Pharmacokinetics of DA-7867, a New Oxazolidinone, after Intravenous or Oral Administration to Rats: Intestinal First-Pass Effect

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Received 31 January 2003/Returned for modification 12 July 2003/Accepted 4 October 2003

During the 1980s and 1990s, the emergence and widespread spread of methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE; Enterococcus faecium and Enterococcus faecalis), vancomycin/glycopeptide-intermediate Staphylococcus aureus (VISA/VISA, penicillin-resistant Streptococcus pneumonia (PRSP), and multidrug-resistant (MDR) coagulase-negative staphylococci were reported (5). Oxazolidinone antibiotics, such as linezolid (PNU-100766), eperezolid (PNU-100592), PNU-177553, DuP-721, DuP-105, VRC-3808, RWJ-334181, RWJ-337813, and AZ02563, have been synthesized (9) to overcome the aforementioned problem of emergence of bacteria resistant to the antibiotics. The oxazolidinones inhibit bacterial protein synthesis (5, 9). Recently, a new oxazolidinone, DA-7867 ((S)-[N-3-(4-(2-(1-methyl-1,2,4-oxadiazol-3-yl)-pyridine-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl acetamide; a basic compound with a molecular mass of 411.39 Da) was synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Ltd., Yongin, Korea). DA-7867 had good antibacterial activities against gram-positive bacteria (including resistant strains of organisms such as MRSA, VRE, and PRSP) and some gram-negative strains (including Haemophilus influenzae and Moraxella catarrhalis). DA-7867 showed four- to eightfold-better antibacterial activities than linezolid against gram-positive and gram-negative pathogens, including MDR bacteria (W. B. Im, T. H. Lee, J. Cho, S. H. Choi, and J. K. Rhee, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F1131, p. 211, 2002). For example, MICs at which 90% of isolates tested are inhibited (MIC₉₀) of DA-7867 were 0.78, 0.2, and 0.39 µg/ml for MRSA (n = 37), VRE (E. faecium; n = 36), and PRSP (n = 33), and the corresponding values for linezolid were 3.13, 1.56, and 1.56 µg/ml. The MIC₉₀ of DA-7867 were 3.13 and 0.78 µg/ml for H. influenzae (n = 37) and M. catarrhalis, respectively, which were four- to eightfold lower than those of linezolid. DA-7867 is being evaluated in preclinical studies as a new oxazolidinone antibiotic. The purpose of the present study was to report the dose-independent area under the plasma drug concentration-time curve from time zero to time infinity (AUC₀-∞) of DA-7867 after intravenous or oral administration to rats of doses of 1, 5, 10, and 20 mg/kg and gastric and intestinal first-pass effects of DA-7867 after intraportal, intragastric, and intraduodenal administration to rats of a dose of 10 mg/kg.

DA-7867, DA-7858 ((S)-[N-3-(4-(2-(1-methyl-1,2,4-oxadiazol-3-yl)-pyridine-5-yl)-3-fluorophenyl]-2-oxo-5-oxazolidinyl]methyl acetamide, an internal standard of high-performance liquid chromatographic (HPLC) analysis), and hydroxypropylmethylcellulose (HPMC [type 2910], Shin-ETSu Chemical Company, Tokyo, Japan) were supplied from the Research Laboratory of the Dong-A Pharmaceutical Company. Dimethylacetamide (DMA) and polyethylene glycol (PEG) 400 were purchased from Sigma Chemical Company (St. Louis, Mo.) and D克斯an Chemical Company (Seoul, Korea), respectively. Other chemicals were of reagent or HPLC grade and therefore were used without further purification. Male Sprague-Dawley rats (weight, 264 to 310 g) were purchased from Charles River Company Korea (Biogenomics, Seoul, Korea). The animals were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea) at a temperature between 20 and 23°C with a 12-h light-dark cycle and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with food (Samyang Company, Seoul, Korea) and water ad libitum. The protocol of the animal study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University. The pretreatment and surgical procedures for intravenous, oral, intraportal, intraduodenal, and intragastric administration were similar to previously reported methods (14). DA-7867 (DA-7867 powder dissolved in DMA-PEG 400-distilled water at 3:5:2 [vol/vol/vol]) at doses of 1 (n = 10), 5 (n = 10), 10 (n = 9), and 20 (n = 10) mg/kg was injected over 1 min via the jugular vein (total injection volume, 2 ml/kg). An approximately 0.12-ml aliquot of blood sample was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of
infusion), 5, 30, 60, 90, 120, 240, 360, 480, 720, 960, 1,440, 2,160, and 2,880 min after intravenous administration of the drug. Blood samples were centrifuged immediately, and a 50-μl aliquot of each plasma sample was stored in a −70°C freezer until HPLC analysis of DA-7867 (1). At 48 h after an intravenous administration, the entire gastrointestinal tract (including its contents and feces) was removed, transferred to a beaker containing 100 ml of methanol (to facilitate extraction of DA-7867), and cut into small pieces with scissors. After manual shaking and stirring with a glass rod, two 50-μl aliquots of the supernatant were collected from each beaker. Urine was collected over a 48-h period. Other procedures were similar to previously reported methods (14). DA-7867 at a dose of 20 mg/kg was also intravenously administered to six rats after bile duct cannulation, and a 24-h bile sample was collected. DA-7867 (DA-7867 powder suspended in 1% HPMC) at doses of 1 (n = 9), 5 (n = 10), 10 (n = 9), and 20 (n = 10) mg/kg was administered orally with feeding tubing (total oral volume, 3 ml/kg). Blood samples were collected at 0, 15, 30, 60, 90, 120, 240, 360, 480, 720, 960, 1,440, 2,160, and 2,880 min. Other procedures were similar to previously reported methods (14).

For an intraportal infusion (n = 6), 1% HPMC (1.0 ml) was instilled into the stomach and duodenum, respectively, with a 23-gauge needle, and DA-7867 (suspended in 1% HPMC) at a dose of 10 mg/kg was infused (1.0 ml) over 15 min via the pyloric vein with the assistance of an infusion pump (model 2400-006, Harvard Instrument, South Natick, Mass.). For intraduodenal instillation (n = 5), 1% HPMC was instilled into the stomach and infused over 15 min via the pyloric vein, and DA-7867 was instilled into the duodenum. For intragastric instillation, (n = 7), 1% HPMC was infused over 15 min via the pyloric vein and instilled into the duodenum, and DA-7867 was instilled into the stomach. Other procedures were similar to previously reported methods (14).

The AUC₀₋∞ was calculated by the trapezoidal rule-extrapolation method; this method employed the logarithmic trapezoidal rule for the calculation of the area during the declining plasma drug-level phase (2) and the linear trapezoidal rule for the rising plasma drug-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma drug concentration by the terminal rate constant. Standard methods (10) were used to calculate the following pharmacokinetic parameters: the time-averaged total body clearance (CL), area under the first moment of plasma drug concentration-time curve (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (Vss), and time-averaged renal (CLR) and nonrenal (CNR) clearances (17). The extent of absolute oral bioavailability (F) was measured by dividing the AUC₀₋∞ values after various oral administrations by AUC₀₋∞ after an intravenous administration of DA-7867 at a dose of 1 mg/kg. The mean values of Vss (3), terminal half-life (t₁/₂) (7), and CL, CLR, and CNR (4) were calculated by the harmonic mean method.

Levels of statistical significance were assessed by the Duncan’s multiple range test of the Statistical Package for Social Sciences (SPSS) posteriori analysis of variance among the three or four means for unpaired data. Significant differences were judged as a P value of <0.05. All results are expressed as means ± standard deviations.

The mean arterial plasma drug concentration-time profiles of DA-7867 after an intravenous administration of various doses to rats are shown in Fig. 1A, and some relevant pharmacokinetic parameters are listed in Table 1. After an intravenous administration to rats of doses of 1, 5, 10, and 20 mg/kg, the concentrations of DA-7867 in plasma declined in a poly-exponential fashion for all four doses studied (Fig. 1A). Note that the dose-normalized (based on 1 mg/kg) AUC₀₋∞ values of DA-7867 were independent of intravenous doses studied; the values were 1,110.9 ± 93.1, 1,120 ± 59.2, 1,170 ± 209, and 1,160 ± 100 μg min/ml for 1, 5, 10, and 20 mg/kg, respectively (Table 1). As expected from AUC₀₋∞ values, the CLs were also independent of intravenous doses of DA-7867 (Table 1). The terminal t₁/₂, CLR, and total amount of unchanged DA-7867
recovered from entire gastrointestinal tract at 48 h (GI48 h; expressed in terms of the percentage of dose) were also independent of intravenous doses studied (Table 1). Although the differences were not considerable (hence the biological importance of those parameters seem to be not considerable), the MRT, Vσ, CLR, and total amount of unchanged DA-7867 excreted in 48-h urine (AUC0-48 h, expressed in terms of the percentage of dose) were also independent of the oral doses: the mean value was 71.1% (Table 2). Moreover, terminal t1/2s, AUC0-48 h, and GI48 h of DA-7867 were not significantly different among four different doses studied (Table 2).

The AUC0-48 h values of DA-7867 after intraduodenal (6,010 ± 1,470 µg·min/ml) and intragastric (5,590 ± 1,360 µg·min/ml) administration at a dose of 10 mg/kg were comparable, indicating that the gastric first-pass effect of DA-7867 could be almost negligible in rats, if any. However, the AUC0-48 h of DA-7867 after an intraduodenal administration was significantly smaller (23.7% decrease) than that after an intraportal administration (7,880 ± 1,050 µg·min/ml), suggesting that the intestinal first-pass effect of DA-7867 was considerable in rats (approximately 24.0%).

Dose-independent pharmacokinetic parameters of DA-7867 were observed after intravenous administration (such as AUC0-48 h and CL values) or oral administration (such as AUC0-48 h values) at doses of 1 to 20 mg/kg (Tables 1 and 2) in rats. Hence, the dose of 10 mg/kg was arbitrarily chosen to measure the first-pass effects of DA-7867 in rats. The F was 70.8% at an oral dose of 10 mg/kg (Table 2). After oral ad-

### TABLE 1. Pharmacokinetic parameters of DA-7867 after a 1-min intravenous infusion at various doses to rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mg/kg (10)</th>
<th>5 mg/kg (10)</th>
<th>10 mg/kg (9)</th>
<th>20 mg/kg (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>278 ± 5.59</td>
<td>260 ± 6.08</td>
<td>285 ± 6.08</td>
<td>284 ± 6.69</td>
</tr>
<tr>
<td>AUC0-48 h (µg·min/ml)</td>
<td>1,110 ± 93.1</td>
<td>5,600 ± 296</td>
<td>11,700 ± 2,090</td>
<td>23,200 ± 2,000</td>
</tr>
<tr>
<td>Terminal t1/2 (min)</td>
<td>820 ± 300</td>
<td>1,070 ± 237</td>
<td>895 ± 106</td>
<td>1,040 ± 192</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>558 ± 124</td>
<td>945 ± 183</td>
<td>1,020 ± 602</td>
<td>854 ± 200</td>
</tr>
<tr>
<td>Vσ (ml/kg)</td>
<td>505 ± 134</td>
<td>820 ± 141</td>
<td>718 ± 392</td>
<td>688 ± 185</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>0.096 ± 0.0751</td>
<td>0.906 ± 0.0535</td>
<td>0.856 ± 0.132</td>
<td>0.804 ± 0.07754</td>
</tr>
<tr>
<td>GI48 h (% of dose)</td>
<td>11.0 ± 2.88</td>
<td>9.54 ± 0.792</td>
<td>6.61 ± 1.60</td>
<td>6.45 ± 0.931</td>
</tr>
<tr>
<td>GI48 h (% of dose)</td>
<td>11.0 ± 2.28</td>
<td>8.74 ± 1.61</td>
<td>9.04 ± 3.79</td>
<td>9.04 ± 1.23</td>
</tr>
</tbody>
</table>

### TABLE 2. Pharmacokinetic parameters of DA-7867 after oral administration at various doses to rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mg/kg (9)</th>
<th>5 mg/kg (10)</th>
<th>10 mg/kg (9)</th>
<th>20 mg/kg (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>271 ± 10.1</td>
<td>258 ± 24.0</td>
<td>258 ± 9.72</td>
<td>251 ± 4.15</td>
</tr>
<tr>
<td>AUC0-48 h (µg·min/ml)</td>
<td>774 ± 114</td>
<td>4,000 ± 863</td>
<td>7,860 ± 1,040</td>
<td>15,900 ± 3,080</td>
</tr>
<tr>
<td>Terminal t1/2 (min)</td>
<td>812 ± 311</td>
<td>711 ± 298</td>
<td>873 ± 145</td>
<td>896 ± 165</td>
</tr>
<tr>
<td>CL (µg/ml·h)</td>
<td>1.05 ± 0.438</td>
<td>4.24 ± 1.10</td>
<td>5.65 ± 0.518</td>
<td>11.4 ± 1.30</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>175 ± 76.9</td>
<td>354 ± 121</td>
<td>373 ± 164</td>
<td>390 ± 129</td>
</tr>
<tr>
<td>GI48 h (% of dose)</td>
<td>0.0778 ± 0.0583</td>
<td>0.0790 ± 0.0244</td>
<td>0.0615 ± 0.0331</td>
<td>0.0577 ± 0.0303</td>
</tr>
<tr>
<td>GLh (% of dose)</td>
<td>6.75 ± 2.24</td>
<td>6.83 ± 3.09</td>
<td>5.05 ± 1.39</td>
<td>4.94 ± 1.82</td>
</tr>
<tr>
<td>P (%)</td>
<td>69.7</td>
<td>72.1</td>
<td>70.8</td>
<td>71.6</td>
</tr>
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</table>

### Notes

- Values are means ± standard deviations.
- Dose-normalized (1 mg/kg) values were compared when statistical analysis was performed.
- The AUC0-48 h values were significantly different (P < 0.05) from the 5-, 10-, and 20-mg/kg results.
ministration of DA-7867 at a dose of 10 mg/kg, the percentage of oral dose of GI48 h was 13.3% (Table 2). It is possible that this unchanged DA-7867, 13.3%, might be partly attributed to the gastrointestinal (including biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics, the mean true fraction of dose unabsorbed (F unabs) in this study could be estimated by the equation (19) \(0.133 = F_{\text{unabs}} + (0.708 \times 0.0711)\), where 0.708 and 0.0711 are the F (Table 2) and mean fraction of intravenous dose of GI48 h at a dose of 10 mg/kg (Table 1), respectively. The calculated F unabs was 8.27% of the oral dose. Since approximately 8.27% of orally administered DA-7867 at a dose of 10 mg/kg was not absorbed from the gastrointestinal tract for up to 48 h, approximately 21% (100 – 70.8 – 8.27) of orally administered DA-7867 at a dose of 10 mg/kg could be eliminated by the first-pass effect.

After intravenous administration of DA-7867 at doses of 1 to 20 mg/kg to rats, the CL values (0.856 to 0.906 ml/min/kg based on plasma data; Table 1) were considerably smaller than the reported cardiac output in rats: 296 ml/min/kg based on blood data (6). This suggested that the first-pass effect of DA-7867 in the lung and heart could be almost negligible, if any, in rats. The hepatic first-pass effect of DA-7867 also seemed to be almost negligible in rats (our unpublished data); the AUC0–∞ values of DA-7867 were not significantly different between intravenous and intraportal administration, and similar results were also obtained between intravenous administration to control rats and rats pretreated with SKF 525-A, a nonspecific hepatic microsomal cytochrome P450 inhibitor.

After intragastric and intraduodenal instillation of DA-7867 at a dose of 10 mg/kg to rats, the AUC0–∞ values of DA-7867 were not significantly different, indicating that the gastric first-pass effect of DA-7867 in rats was almost negligible. However, the AUC0–∞ after an intraduodenal administration was 76.3% of that after an intraportal administration, suggesting that the intestinal first-pass effect of DA-7867 could be approximately 21.8% [(100 – 76.3) \times (1 – 0.0827)] of the oral dose. The considerable intestinal first-pass effects of furosemide (12), azosamide (14), YH439 (a new hepatoprotective agent) (15), YJA-20379-8 (a new reversible proton pump inhibitor) (16), iriflavone (18), bumetanide (11), KR-31543 (a new hepatoprotective agent for ischemia-reperfusion damage) (20), SR-4668 (a candidate for diabetic neuropathy) (13), KR-60436 (a new reversible proton pump inhibitor) (22), and oltipraz (our unpublished data) in rats and midazolam (21) and saquinavir (8) in humans have been reported.

In conclusion, after oral administration of DA-7867 at a dose of 10 mg/kg to rats, approximately 8.27% of the oral dose was not absorbed, the intestinal first-pass effect was approximately 21.8% of oral dose, and F was 70.8%.

This work was supported in part by a grant from the Korea Ministry of Health & Welfare (01-PJ1-PG4-01PT01-0005), 2001-2004.

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