Short Communication

Metabolic Disposition of the New Fluoroquinolone Antibacterial Agent DW116 in Rats

ABSTRACT:

The metabolic disposition of the new fluoroquinolone antibacterial agent DW116 has been studied in Sprague-Dawley rats. The compound was well absorbed and demonstrated excellent oral bioavailability. The plasma kinetic profiles were characterized by monoexponential elimination with an elimination half life of 3-4 hr. The apparent mean total clearance (CLt) and the volume of distribution (Vss) ranged from 221 ± 55 to 274 ± 27 ml/hr/kg and 1.0 ± 0.1 to 1.5 ± 0.2 l/kg, respectively, and were independent of dose between 4 and 20 mg/kg levels. The renal (CLR) clearance was 64.5 ml/hr/kg and the biliary (CLb) clearance was 33.8 ml/hr/kg. The combined value accounted for approximately one-half of the total clearance, indicating that the remaining one-half of the administered dose was eliminated via hepatic clearance. The major metabolite excreted in the bile was identified as the glucuronide ester of parent drug using base-hydrolysis of the conjugate metabolite followed by co-HPLC with standard compound, 13F-NMR and LC-MS methods. 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. The mass balance study after a single (100mg/kg) oral administration of [14C]-DW116 indicated complete recovery of radioactivity over a 7-day period, accounting for approximately 60-70% in feces and 30-40% in urine. [14C]-DW116 extensively distributed during a prolonged process into all tissues with a rather slower penetration into the brain, lung, and muscle. The compound also readily crossed the placenta and was transferred to the fetus.

Fluoroquinolone antibiotics, which interrupt the DNA gyrase-mediated DNA cleavage and reseling cycle at the cleavage step, possess a broad spectrum of antibacterial activity (1–3). DW1161 has been synthesized to provide improved oral bioavailability and is currently being evaluated as a new fluoroquinolone antibiotic agent. Early pharmacokinetic studies revealed that the compound was rapidly and well absorbed after oral administration. The protecting effects of DW116 against systemic infection of various Gram-positive and Gram-negative organisms in mice were more potent than rifloxacin and comparable with ofloxacin, ciprofloxacin, and sparfloxacin (4). In this communication, we present the results of the following disposition studies of DW116 in rats: 1) absolute bioavailability; 2) kinetics in plasma, urine, and bile; 3) mass balance; 4) biotransformation; 5) whole-body autoradiography; 6) milk excretion; and 7) fetus transfer.

Materials and Methods. DW116 and internal standard (see structures in fig. 1) were obtained from Dong Wha Pharmaceutical Industry Co. (Anyang, Korea). [14C]-labeled DW116 (asterisk in fig. 1 indicates site of 14C label) was synthesized with a specific activity of 32.3 μCi/mmol or 74 μCi/mg in SRI International (Menlo Park, CA). All chemicals and reagents used were analytical grade, and the chromatographic solvents used were HPLC grade. For kinetic studies in plasma, urine, and bile, male Sprague-Dawley rats (200–280 g) were purchased from the Genetic Engineering Research Institute (Seoul, Korea) for kinetic and excretion studies; Simonsen Laboratories, Inc. (Gilroy, CA), for mass balance, milk excretion, and placental studies; and Charles River Laboratories, Groton, CT, for analytical and excretion studies.

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1 Abbreviations used are: DW116, 1-(5-fluoro-2-pyridyl)-6-fluoro-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride; HP, Hewlett-Packard; AUC, area under the plasma concentration vs. time curve; CLR, renal clearance; CLb, biliary clearance; CLt, total clearance; Vss, volume at steady-state.

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0.01% tetrabutyl ammonium chloride and 1% l-heptane sulfonic acid that was adjusted to pH 2.5 with phosphoric acid (B). A linear gradient was used to change the composition from 10% A to 25% A in 7 min and to hold 25% A and 75% B for 10 min. The flow rate was 1.0 ml/min, and the column effluent was monitored by a UV detector at 280 nm. Plasma (100 μl) was deprotenized using acetonitrile (150 μl) and urine, or bile was analyzed directly after simple centrifugation. In all analysis, internal standard was added to each sample. Aliquots were analyzed by HPLC at the conditions described herein. The concentration of DW116 in each sample was obtained from standard calibration curve. Pharmacokinetic parameters were calculated by PCNONLIN (SCI Software, Lexington, KY) according to monoexponential elimination. To analyze total (unmetabolized plus conjugate) DW116, plasma, urine, and bile (100 μl) were hydrolyzed using 0.1 N NaOH (100 μl) at 37°C for 30 min, then adjusted to pH 7 using 0.1 N HCl. An aliquot of the supernatant was analyzed in the same manner described previously for unmetabolized drug assay.

For the profile of radioactivity, urine and plasma were examined without any treatment. Water homogenates (1:3, w/w) of feces were centrifuged after methanol was added (1:5, v/v), and the supernatant fraction was evaporated using a nitrogen stream. The residue was dissolved in the mobile phase. Chromatography was performed using an HP 1090 HPLC system coupled to a radioactivity flow detector (Beta-Ram, I.N.U.S. Systems, Inc., Tampa, FL), 5 μm Beckman Ultrasphere C18 column and with a linear gradient from 10 to 70% of acetonitrile and 10 mM K2HPO4 in 0.2% acetic acid (pH 3.0) in 25 min with the flow rate of 1 ml/min.

**Identification of the Conjugated Metabolite.** Aliquots of untreated and base-hydrolyzed 6-hr bile samples after a single (20 mg/kg) oral administration of DW116 were examined by 19F-NMR (Varian Unity plus 300) equipped with a broadband probe and LC/MS (HP 5988A LC-TSP-MS system equipped with an HP 5955-7598 thermospray interface). In LC/MS analysis, both positive- and negative-ion chemical ionization modes were conducted. The column was Shisheido 5 μm type UG 120Å capcell pak C18. 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.

**Results and Discussion.** The plasma concentration vs. time profiles of unmetabolized DW116 are shown in fig. 2, and pharmacokinetic parameters are presented in table 1. The compound was absorbed rapidly after oral administration. The first sampling time.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>4, iv</th>
<th>4, po</th>
<th>8, po</th>
<th>20, po</th>
</tr>
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<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/ml)</td>
<td></td>
<td>3.54 ± 0.16</td>
<td>3.44 ± 0.33</td>
<td>7.62 ± 0.12</td>
<td>14.83 ± 1.74</td>
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<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td></td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.06</td>
<td>0.46 ± 0.11</td>
<td>0.92 ± 0.47</td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</td>
<td></td>
<td>3.9 ± 0.3</td>
<td>3.8 ± 0.5</td>
<td>3.1 ± 0.6</td>
<td>3.6 ± 0.2</td>
<td></td>
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<tr>
<td>AUC</td>
<td></td>
<td>14.7 ± 1.4</td>
<td>17.8 ± 2.8</td>
<td>39.1 ± 7.1</td>
<td>75.6 ± 3.7</td>
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<tr>
<td>MRT (hr)</td>
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<td>5.1 ± 0.6</td>
<td>5.8 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>5.4 ± 0.3</td>
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<tr>
<td>CL&lt;sub&gt;T&lt;/sub&gt; (ml/hr/kg)</td>
<td></td>
<td>274 ± 27</td>
<td>239 ± 54</td>
<td>221 ± 55</td>
<td>266 ± 22</td>
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<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt; (liters/kg)</td>
<td></td>
<td>1.63 ± 0.22</td>
<td>1.35 ± 0.0</td>
<td>97 ± 0.05</td>
<td>1.43 ± 0.01</td>
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</tbody>
</table>

C<sub>max</sub>; maximum concentration; t<sub>max</sub>; time to maximum concentration; t<sub>1/2</sub>, half-life; MRT, mean residence time; iv, intravenous; po, oral.

<sup>a</sup>The fraction of dose absorbed was assumed to be unity, because the oral bioavailability of DW116 was >100%.

**TABLE 1 Mean pharmacokinetic parameters of DW116**

**FIG. 1.** Structures of DW116 and internal standard.

*Asterisk indicates site of 14C label.

**FIG. 2.** Mean plasma unmetabolized DW116 concentration vs. time profiles after the 4 mg iv (▲), 4 mg po (▲), 8 mg po (●), and 20 mg po (○) single doses.

CONC., concentration.

**FIG. 3.** Mean biliary excretion of unmetabolized (○) and total (unmetabolized plus conjugated; (●) DW116 after a single (4 mg/kg) oral administration.

Symbols and bars represent mean ± SD for three rats.
administration and demonstrated excellent oral bioavailability, compared with AUC values obtained after 4 mg/kg intravenous and oral administration. The plasma peak concentration and AUC value after oral administration of 4, 8, and 20 mg/kg of DW116 increased in proportion to the administered dose, and the terminal elimination rate of DW116 was independent of dose. The peak time ranged from 0.4 to 0.9 hr, with a rather slower time at the highest dose.

DW116 was also present the glucuronide ester form in the plasma. The conjugate accounted for 13–40% of total drug after a single 8 mg/kg oral administration. The percentage of the conjugate, which increased by ~2-fold (20% → 40%) after 2 hr did not change until 8 hr postdose.

The urinary recoveries of unmetabolized and total drug accounted for 28.6 ± 2.7% and 36.4 ± 1.8% of the administered dose, respectively. The corresponding mean biliary recoveries were 14.4 ± 5.5% and 37.0 ± 7.6% of the administered dose, respectively. The conjugate is at a much lower level in urine than bile. Combined, these two routes of elimination accounted for ~43% of the dose for unmetabolized drug and 73% of the dose for total drug within 22 hr. The excretion rate of both unmetabolized and total drug was greatest between 4 and 8 hr in urine and for the first 1 hr in bile, then gradually decreased (fig. 3). Mean CL_{ur} of DW 116 was 73.4 ml/hr/kg and the CL_{br} was 83.3 ml/hr/kg. CL_{ur} is greater than the combined value of CL_{ur} and CL_{br}. This indicates that most of the administered dose is eliminated via hepatic clearance and CL_{br}. The mean terminal biliary elimination half-life of unmetabolized DW116 was ~3 hr. DW116 readily crosses the placenta, and radioactivity in the placenta, fetal liver, and fetus were highest at 4 hr post dose. Fetal liver concentrations were higher than the other two organs. Radioactivity in milk were also highest at 4 hr.

The mass balance study indicated complete recovery of radioactivity over a 7-day period, accounting for 57.6 ± 9.0% (male) to 71.3 ± 1.9% (female) in the feces and 33.6 ± 1.9% (female) to 39.2 ± 3.6% (male) in the urine. No organs and tissues have a significant level of radioactivity during 7 days postdose. The majority of the total recovery of radioactivity is eliminated in the first 24 hr postdose. The radioactivity profile of fecal extract reveals that the parent is the major component and that glucuronide is not detected. It is likely that the glucuronide ester excreted in the bile is hydrolyzed before it is eliminated in the feces. We have observed the same phenomenon for a ciprofloxin (5). Three and five minor metabolite (or degradation) peaks were detected in the urine bile extracts, respectively, but not identified. If the minor metabolites can be quantified, the recovery will reach 100% within 24 hr after drug administration in the urine and bile.

The 19 F-NMR analysis of the base-hydrated bile sample also showed the disappearance of two major resonances centered at 40.80 and 41.83 ppm, which were observed in the untreated bile sample. The resonance peaks that centered at 42.45 and 45.54 ppm in both untreated and base-hydrated bile samples, respectively, were confirmed to be the only resonance of two fluorines of the DW116 molecule because the signals coresonated with that of standard DW116.

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. 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. 4). In the negative mode, the molecular ion of glucuronic acid was also detected at m/z 176. This, along with the results of HPLC and 19 F-NMR analyses, confirmed that the metabolite was the glucuronide ester of DW116.

From the whole-body autoradiogram, as expected with a large V_{ur} (1.0–1.6 liters/kg), it is known that 14 C-DW116 extensively distributes into most tissues in a prolonged process. The penetration into the brain, lung, and muscle is slower than other tissues. Most tissue concentrations exceed blood concentrations. The protein binding with DW116 is relatively low (4). In summary, these data indicate that DW116 shows excellent oral bioavailability and linear pharmacokinetics, with a half-life of 3–4 hr. The compound extensively distributes into organs and tissues in a prolonged process, and is excreted as parent drug and its glucuronide ester in both urine and bile.

References