Penetration of Pazufloxacin into the Fluid of Suction Blisters Induced in Human Skin

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We investigated the pharmacokinetics of pazufloxacin (PZFX) in the fluid of suction blisters induced in the skin of 6 healthy male volunteers after a 200 mg oral dose of PZFX. The time to reach the maximum concentration (t_{max}) of PZFX in plasma was 0.7 hours, and the maximum concentration (C_{max}) was 2.95 µg/mL. The half-life of the elimination phase (t_{1/2}) in plasma was 1.75 hours, and the area under the concentration-time curve (AUC) was 6.70 µg.h/mL. The C_{max}, t_{1/2} and AUC values in the suction blister fluid were 2.0 hours, 1.52 µg/mL, 1.95 hours and 6.28 µg.h/mL, respectively. The ratio of the AUC of PZFX in blister fluid to the AUC in plasma was almost 1.0. Thus, excellent penetration of PZFX into suction blister fluid was observed.

Key words: pazufloxacin, suction blister fluid, pharmacokinetics

INTRODUCTION

Pazufloxacin (PZFX) is a new oral quinolone antimicrobial agent which has a broad spectrum and shows superior antimicrobial activity against both gram-positive and gram-negative bacteria including Pseudomonas aeruginosa.¹ In a phase I clinical study, the maximum concentration (C_{max}) of PZFX in blood was higher than that of the other quinolones, the half-life in the elimination phase in the blood (t_{1/2}) was relatively short, and a high urinary excretion rate was observed.² Furthermore, the ability of PZFX to penetrate into human tissue culture cells is lower than that of other quinolones.³ While the presence of antibiotics in infectious foci is an important factor regarding their efficacy in infectious diseases, it is difficult to investigate the penetration of antibiotics into infectious foci in a clinical study. We therefore created a human skin blister model to examine the penetration of PZFX into extracellular fluid, postulating that this model would accurately reflect clinical penetration into infectious foci.

MATERIALS AND METHODS

Human subjects

Six healthy male volunteers, 35 to 45 years old, whose body weight ranged from 58 to 71 kg participated in the study. Their written informed consent was obtained prior to beginning the study. No abnormal findings were detected in physical, biochemical, hematological and urinary examinations carried out before the administration of PZFX.

Drug administration

100 mg PZFX tablets were provided by Toyama Chemical Co Ltd (Tokyo, Japan). PZFX was administered in a single oral dose of 200 mg (100 mg tablet x 2) to fasting male volunteers.

Blood samples

Blood samples were collected in test tubes containing sodium heparin at 0, 0.5, 1, 2, 3, 4 and 6 hours after the administration of PZFX. The specimens were centrifuged at 1600 rpm and the plasma was stored at −70°C until assayed.

Blister fluid samples

Seven blisters were produced on the forearm of each volunteer by the method of Totsuka et al.⁴ A plastic tube (diameter, 8.5 mm) connected to a vacuum source was fixed tightly to the midvolar area of the forearm. Blisters were formed by suction for 2 hours at a negative pressure of 225–250 mm Hg. The volunteers were randomly assigned to 2 groups of 3 men each. Fluid samples were collected at 0, 0.5, 2 and 4 hours after the administration of PZFX in 1 group and at 0, 1, 3 and 6 hours after the dose in the other group. The samples were stored at −70°C until assayed.

Clinical chemistry analysis

Plasma albumin and phospholipid levels were determined with an automatic analyzer (Hitachi model 705, Tokyo, Japan). Albumin levels in the suction blister fluid were calculated from albumin/total protein ratios based
on the results obtained from cellulose acetate electrophoresis. Total protein concentrations were determined by the pyrogallol red method, sodium (Na⁺) and potassium (K⁺) levels determined with a flame photometer (Japan Spectroscopic FLAME-30C, Tokyo, Japan) and chloride (Cl⁻) levels determined by coulometric titration (Jookoo cholorimeter C-200AP, Tokyo, Japan).

**PZFX concentrations**

Concentrations of PZFX were determined by high-performance liquid chromatography. Plasma samples (0.2 mL) and suction blister fluid (0.2 mL) were deproteinized with 0.2 mL of methanol, centrifuged at 3000 × g for 10 minutes, and 20–50 μL of the supernatant was injected into the column. The column was packed with STR ODS (4.6 mm Φ × 150 mm; Shimadzu Techno Research, Tokyo, Japan) with a guard column (4.6 mm Φ × 50 mm). The mobile phase consisted of 90 mL of acetonitrile, 50 mL of 0.2 mol/L phosphate buffer (pH 7.0), 860 mL of distilled water, and 6.8 g of tetra-n-butylammonium hydrogen sulfate (Tokyo Kasei, Tokyo, Japan). The flow rate was 1.0 mL/min, and the UV detection wavelength was set at 330 nm. All separation procedures were carried out at 25°C. A phosphate buffer solution was prepared by mixing 0.2 mol/L potassium dihydrogenphosphate and 0.2 mol/L disodiumhydrogenphosphate, pH 7.0.

**Pharmacokinetic analysis**

Pharmacokinetic parameters in the plasma and suction blister fluid were estimated using the PAG-CP computer program package (Asmedica, Osaka, Japan). The areas under the plasma and the blister fluid concentration-time curves (AUCs) were calculated by the trapezoidal rule method.

**RESULTS**

The concentrations of albumin, phospholipid, Na⁺, K⁺, and Cl⁻ in suction blister fluid and plasma were determined, and the blister fluid/plasma concentration (B/P) ratio of each component calculated. The B/P ratios for albumin and phospholipid were 0.30 and 0.26, respectively, and the concentrations of Na⁺, K⁺, and Cl⁻ in suction blister fluid were identical to that in plasma. The concentrations of PZFX in suction blister fluid and plasma after a single oral 200 mg dose are shown in Table 1. The peak plasma level of PZFX was 2.88 ± 1.05 μg/mL at 0.5 hours after the oral dose, and the peak suction blister fluid level of PZFX was 1.52 ± 0.10 μg/mL at 2.0 hours after administration. The pharmacokinetic parameters in suction blister fluid and in plasma were calculated, and resulted in a Cmax in blister fluid and plasma of 1.52 and 2.95 μg/mL, a tmax of 2.0 and 0.7 hours, respectively, and a t1/2 in blister fluid which was almost the same as in plasma. The AUC of PZFX in blister fluid and in plasma was 6.70 and 6.28 μg·h/mL, respectively, and the concentrations of Na⁺, K⁺, and Cl⁻ in suction blister fluid and plasma were calculated, and resulted in a Cmax in blister fluid and plasma of 1.52 and 2.95 μg/mL, a tmax of 2.0 and 0.7 hours, respectively, and a t1/2 in blister fluid which was almost the same as in plasma. The AUC of PZFX in blister fluid and in plasma was 6.70 and 6.28 μg·h/mL, respectively, and the AUC ratio of blister fluid/plasma was 0.94.

**DISCUSSION**

Concentrations of antimicrobial agents in tissue homogenates have often been measured and regarded as predictors of efficacy. However, it is generally assumed that most bacterial infections occur in what is considered the extracellular or interstitial space, although certain infections are known to be caused by some intracellular pathogens. Baldwin et al. suggested that clinical efficacy should be directly related to the concentration of drugs at the site of the pathogen, and that the penetration of antibiotics into foci of inflammation is the most important factor in determining efficacy. Nix et al. found that antibiotics should be present in the extracellular fluid to be effective in treating bacterial infections, and suggested that the low and high tissue homogenate/serum ratios of antimicrobial agents are potentially misleading when used to describe the responsiveness of extracellular gram-negative bacteria. It has been suggested that blister fluid obtained by mild suction is representative of interstitial fluid. The blister fluid/plasma concentration ratio for albumin and phospholipid obtained in our study was similar to that reported by Vermeer et al., and therefore, the suction blister fluid in our study may be regarded as interstitial fluid.

In human studies, Aoyama et al. showed that the Cmax and AUC of a 300 mg po dose of ofloxacin (OFLX) in suction blister fluid was 1.59 μg/mL and 10.1 μg·h/mL, and Catchpole et al. reported Cmax and AUC values for a 750 mg po dose of ciprofloxacin (CPFX) in cantharides-induced blister fluid of 2.3 μg/mL and 20.3 μg·h/mL. Although the experimental conditions were different, the Cmax of a 200 mg po dose of OFLX and a 200 mg po dose of CPFX would be expected to be lower than the 1.52 μg/mL level obtained with PZFX in this study, and the AUC of a 200 mg po dose of OFLX has been suggested to be higher than the 6.28 μg·h/mL level of PZFX found in this study.

Cmax and AUC are acknowledged to be the pharmacokinetic parameters that best predict the efficacy of PZFX in blister fluid.