithelium cells (ARPE-19).^[15] A carrier-mediated uptake of L-lactic acid (known MCT substrate) on ARPE-19 cells represents a potential mechanism by which monocarboxylic acid drugs eliminates from vitreous humour to retina.

The aim of the present study was to evaluate moxifloxacin recognition by carrier-mediated MCT as a possible mechanism of its vitreous elimination. Significant reduction of moxifloxacin uptake in the presence of competitive MCT substrates (L-lactic acid, ofloxacin and salicylic acid) on ARPE-19 cells suggests possible interaction with MCT (Figure 4). However, moxifloxacin uptake inhibition was not observed by di- and tri-carboxylic acids in ARPE-19 cells (data not shown).^[9,36–38] This observation further suggests that cellular accumulation of moxifloxacin is mediated by MCT influx transporter. The uptake of moxifloxacin on ARPE-19 cells was found saturable (Figure 3). The kinetics determination of moxifloxacin uptake on ARPE-19 cells yielded V_{max} and K_m values of 0.58 ± 0.16 $\mu M/min/mg$ protein and 1.56 ± 0.32 μM , respectively.

Furthermore, significant reduction of moxifloxacin uptake by quercetin and pCMBA (classical MCT inhibitors)^[9,39,40] confirms active involvement of MCT during moxifloxacin uptake across ARPE-19 apical cell monolayer (Figure 5). The cell-impermeant organomercurial reagent such as pCMBS reportedly causing oligomerization of CD147, an accessory protein responsible for MCT1 expression at the cell surface.^[41,42] Reduction of moxifloxacin uptake (Figure 5) by pCMBS (a potent indirect MCT1 inhibitor) because of CD147 conformational changes states that MCT1 but not MCT2^[15] is involved in the moxifloxacin uptake process. In addition, Owens *et al.* have reported that expression of MCT1 but not MCT4 in *Xenopus laevis* oocytes was significantly inhibited by AR-C155858 at different concentration levels (0.1 and 10 μM).^[43] The binding site for AR-C155858 is contained within the C-terminal half of the MCT1 and involves 7–10 transmembrane domains.^[43] This study indicates 8- to 10-fold reduction of L-lactic acid uptake by MCT1 in the presence of AR-C155858 (0.1 μM) in *Xenopus laevis* oocytes. However, no such reports are available showing AR-C155858 inhibitory potential for MCT3, MCT4 or MCT8 expressed on RPE. Therefore, we exposed ARPE-19 cells with different concentration of AR-C155858 to perform uptake of Moxifloxacin. The uptake was reduced by ~58% and ~64% in presence of 0.1 and 10 μM AR-C155858, respectively (Figure 5). Moreover, sulfhydryl group modifying agents (NEM and DTT) have demonstrated significant reduction of moxifloxacin uptake (Figure 6) suggesting the role of sulfhydryl groups and MCT1 in moxifloxacin translocation, and this observation was found consistent with the earlier report.^[44] Our study provides direct evidence of proton-coupled mediated transport of moxifloxacin across ARPE-19 cells and obtained results were found consistent with the previously published reports.^[8,15] In addition, metabolic inhibitors such as sodium azide and

ouabain (Na^+/K^+ ATPase inhibitor) have shown significant reduction of moxifloxacin accumulation in ARPE-19 cells (Figure 8), and this observation was found consistent with the characteristics of Na^+/K^+ ATPase polarization on the apical membrane of ARPE-19 cells.^[38] Although complete inhibition of moxifloxacin was never achieved in the presence of any MCT inhibitors, this may be attributed due to the passive drug diffusion (nonsaturable kinetics) across the cell membrane. Because presence of a protonophore (DNP) hinders MCT transporter activation by proton binding, therefore, significant reduction of transcellular moxifloxacin accumulation was observed (Figure 6). Furthermore, translocation of moxifloxacin was found proton-dependent (highest at pH 5.0) on ARPE-19 cells (Figure 7).

Conclusion

In conclusion, this study was attempted to demonstrate moxifloxacin interaction with Na^+ - and H^+ -coupled monocarboxylate transporter, most likely MCT1. Knowledge of elimination pharmacokinetics from vitreous humour is vital for designing dosage regime for posterior segment diseases. Therefore, interaction of moxifloxacin with MCT expressed on retina (a major elimination route for intravitreal drugs) may offer better understanding of its vitreous clearance. We anticipate that lowest vitreal half-life of intravitreally injected moxifloxacin compared with other fluoroquinolones may be due to its interaction with MCT influx transporter. Because moxifloxacin is clinically delivered intravitreally for treating postoperative bacterial endophthalmitis, information about such interactions will be crucial in the clinical settings and can be further explored to improve its vitreous half-life and therapeutic efficacy.

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Figure 1.
Chemical structure of moxifloxacin.

Figure 2.
Liquid chromatography–mass spectrometry mass spectrum of moxifloxacin (m/z 402.2→384.2) using levofloxacin (m/z 362.2→344.2) as an internal standard.

Figure 3.

Concentration dependent uptake of moxifloxacin (0.5–120 μM) on ARPE-19 cells. Values are expressed as mean $\pm$ standard deviation ($n = 4$). Open squares represents total uptake at 37 °C and open triangle represents saturable component (i.e. total uptake at 37 °C – nonsaturable component at 4 °C).

Figure 4.

Cellular accumulation of moxifloxacin alone and in presence of salicylic acid, ofloxacin and L-lactic acid across ARPE-19 cells. Values are expressed as mean $\pm$ standard deviation ($n = 4$). *Data were considered statistically ($P < 0.05$) different from the control group. One-way analysis of different treatment group did not show any statistical difference.

Figure 5.

Inhibition of moxifloxacin uptake by monocarboxylate transporter inhibitors. Values are expressed as mean $\pm$ standard deviation ($n = 4$). *Data were considered statistically (P 0.05) different from the control group. One-way analysis of different treatment group did not show any statistical difference.

Figure 6.

Inhibition of moxifloxacin uptake by protonophore (2,4-dinitrophenol), organic anion transporter inhibitor (4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid) and sulfhydryl-modifying agents (N-Ethylmaleimide (NEM) and Dithiothreitol (DTT)). Values are expressed as mean $\pm$ standard deviation ($n = 4$). *Data were considered statistically ($P < 0.05$) different from the control group. One-way analysis of different treatment group revealed that treatment groups (with NEM, DTT and 2,4-dinitrophenol) were statistically different from treatment group (4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid) with $P < 0.05$, $P = 0.023$.

Figure 7. Effect of pH on cellular accumulation of moxifloxacin across ARPE-19 cells. Values are expressed as mean $\pm$ standard deviation ($n = 4$).

Figure 8.

Inhibition of moxifloxacin uptake by metabolic inhibitors (ouabain and sodium azide) across ARPE-19 cells. Values are expressed as mean $\pm$ standard deviation ($n = 4$). *Data were considered statistically significant for $P < 0.05$.