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Enhanced penetration of moxifloxacin into rat prostate tissue evidenced by microdialysis

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ABSTRACT

Moxifloxacin is reported to have increased distribution into the prostate compared with older fluoroquinolones such as norfloxacin and ciprofloxacin, being able to reach tissue-to-plasma concentration ratios greater than unity. However, most of these studies use tissue homogenates derived from biopsy samples, which can lead to overestimation of free concentrations as fluoroquinolones tend to accumulate in the intracellular space. The aim of this study was to investigate moxifloxacin pharmacokinetics in rat prostate interstitial fluid by microdialysis. Tissue pharmacokinetics was assessed by implanting a small microdialysis catheter in the prostate gland. Blood samples were simultaneously collected for assessing plasma pharmacokinetics. Analysis of plasma ($N = 154$) and microdialysis ($N = 344$) concentrations after a single intravenous dose of 6 or 12 mg/kg moxifloxacin was conducted in the non-linear mixed-effect modelling software NONMEM v.6 as well by a non-compartmental approach. Moxifloxacin showed a significant tissue distribution in the prostate ($AUC_{prostate,ISF}/f_u \cdot AUC_{plasma} = 1.24 \pm 0.37$), 59% higher than the value obtained for levofloxacin in a previous study. A three-compartment model with non-linear kinetics could adequately describe moxifloxacin pharmacokinetics in terms of curve fitting and precision in parameter estimation. The developed pharmacokinetic model indicates that passive diffusion and active transport are the mechanisms involved in moxifloxacin distribution to the prostate. These findings suggest that moxifloxacin could be a better alternative to levofloxacin for the treatment of chronic bacterial prostatitis owing to its enhanced tissue penetration and higher AUC_{tissue}/MIC ratios, even though it is not yet approved by the US FDA for this indication.

1. Introduction

It has been well documented that chronic bacterial prostatitis (CBP) is frequently a difficult-to-treat condition, mainly due to poor penetration of many antimicrobial agents into prostatic tissue, which may lead to subtherapeutic concentrations and the development of bacterial resistance [1]. To promote bacterial eradication and avoid recurrent infections, tissue levels need to be high enough in consistency with the minimum inhibitory concentration (MIC) of the pathogen. Fluoroquinolones (FQs) are the first-choice antimicrobial agents both for acute bacterial prostatitis and CBP owing to their favourable pharmacokinetic (PK) properties, such as extent of tissue distribution and bactericidal activity against a wide range of pathogens involved in this infection [2]. In a review by Lipsky et al. [3], the recommended antibiotic therapy for CBP was ciprofloxacin 400 mg intravenous (i.v.) every 12 h or levofloxacin 500 mg i.v. every 24 h when the causative pathogen is Enterobacteriaceae or *Enterococcus* spp., whilst azithromycin 500 mg orally once daily is recommended when CBP is due to *Staphylococcus* spp. In both cases, the suggested duration of therapy is 4–6 weeks. Such dosing regimens may, however, be associated with unwanted side effects due to prolonged daily exposure to FQs or macrolides.

Moxifloxacin is a fourth-generation FQ used in the treatment of respiratory tract infections (acute bacterial sinusitis, chronic bronchitis and community-acquired pneumonia), skin infections and complicated intra-abdominal infections. Moxifloxacin is reported to have an increased tissue distribution compared with older FQs, such as norfloxacin and ciprofloxacin, reaching tissue-to-plasma concentration ratios greater than unity in some cases [4]. Moxifloxacin is not approved to treat CBP, although drug penetration in the prostate has been shown to be high [5].

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In a previous microdialysis study, we have shown that the distribution of levofloxacin in rat prostate is likely to be influenced by active efflux transporters and that free prostate interstitial fluid (ISF) concentrations are on average 22% lower than the respective free plasma levels [6]. The fitted population PK model could adequately describe the mechanism involved in the distribution of levofloxacin between plasma, peripheral and prostate compartments.

FQs differ in their capacity to penetrate into the prostate. Literature data on the ratio of prostatic fluid-to-plasma concentrations of FQs show marked differences: 0.10 for norfloxacin [7]; 0.20 for ciprofloxacin [8]; 0.29 for levofloxacin [5]; 0.48 for lomefloxacin [7]; 1.23 for gatifloxacin [8]; and 1.60 for moxifloxacin [5]. These differences in prostatic fluid-to-plasma ratios are hypothesised to be influenced by two mechanisms: the molecule lipophilicity, which drives the passive diffusion capacity through membranes; and ion-trapping mechanisms [4]. A third mechanism driven by efflux transporters [e.g. P-glycoprotein (P-gp), multidrug resistance protein (MRP) family transporters] may also be involved. Brillault et al. [9] investigated the relative contribution of active transport and passive permeability of six FQs in a Calu-3 lung epithelial cell model, which mimics the human lung epithelium. The authors concluded that active transport is predominant for FQs with relatively low lipophilicity (e.g. ciprofloxacin and norfloxacin) but its influence is minor for FQs with higher lipophilicity such as perfloxacin and moxifloxacin. In a previous study, however, the same authors showed that moxifloxacin is subject to active transport in the Calu-3 lung epithelial cell model and that this transport is mainly P-gp-dependent [10].

Considering the previous reports on FQs tissue penetration and their importance for the treatment of prostatitis, this study aimed at comparing moxifloxacin pharmacokinetics in prostate ISF determined by microdialysis with its time course in plasma. A population PK model was used to fit moxifloxacin concentrations in plasma and tissue simultaneously. These findings will show that moxifloxacin, although not approved by the US Food and Drug Administration (FDA) for genitourinary infections, is a good candidate to treat prostatitis, with a prostate penetration ratio higher than levofloxacin.

2. Materials and methods

2.1. Chemicals

Moxifloxacin hydrochloride was kindly supplied by Bayer Pharma AG (Leverkusen, Germany) and gatifloxacin (internal standard) was provided by Bristol-Myers Squibb (São Paulo, Brazil). Urethane (ethyl carbamate, ≥99%) was purchased from Sigma–Aldrich (St Louis, MO). Heparin 5000 IU/mL was purchased from Cristália Produtos Químicos Farmacêuticos Ltda (São Paulo, Brazil). High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol and triethylamine were purchased from Tedia (Fairfield, OH). Formic acid was purchased from Fluka Chemie GmbH (Buchs, Switzerland) and phosphoric acid from Merck (Darmstadt, Germany). Water was purified using a Millipore® Milli-Q system (Bedford, MA). All other chemicals used were of pharmaceutical or analytical grade.

2.2. Microdialysis apparatus

The microdialysis system consisted of a PHD 2000 syringe pump (Harvard Apparatus, Holliston, MA), and 500 μL gas-tight syringes (Hamilton Company, Reno, NV) were employed to deliver the perfusion fluid through the microdialysis probes. The perfusion fluid consisted of Ringer's solution pH 7.2 (147 mM NaCl, 1.3 mM $CaCl_2$, 4 mM KCl) prepared according to the literature [11].

2.3. In vitro microdialysis experiments

Relative recovery (RR) of the microdialysis probes was previously determined in vitro to check any influence of drug concentration on probe recovery and drug binding to the plastic probe components as it is likely to occur with more lipophilic molecules. Dialysis and retrodialysis experiments were carried out using Ringer's solution at 37 ± 2 °C as perfusion fluid and gentle magnetic stirring of the probes' external media in order to maintain sink conditions. Three different moxifloxacin concentrations in the range of 150–3000 ng/mL were investigated, either in the periprobe solution (dialysis) or in the perfusion solution (retrodialysis). The perfusion solution was pumped at a flow rate of 1.5 μL/min throughout the in vitro experiments.

After each change in solution, the probes were allowed to stabilise with the new solution for 1 h. Three consecutive dialysate samples were collected at 30-min intervals and were assayed by HPLC. RR calculated by dialysis and retrodialysis was determined according to Eqs. (1a) and (1b), respectively.

$$RR_D\,(\%) = \left(\frac{C_{dial}}{C_{perf}}\right) \times 100 \quad (1a)$$

$$RR_{RD}\,(\%) = \frac{(C_{perf} - C_{dial})}{C_{perf}} \times 100 \quad (1b)$$

where RR_D is the relative recovery by dialysis (gain), RR_{RD} is the relative recovery by retrodialysis (delivery), C_{dial} is the concentration in the dialysate and C_{perf} is the concentration in the perfusion fluid.

2.4. In vivo probe calibration and pharmacokinetic experiments

Plasma pharmacokinetics and tissue microdialysis were performed in the same rat simultaneously to minimise interindividual variability. Male Wistar rats weighing 0.25–0.35 kg (N = 14) were anaesthetised with 1.25 g/kg urethane intraperitoneally and were placed in a supine position. A small incision was made in the abdomen of the rat and the two lateral lobes of the prostate gland were exposed with minimal dissection. A CMA 20 microdialysis probe (4 mm membrane length, 20 000 Da cut-off) was implanted in the tissue with the help of a guide needle. A FEP flexible cannula (1.19 mm OD × 0.63 mm ID; BASi Analytical Instruments, West Lafayette, IN) was inserted in the right carotid artery for blood sampling. The cannula was filled with heparin solution to avoid clotting (100 IU/mL heparin in saline). Moxifloxacin 500 ng/mL in Ringer's solution was perfused through the microdialysis probe at a flow rate of 1.5 μL/min and was allowed to equilibrate for 1 h. Then three dialysate samples were collected at 30-min intervals for determination of the relative drug loss according to Eq. (1b). The syringe was then switched to plain Ringer's solution and flushed for an additional hour for washing the drug out of the probe and tissue.

A test sample was collected and assayed after the wash-out period in order to make sure that no drug could be detected in the dialysate. Rats were given an i.v. dose of moxifloxacin 6 mg/kg or 12 mg/kg through the femoral vein of the left hind leg. These doses were based on the standard moxifloxacin 400 mg/day dose in humans considering a 70-kg male adult. A period of 5 min was allowed before collecting the first microdialysis sample owing to the dead volume of 7.1 μL between the probe membrane and the end of the outlet tubing. Dialysate samples were collected every 30 min for up to 12 h after dosing. Blood samples (ca. 200 μL) were withdrawn at 5, 15 and 30 min and 1, 2, 3, 4, 6, 8, 10 and 12 h after administration in tubes containing heparin and were immediately centrifuged for plasma separation (9400 × g for 10 min at 4 ± 1 °C). Plasma and dialysate samples were stored in polypropylene tubes at −80 ± 2 °C until HPLC analysis. For data analysis purposes, the