Arch. Pharm. Res. Vol.20, No.4, pp. 358-362, 1997

Metabolism and Excretion Study of DW116, A New Fluoroquinolone, in Rats

Byung Hwa Jung, Young Han Park, Jongsei Park[1] and Bong Chul Chung*

Doping Control Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul, 130-650, Korea and [1]Korea Food and Drug Administration, 5 Nokbundong, Eunpyunggu, Seoul 122-020 Korea

(Received June 4, 1997)

Metabolite identification and urinary and biliary excretion of the new fluoroquinolone antibacterial agent DW116 [1-(5-fluoro-2-pyridyl)-6-fluoro-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, hydrochloride] after oral administration have been studied in Sprague-Dawley rats . The excretion kinetics were monoexponential. Most of the drug was eliminated via the hepatic and renal routes. Mean renal clearance of DW116 was 73.4 ml/hr/kg and mean biliary clearance was 83.8 ml/hr/kg. The major metabolite excreted in the bile was identified as the glucuronide ester of the parent drug using base-hydrolysis of the conjugate metabolite followed by co-HPLC with standard compound, ^{19}F-NMR and LC-MS methods. The glucuronide conjugate was also found in urine. The mean urinary recoveries of free and total (free plus glucuronide ester) DW116 were 28.6±2.7% and 36.4±1.8% of the administered dose and the corresponding biliary recoveries were 14.4±5.5% and 37.0±7.6%, respectively.

Keywords : Fluoroquinolone, DW116, Metabolism, Pharmacokinetics

INTRODUCTION

Fluoroquinolones are potent broad spectrum antibacterial agents and have excellent activity against various bacteria, a low frequency of adverse effects, and good absorption on oral administration. They have similar chemical structures but exhibit wide differences in their pharmacokinetics and metabolic profiles. Ofloxacin is almost (70%) exclusively eliminated by the kidney (Marchbanks *et al.*, 1992), whereas perfloxacin is predominently cleared by the liver. Some fluoroquinolones, such as norfloxacin, ciprofloxacin, enoxacin, fleroxacin, temafloxacin, and lomefloxacin are eliminated via both renal and hepatic routes. (Blum, 1992). Most of quinolones were metabolized to the corresponding oxo derivatives through microsomal oxidative pathway by cytochrome P-450. Barofloxacin (Nakagawa *et al.*, 1995) and sparfloxacin (Montay *et al.*, 1994) were excreted as glucuronide conjugates in urine and the new fluoroquinolone, T-3761, is excreted both in urine and bile as a glucuronide conjugate (Tai *et al.*, 1995). Some fluoroquinolones also have an active metabolite, N-desmethyl metabolite (Nakagawa *et al.*, 1995, Lombardi *et al.*, 1992, Stuck *et al.*, 1992).

Correspondence to: Bong Chul Chung, Doping Control Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul, 130-650, Korea

In these experiments, we identified a major metabolite of the new fluoroquinolone, DW116, in urine and bile after oral administration to rats. The excretion profile of this new antibacterial drug was investigated.

MATERIALS AND METHODS

Materials

DW116 (Fig. 1) was supplied from Dong Wha Pharmaceutical (Anyang, Korea). All other chemicals were of analytical grade or HPLC grade.

Animals

Male Sprague-Dawley rats, weighing 200~280 g (5-

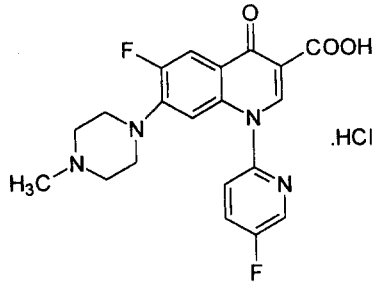

Fig. 1. Chemcial structure of DW116.

10 weeks of age) were purchased from Genetic Engineering Research Institute (Seoul, Korea). Water and food were supplied *ad libitum*.

Animal treatments and sample collections

DW116 was dissolved in water for oral administration and in saline for i.v. injection. All collected samples by the time schedule described below were stored at -20°C until analyzed.

Urinary excretion studies

DW116, 4mg/kg, was administered to rats (n=4) and housed individually in each metabolic cage. Urine was collected overnight prior to dosing (0), 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-22 hr post dose period.

Biliary excretion studies and identification of metabolite

Three rats were anethesized with ether and the bile duct was cannlated using PE 10 tube (Natume, Japan) through abdominal midline incision. The catheter was tightened by black silk sutures and then skin incision was closed. After recovered from anesthesia, they were orally administered 4mg/kg doses of DW116. Bile samples were collected prior to dosing (0), 0-1, 1-2, 2-3, 3-4, 4-6, 6-8, 8-10 and 10-22 hr post dose. Separately, a 20 mg/kg dose of DW116 was orally administered to rat and bile samples were collected prior to dosing (0) and 0-6 hr post dose period.

HPLC assay of DW116

The HPLC system consisted of a Hewlett Packard (HP) 1090 Liquid Chromatograph and a HP 3392 integrator. A Lichrosorb RP-18 column (5 μm, 20 cm, l ×0.46 cm, i.d., Hewlett Packard) and ODS-Hypersil guard column (5 μm, 2 cm, l×0.21 cm, i.d. Hewlett Packard) were used for all analyses. Mobile phase was a mixture of acetonitrile (A) and 0.1 M potassium dihydrogen phosphate containing 0.01% tetrabutyl ammonium chloride and 1% 1-heptane sulfonic acid which was adjusted to pH 2.5 with phosphoric acid (B). Isocratic system (A:B=15:85, v/v) for the detection of metabolites and gradient system for quantification of DW116 (10% A to 25% A in 7 min and to hold 25% A and 75% B for 10 min) was used. The flow rate was 1.0 ml/min and the column effluent was monitored by a UV detector at 280 nm.

Analysis of the free (Unconjugated) DW116 in urine and bile

A portion (100 μl) of urine or bile was taken after centrifugation, 10 μl of internal standard [1-(2,4-fluorophenyl)-6-fluoro-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, 600 μg/ml, dissolved in methanol) was added. After vortexed for 1 min, an aliquot (5 μl) were then injected directly into HPLC column. Both urine and bile standards were prepared at the concentrations of 5, 10, 20, 50, 100, 200, and 500 μg/ml.

Analysis of total DW116 in urine and bile

To a 100 μl aliquot of urine or bile sample, 10 μl of internal standard (600 μg/ml) and 10 μl of 0.1 M NaOH were added and incubated at 37°C for 30 min. After cooling, 10 μl of 0.1M HCl was added to the hydrolysate, vortexed and centrifuged. An aliquot (5 μl) of the supernatant was analyzed as described for the free drug analysis in urine or bile.

Under these conditions the retention times of DW 116 and internal standard were 4.8 min and 7.5 min, respectively. The lower detection limit was 0.3 μg/ml. Intraday and interday coefficient of variance (CV, %) was < 9% at 5 μg/ml and < 0.7% at 500 μg/ml (n=3).

Identification of the conjugated metabolite

Aliquots of untreated and base-hydrolyzed 6 hr bile samples obtained after a single (20 mg/kg) oral administration of DW116 were examined by both HPLC and ^{19}F-NMR (Varian Unity plus 300) equipped with a broad-band probe. The HPLC conditions were the same as described for the assay of DW116 in urine and bile except that the mobile phase system was isocratic, consisting of 15% A and 85% of B. D_2O (10%) was added to the bile sample for NMR locking. The proton channel was not decoupled and the spectrum was acquired at 298 K. The spectral width and number of points acquired were 100,000 Hz and 128 K complex points. The FID data was apodized with the 3~10 Hz line broadening. ^{19}F Chemical shifts were given in ppm from CF_3COOD.

To detect the molecular ion of the conjugated metabolite, aliquots of the untreated 6 hr bile were also analyzed by LC-MS (Hewlett-Packard HP 5988A LC-TSP-MS system equipped with HP 5955-7598 thermospray interface). The column was Type UG 120Å capcell pak C_{18} (5 μm, 15 cm, l×0.46 cm, i.d., Shisheido, Japan). The mobile phase composition was 0.2 M ammonium formate: methanol (85:15) for the first 10 min followed by a linear gradient (75:25) for 10 min, then isocratic for 5 min. The flow rate was 1.0 ml/min. The TSP interface auxiliary pump maintained a pressure of 13.3~26.6 Pa ion source and two spectrometer diffusion pumps maintained at $2.66\sim5.32\times10^{-5}$ Pa. The ion source temperature was 276°C. The filament-on mode (ionization by electron beam) was used. Both positive and negative ion chemical ionization modes were carried out.

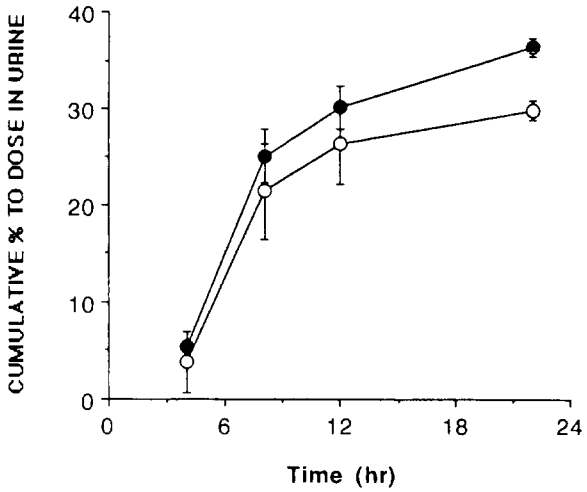

Fig. 2. Mean cumulative percentages of DW116 excreted in urine as free (○) and total (free plus conjugated: ●) DW 116 following a single (4 mg/kg) oral administration to rats (n=4). Bars represent standard deviation.

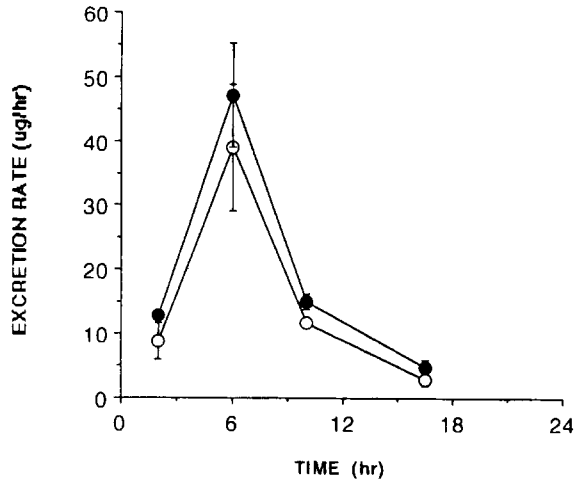

Fig. 4. Mean urinary excretion rate of free (○) and total (free plus conjugated: ●) DW116 following a single (4 mg/kg) oral administration to rats (n=4). Bars represent standard deviation.

RESULTS AND DISCUSSION

Excretion

The mean cumulative percentage of dose excreted in urine and bile as a function of time are shown in fig 2 and 3, respectively. The urinary and biliary excretion rate versus time plots following a single (4 mg/kg) oral administration of DW 116 are shown in Fig. 4 and 5, respectively. The urinary recoveries of free and total DW116 accounted for 28.6±2.7% and 36.4±1.8% of the administered oral dose, respectively (Fig. 2) and the corresponding mean biliary recoveries were 14.4±5.5% and 37.0±7.6% (Fig. 3). The total values, sum of these two routes of elimination up to 22 hr accounted for approximately 43% of the dose for free drug and 73% of the dose for total drug. This indicates that most of DW 116 is excreted via the hepatic and renal routes either by free or conjugated form. The excretion rate of both free and total drug was the greates during 4~8 hr period in urine and for the first one hour period in bile (Fig. 4). By 8 hr post dose urinary recovery reached 69% of the total excretion and similarly 68% of the total biliary excretion was accomplished by 4 hr post dose. The urinary and biliary elimination of free DW116 was monoexponential.

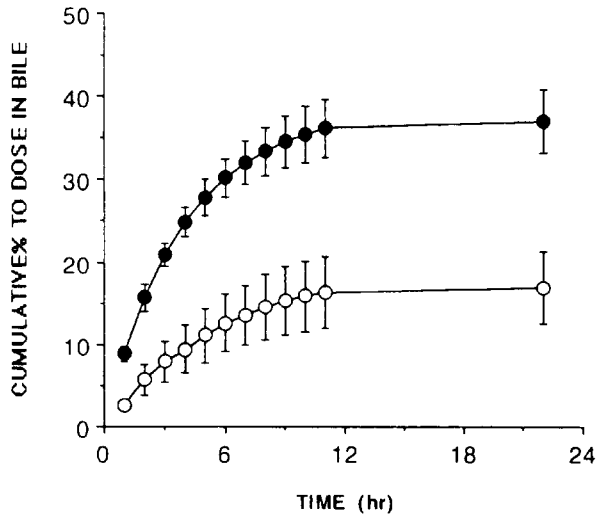

Fig. 3. Mean cumulative percentages of DW116 excreted in bile as free (○) and total (free plus conjugated: ●) DW 116 following a single (4 mg/kg) oral administration to rats (n=3). Bars represent standard deviation.

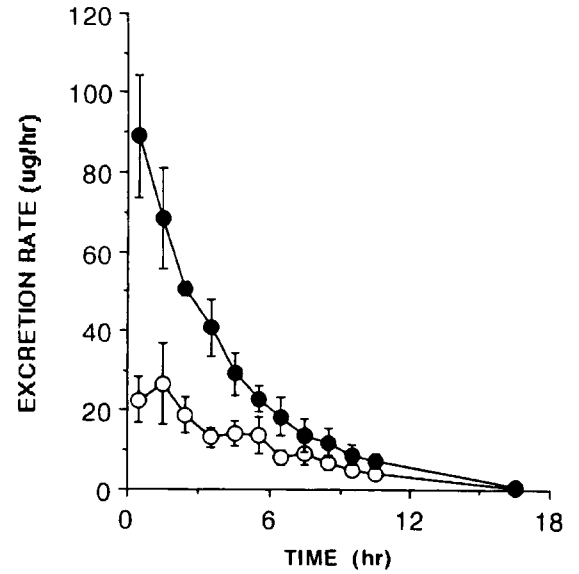

Fig. 5. Mean biliary excretion rate of freefree (○) and total (freefree plus conjugated: ●) DW116 following a single (4 mg/kg) oral administration to rats (n=3). Bars represent standard deviation.

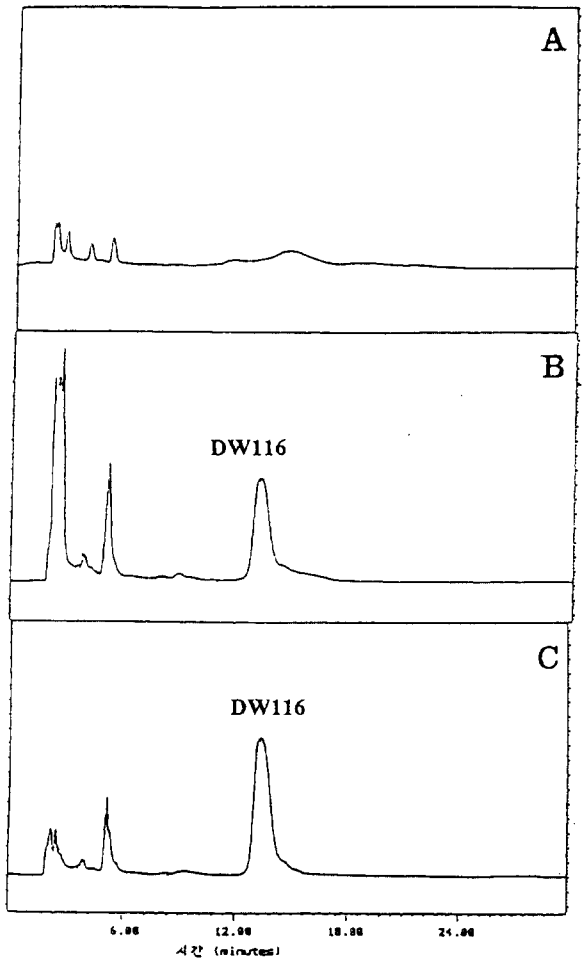

Fig. 6. Typical HPLC chromatograms of blank bile (A), untreated 6 hr bile (B) and base-hydrolyzed 6 hr bile (C) samples after a single (20 mg/kg) oral administration of DW 116.

The mean renal clearance (Cl_R) of DW 116 was 73.4 ml/hr/kg and the biliary clearance (Cl_B) was 83.8 ml/hr/kg. Cl_R and Cl_B were calulated as $X_{U\ 0\text{-}22h}$ (total amount of DW116 excreted in urine during 0-22 hours) and $X_{B\ 0\text{-}22h}$ (total amount of DW116 excreted in bile during 0-22 hours) divided by AUC (Chung, B.C., *et al.*, 1995). The mean terminal biliary elimination half-life of free DW116 was approximately 3 hr.

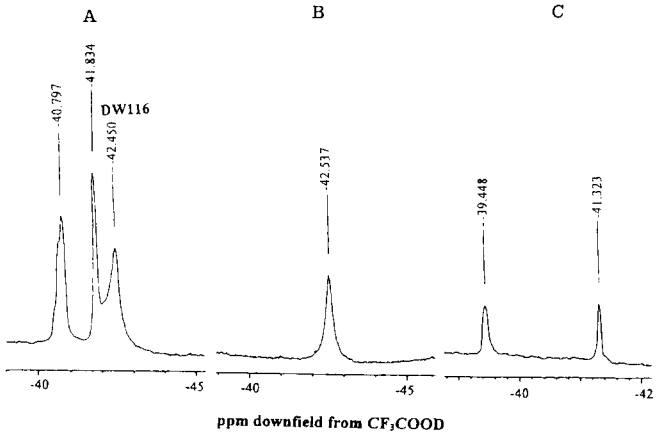

Fig. 7. Typical ^{19}F-NMR spectra of 6 hr bile samples after a single (20 mg/kg) oral administration of DW 116. (A) untreated bile at pH 8.6, (B) base- hydrolyzed bile at pH 8.6, (C) base-hydrolyzed bile at pH 2.4.

Identification of the conjugated metabolite

HPLC analysis of untreated, base-hydrolyzed, and blank bile samples are shown in Fig. 6. The peak eluted at the solvent front in the untreated bile (Fig. 6B) was greatly decreased and consequently the free drug peak was increased after base hydrolysis (Fig. 6C). This indicated that the polar component eluted with

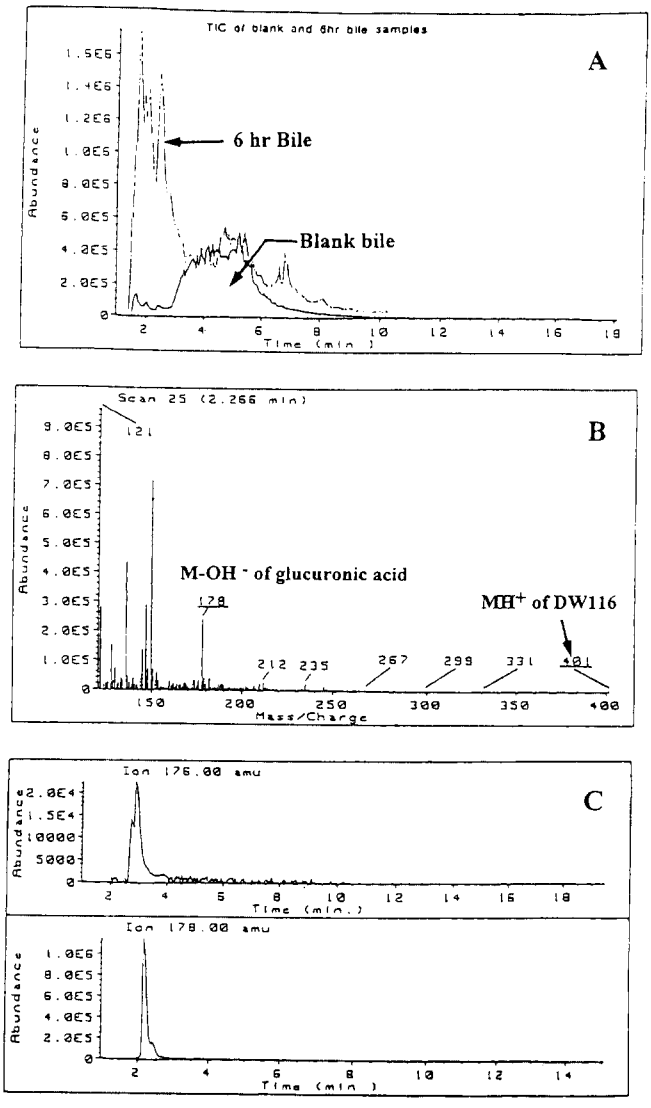

Fig. 8. LC-MS profiles of blank and untreated 6 hr bile samples after a single (20 mg/kg) oral administration of DW116. TIC of blank and 6 hr bile samples in the positive mode (A), MS of the peak, at 2.27 min (B), selected ion chromatogram at m/z 176 and MS of the peak at 2.87 min (C).

the solvent peak was a conjugated metabolite. The glucuronide ester was not easily hydrolyzed with either β-glucuronidase or hydrochloric acid. The ^{19}F-NMR analysis of the base-hydrolyzed bile sample also showed the disappearance of two major resonances centered at 40.80 ppm and 41.83 ppm which were observed in the untreated bile sample (Fig. 7A and B). The resonance peaks which centered at 42.45 and 42.54 ppm in both untreated and base-hydrolyzed bile samples, respectively, were confirmed to be the only resonance of two fluorines of the DW116 molecule because the signals co-resonanced with that of standard DW116. The chemical shift of the two fluorines in the DW116 molecule were dependent on the pH of the fluid: the one resonance centered at 42.54 ppm at pH 8.6 resolved as two resonances at 39.45 ppm and 41.32 ppm at pH 2.4 (Fig. 7C). The LC-MS analyses of blank and 6 hr bile samples revealed the presence of a polar metabolite centered at 2.2 min only in the 6 hr bile sample (Fig. 8A). In the positive ion mode the mass spectrum of this peak showed ion peaks at m/z 178 and m/z 401, which match the molecular ions of glucuronic acid and parent drug (Fig. 8B). In the negative mode, the molecular ion of glucuronic acid was also detected at m/z 176 (Fig. 8C). This, along with the results of HPLC and ^{19}F-NMR analyses, confirmed that the metabolite was the glucuronide ester of DW116.

ACKNOWLEDGEMENT

We thank Dong Wha Pharmacentical Ind., Co. for the generous supply of DW116, and Dr. Kang-Bong Lee and Dr. Yunje Kim for their valuable technical assistance for ^{19}F-NMR and LC-MS analyses, respectively.

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