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Original article

Effect of urinary excretion on the bladder tissue distribution of fluoroquinolones in rats

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ABSTRACT

The purpose of this study was to evaluate which of blood or urine has the greater effect on bladder tissue concentrations of fluoroquinolones important for the treatment of urinary tract infections by measuring concentrations of fluoroquinolones in the vesical tissue (chemically and immunohistochemically) and intravesical space (chemically). Thirty-minute incubation of isolated rat bladders with fluoroquinolones showed only a 1.9-fold difference in transferability among norfloxacin, levofloxacin, ciprofloxacin and sparfloxacin. Intravesical instillation of norfloxacin and sparfloxacin in rats yielded similar vesical tissue distributions. Thus, there were no large differences in vesical tissue transfer among the four fluoroquinolones. The bladder tissue/plasma concentration ratios of norfloxacin (high urinary excretion-type) and sparfloxacin (low urinary excretion-type) at 1 h after a single oral dose (10 mg/kg) to rats were 15.4 and 1.3, respectively. The bladder tissue/plasma concentration ratios of norfloxacin after an intravenous injection (10 mg/kg) to ureter-catheterized and sham-operated rats were 1.36 and 57.8. Thus the bladder tissue distribution was significantly higher in the urine-exposed bladder. Immunohistochemical examination of the vesical tissue localization of norfloxacin in rats given a single intravenous dose revealed the presence of the drug-positive image in the cytoplasm of surface layer cells (both in umbrella and cover cells) of the bladder transitional epithelium. In conclusion, the results suggest that norfloxacin and other fluoroquinolones are excreted into urine and then transferred to the surface layer of the bladder transitional epithelium. Therefore, the urine levels have a greater effect on the vesicle tissue distribution of fluoroquinolones than the plasma levels in rats.

1. Introduction

The urinary bladder urothelium is one of the most impermeable barriers [1] and is considered not to reabsorb drugs. Generally, in pharmacokinetics the bladder is assumed to be and is treated simply as a non-returning compartment or storage site for renally excreted drugs and metabolites. However, some experimental data have suggested that drugs may sometimes be re-absorbed through the bladder. Wayne et al. [2] reported the in vivo absorption of saccharin through rat bladders, and Dalton et al. [3] showed that when mitomycin C was instilled into the intravesical space in rats, this antineoplastic agent was absorbed through the bladder into blood in normal, nontumor-bearing rats. Weakly acidic drugs such as sodium salicylate and neutral drugs such as antipyrines are also absorbed from the bladder, and the bladder may represent a site for drug re-absorption into the systemic circulation [4,5]. Tringali et al. [6] showed that a novel paclitaxel derivative was transported into the urothelium after instillation in isolated rabbit whole bladders as a new pre-clinical model of the kinetics of locally administered anticancer agents. Matsumoto et al. [7] induced bladder overactivity in whole animals by intravesical instillation of oxotremorine-M (a nonselective muscarinic acetylcholine receptor agonist) and then blocked the overactivity by intravesical instillation of muscarinic acetylcholine receptor antagonists. Chuang et al. [8] reported that carbachol-induced bladder overactivity in female rats was inhibited by intravesical instillation of human urine containing excreted solifenacin (muscarinic acetylcholine receptor antagonist) after oral administration, showing that the vesical

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tissue distribution of this drug is of some therapeutic benefit for the treatment of overactive bladder syndrome.

Antibiotic pharmacokinetic/pharmacodynamic (PK/PD) analyses have been used to estimate optimal parameters for maximizing the antibacterial activity of these agents, for preventing the development of drug resistance, and for minimizing the toxicity [9,10]. Based on the results, some general pharmacokinetic indices have been proposed, such as for prediction of the efficacy of antibiotic agents. These indices include the ratio of the maximum plasma concentration (Cmax) of an antibiotic to the minimum inhibitory concentration (MIC), the area under the concentration-time curve (AUC) to the MIC and the duration of time during which plasma concentrations exceed the MIC of the antibiotic against bacteria [11]. However, although many PK/PD analyses have examined the treatment of respiratory tract infections and bacteremia [12,13], only a few PK/PD studies have investigated the treatment of urinary tract infections (UTIs). Stamey et al. [14] showed that clinical cure in the treatment of UTIs was more closely associated with urine antibiotic concentration, rather than plasma concentration. Recently, Deguchi et al. [15] performed a traditional PK/PD analysis in patients with UTIs treated with 500 mg of levofloxacin (LVFX) every 24 h for 7–14 days, and they suggested that, in addition to its plasma concentration, a high concentration of LVFX in the urine might play a key role in eradicating bacteria [15]. However, it remains unclear whether and to what extent the urinary concentrations of fluoroquinolones affect their bladder tissue concentrations in patients being treated for UTIs.

Therefore, this study was designed to evaluate which of systemic or urinary exposure more greatly affects the bladder tissue concentrations of fluoroquinolones important for the treatment of UTIs. For this purpose, the concentrations of these fluoroquinolones were determined in the vesical tissue and the intravesical space.

2. Materials and methods

2.1. Drugs and reagents

Norfloxacin (NFLX), sparfloxacin (SPFX), ciprofloxacin (CPFX) and LVFX were purchased from Sigma–Aldrich Japan K.K. (Tokyo, Japan). Sheep anti-NFLX polyclonal antibody and sheep Ig fraction antibody were purchased from GenWay Biotech Inc. (San Diego, CA). A Vectastain Elite ABC Sheep IgG Kit and Simple Stain DAB solution were purchased from Vector Laboratories, Inc. (Burlingame, CA) and Nichirei Corporation (Tokyo, Japan), respectively. All other reagents used were of commercially available special grade.

2.2. Animals

Rats (7–8 week-old male Sprague Dawley rats) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). All animals were cared for and handled throughout the study in accordance with the institutional protocol and standard operating procedures approved by the institutional animal care/use committee at Kyorin Pharmaceutical Co., Ltd.

2.3. In vitro bladder tissue distribution of fluoroquinolones

Twelve rats (i.e., three per group) under isoflurane anesthesia were sacrificed by exsanguinations from the abdominal aorta and the bladders were excised immediately. The isolated bladders were placed in 2.0 mL Eppendorf Tubes®, 1 mL of saline containing NFLX, LVFX, CPFX and SPFX (100 μg/mL, pH 6–7.7) was added, and the tubes were incubated at 37 °C for 30 min. After incubation, the bladder tissues were rinsed three times with saline for transfer into another tube and then a two-fold volume of methanol and a stainless steel ball (5.0 mm ϕ.) were added to the tissue sample, and the tube was shaken to homogenize the bladder for 5 min using cell disruption equipment (Shake Master, Bio Medical Science Inc.). The resultant homogenate was centrifuged at 6500 × *g* for 5 min. The supernatant layer was injected into the HPLC-UV system to determine the bladder concentration of each fluoroquinolone.

2.4. Urinary excretion of fluoroquinolones after intravenous injection

Individual doses of NFLX, LVFX, CPFX and SPFX were intravenously cassette-injected into rats (2.5 mg/kg), after which the rats were individually housed in metabolic cages. Urine was collected up to 48 h after injection. An aliquot of urine was diluted with water and mixed with acetonitrile/methanol (1:1). The sample was vortexed and allowed to stand for 10 min, followed by centrifugation at 6500× *g* for 5 min. The supernatant layer was injected into a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system to determine the concentration of each drug.

2.5. Bladder tissue distribution of NFLX and SPFX after oral treatment

Plasma and bladder-tissue concentrations of fluoroquinolones (unchanged parent form) were determined after single oral dosing of NFLX and SPFX (10 mg/kg each) to rats that had been fasted overnight. At 1 h after dosing, the rats were sacrificed under isoflurane anesthesia and the plasma and bladders were collected immediately. A two-fold volume of acetonitrile was added to one volume of plasma sample for deproteinizing. After standing for 10 min, the sample was centrifuged at 6500× *g* for 5 min. The supernatant layers from plasma and bladder samples were injected into the HPLC-UV system to determine the concentrations of each unchanged form.

2.6. Bladder tissue transfer of NFLX and SPFX after intravesical space instillation

Rats were anesthetized with urethane (1.5 g/kg, sc) to ligate the penis. Then, after abdominal incision, the bilateral ureters were ligated. After forced aspiration of urine in the bladder using a syringe (30 G), 0.5 mL of saline containing a mixture of NFLX or SPFX (50 μg/mL, each) was instilled into the intravesical spaces of three rats. At 30 min after instillation, the bladder was excised and rinsed with saline, and then the bladder tissue concentration of each unchanged form was determined with an HPLC-UV system as described above.

2.7. Bladder tissue distribution of NFLX after intravenous injection to ureter-catheterized rats

As shown in Fig. 1, rats fasted overnight were cut with a small incision along the abdominal midline under urethane anesthesia and catheterized in the bilateral ureters using an indwelling needle (24G) and polyurethane tube (0.64 mm o.d., 0.30 mm i.d.). The femoral vein was catheterized with a polyurethane tube (PE50) and then the abdominal opening was closed. (Sham-operated rats were incised at the abdominal midline and catheterized in the abdominal vein.) A single dose of NFLX was intravenously injected at 10 mg/kg to ureter-catheterized and sham-operated rats to determine the plasma and bladder concentrations of the unchanged form. At 1 h after dosing, the rats were sacrificed by exsanguinations from the abdominal aorta to collect the plasma and isolate the bladder. The concentrations of the unchanged forms in plasma and