Pharmacokinetics and Safety of Ascending Single Doses of DZ-2640, a New Oral Carbapenem Antibiotic, Administered to Healthy Japanese Subjects

Makoto Tanaka,1,* Kinuyo Kato,1 Hideo Hakusui,1 Yoichi Murakami,2 Kenichi SATO,2 Yasuhiro ITO,1 and Keiji Kawamoto1

Drug Metabolism and Analytical Chemistry Research Laboratory,1 New Product Research Laboratories I,2 Global Medical Planning Department,3 and Medical Development Department I,4 Daiichi Pharmaceutical Co. Ltd., Tokyo 134-8630, Japan

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DZ-2640 is the ester-type oral carbapenem prodrug of an active parent compound, DU-6681. The pharmacokinetics and safety of DU-6681 were investigated in six studies after oral administration of a single dose of DZ-2640 to healthy male Japanese volunteers at doses of 25, 50, 100, 200, and 400 mg (as the equivalents of DU-6681) in the fasted state. The same volunteers received the drug at a dose of 100 mg in the fasted and fed states to examine the effect of food intake on the bioavailability of DZ-2640. The concentrations of DU-6681 in plasma and urine were determined by a validated high-performance liquid chromatography method and a bioassay. A good correlation between both methods was seen, indicating an absence of major active metabolites. The mean maximum concentrations of DU-6681 in plasma (\(C_{\text{max}}\)) ranged from 0.263 μg/ml (25-mg dose) to 2.489 μg/ml (400-mg dose) and were reached within 1.5 h following drug administration. After reaching the \(C_{\text{max}}\), plasma DU-6681 concentrations declined in a monophasic manner, with a half-life of 0.47 to 0.89 h. The area under the concentration-time curve (AUC) and \(C_{\text{max}}\) increased almost linearly with the dose up to the 200-mg dose. The AUC and \(C_{\text{max}}\) increased less than proportionally after administration of the 400-mg dose, suggesting a reduction in drug absorption. The plasma protein binding of DU-6681 was in the range of 23.3 to 25.6%. The cumulative urinary recoveries (0 to 24 h) were in the range of 31.9 to 44.9%. The AUC was slightly but statistically significantly reduced by food intake. However, the \(C_{\text{max}}\) half-life, and recovery in urine were not affected by food intake. The renal clearance (402 to 510 ml/min) was much greater than the mean glomerular filtration rate (ca. 120 ml/min), which indicated active tubular secretion of the drug. A mild transient and moderate diarrhea was observed in two of six volunteers in the study with a single dose of 25 mg. Mild soft stools were observed in two of six volunteers who received a 400-mg dose of the drug.

MATERIALS AND METHODS

Chemicals and reagents. DU-6681, (4R,5S,6S)-3-[[6S,6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-6-yl][thio]-6-[[1(R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (DU-6681, the active parent drug). The bioavailability of DU-6681 was greatly improved by esterification of the carboxy group at the C-2 position of the carbapenem ring with POM. DZ-2640 is believed to be hydrolyzed by non-specific esterases in the intestinal tracts of humans to produce DU-6681, pivalic acid, and formaldehyde in a manner similar to that for other prodrugs (1–3, 6). DU-6681 has a high degree of in vitro activity against a broad spectrum of gram-positive and gram-negative organisms (4). The chemical structures of DZ-2640 and DU-6681 are shown in Fig. 1.

This paper describes the single-dose safety and pharmacokinetics of DU-6681 following oral administration of single ascending doses of DZ-2640 (25 to 400 mg) as part of the clinical evaluation of this drug.

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1 Corresponding author. Mailing address: Drug Metabolism and Analytical Chemistry Research Laboratory, Daiichi Pharmaceutical Co. Ltd., 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan. Phone: 81-3-3680-0151. Fax: 81-3-5696-8332. E-mail: LDP04207@nifty.ne.jp.
prior to dosing) states to examine the effect of food intake on the bioavailability of DZ-2640. One subject accidentally received placebo instead of DZ-2640 in the fed state, and data for this subject were excluded from the analysis of the effect of food intake. The washout phase between two treatments was 3 weeks. The standard breakfast consisted of two rolls (80 g), margarine (10 g), cheese (25 g), orange juice (100 ml), a medium boiled egg (50 g), and low-fat milk (150 ml). This breakfast contained 27 g of protein, 27 g of fat, and 96 g of carbohydrate.

Each subject was assessed by the principal investigator to ensure that he was fit and well prior to discharge from the clinic. The subjects returned to the clinic at approximately 7 days after dosing for poststudy assessment which included tests for blood pressure, heart rate, body temperature, electrocardiogram, and laboratory findings. Subjects were not allowed to take any medication without an investigator’s permission until poststudy assessment.

Sample collection. Blood samples of 5 ml were collected in heparinized containers at predosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after oral administration of DZ-2640. The blood samples were immediately centrifuged at 2,000 × g for 10 min to separate the plasma. The separated plasma (2.0 ml) was transferred to a polypropylene tube and was diluted with 1 M MOPS buffer (pH 7.0; 2.0 ml) to stabilize the DU-6681. After dilution, the plasma sample was immediately frozen in a dry ice-ethanol bath and was stored at −80°C until analysis.

Urine samples were collected from 12 h to 0, to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, and 12 to 24 h after drug administration. The urine samples were kept at 2 to 4°C during the sampling interval. The total weight of each sampling interval was recorded, and the total volume was calculated by assuming that the specific gravity of urine was 1.0. The urine sample (20 ml) was transferred to a polypropylene tube and was diluted with 1 M MOPS buffer (pH 7.0; 2.0 ml) to stabilize the DU-6681. After dilution, the urine sample was immediately frozen in a dry ice-ethanol bath and was stored at −80°C until analysis.

Plasma protein binding. The plasma protein binding of DU-6681 was determined ex vivo by an ultrafiltration method (Centrifree; Grace Japan, Tokyo, Japan). The binding study was conducted with plasma samples obtained from the volunteer at time points (2, 4, and 8 h) in the 200-mg dose study. The concentration of unbound drug in the ultrafiltrate was measured. DU-6681 did not show significant adsorption to the membrane. The unbound fraction (FU) in plasma was calculated as the concentration in the filtrate divided by the original concentration in plasma. The plasma protein binding rate was calculated as (1 – FU) × 100.

Analytical methods. All drug assays were performed by Daiichi Pharmaceuticals Co. Ltd. in the Drug Metabolism and Analytical Chemistry Research Laboratory, Tokyo, Japan. The concentrations of DU-6681 in plasma and urine were determined with the use of a modification of a column-switching semi-micrcolumn HPLC method as previously reported by Tanaka and Kato (5).

Briefly, human plasma diluted with an equal volume of 1 M MOPS buffer (pH 7.0) was filtered through an Ultrafree-MC (10,000 MWLI; Millipore, Bedford, Mass.) by centrifugation at 5,000 × g for 90 min at 4°C. The resulting filtrate was injected without further cleanup onto the HPLC system. Precolumn packed with Inertsil ODS-3 (35 by 1.5 mm [inner diameter]; GL Science, Tokyo, Japan) was used to remove interfering endogenous substances in plasma. Following online solid-phase sample cleanup with a column-switching device, the analyte was chromatographed on a reversed-phase analytical column, Inertsil ODS-3 (250 by 1.5 mm [inner diameter]; GL Science), with a mixture of 5 mM phosphate buffer (pH 6.5) containing 1 mM tetrabutylammonium bromide and acetonitrile (80:20, vol/vol) as the analytical mobile phase at a flow rate of 0.1 ml/min.

Human urine diluted with an equal volume of 1 M MOPS buffer (pH 7.0) was directly injected onto the HPLC system. Precolumns packed with LiChrosorb NH2 (10 by 4.0 mm [inner diameter]; GL Science) were used for sample pretreatment. A short intermediate column, Inertsil ODS-3 (35 by 1.5 mm [inner diameter]; GL Science), was placed between the precolumn and the analytical column to minimize the loss of separation efficiency. Following online solid-phase sample cleanup with a column-switching device, DU-6681 was chromatographed on a reversed-phase analytical column, Inertsil ODS-3 (150 by 1.5 mm [inner diameter]; GL Science), with a mixture of 100 mM phosphate buffer (pH 6.5) and acetonitrile (94:6; vol/vol) as the analytical mobile phase at a flow rate of 0.1 ml/min.

DU-6681 was detected by monitoring the column effluent with UV light at a wavelength of 300 nm, which resulted in the limit of quantitation of 0.016 μg/ml of plasma and 0.21 μg/ml of urine. Calibration curves were linear in the range of 0.016 to 7.64 μg/ml in plasma and 0.21 to 101 μg/ml in urine. The intra- and interday accuracy and precision of the assay for DU-6681 in plasma were <10% and <15%, respectively. At the quantitation limit of 0.016 μg/ml, the method showed an acceptable precision and accuracy (<16%). The intra- and interday accuracy and precision of the assay for DU-6681 in urine were <15% at concentrations above 0.21 μg/ml. At the quantitation limit of 0.23 μg/ml, the method showed an acceptable precision and accuracy (<9%). The validity of the concentration results was verified by assaying quality control samples produced from blank plasma and urine spiked with known concentrations of DU-6681.

Additionally, concentrations in plasma and urine were determined by the thin-layer paper disk method with Bacillus subtilis ATCC 6633 as the test strain. The limit of quantification was 0.10 μg/ml. To assess the comparability of the bioassay and the HPLC method and to detect the activities of potential microbially active metabolites relevant in humans, the concentrations determined by both methods were correlated.

Pharmacokinetic and statistical analyses. The software used for the pharmacokinetic analysis was TopFit (7), which was run on an International Business Machines compatible personal computer. The pharmacokinetic analysis was determined by a model-independent method. The elimination rate constant (κ) was determined by least squares regression of the logarithm of the concentration in plasma with time over the terminal phase. The half-life (t1/2) was calculated as 0.693/κ. The maximum concentration in plasma (Cmax) before reaching Cmax (Tmax) were read from the observed values. The area under the concentration-time curve (AUC) was determined to the last quantifiable concentration in plasma by using the linear trapezoidal rule and was extrapolated to infinity by using the terminal-phase rate constant. The mean residence time (MRT) was calculated as the ratio of the area under the first moment curve from time zero to infinity (AUMC0–∞) to the AUC from 0 to infinity (AUC0–∞).

The cumulative excretion of DU-6681 over 24 h (Xc) was calculated in each subject and was expressed as a percentage of the dose given. The renal clearance (CLR) was calculated by using the equation

CLR = D/AUC,

where D is the dose and AUC is the area under the concentration-time curve.

For statistical comparison of the pharmacokinetic parameters obtained in the fasted state and those obtained in the fed state, the Wilcoxon signed rank test was used, with a P value of 0.05 given as the minimal level of significance. To evaluate the correlation between the doses ranging from 25 to 400 mg and the resulting AUC and Cmax, linear regression analysis was performed. All data are expressed as means ± standard deviations (SDs).

RESULTS

Safety. DZ-2640 given as a single oral dose ranging from 25 to 400 mg in the fasted state and 100 mg after a meal was safe and well tolerated. All 45 subjects completed the whole study, and there was no clinically significant changes in findings from physical examinations, vital signs, clinical laboratory findings, or electrocardiograms.

In the study with the 25-mg dose, one case of mild transient diarrhea and one case of moderate diarrhea were observed in two of six subjects who received DZ-2640. At the 400-mg dose, two of six subjects experienced mild soft stools. All other adverse events were judged to be not related to the DZ-2640 treatment.

Single-dose pharmacokinetics. The mean concentrations of DU-6681 from the plasma versus-time profiles obtained after oral administration of a single dose of DZ-2640 (25, 50, 100, 200, and 400 mg as DU-6681) to fasted male Japanese volunteers are shown in Fig. 2. The results of the noncompartmental pharmacokinetic analysis derived from the concentrations in plasma and urine are summarized in Table 1. The absorption of DZ-2640 from the empty gastrointestinal tract was rapid, and Cmax values of 0.263, 0.679, 0.999, 2.025, and 2.489 μg/ml of plasma appeared 1.00 to 1.42 h after oral administration. After Cmax was reached, the plasma drug level decreased monophasically, with elimination t1/2S of 0.47 to 0.89 h. The
intake had no significant influence (P > 0.05; n = 5) on Cmax, t1/2, and cumulative recovery in urine (0 to 24 h). There was a significant (P < 0.05) reduction in the AUC for fed subjects (1.56 ± 0.473 μg·h/ml) compared to that for fasted subjects (1.713 ± 0.434 μg·h/ml) (Table 2).

To investigate the effect of food on the pharmacokinetics of DU-6681 after intake of a light meal, DZ-2640 (100 mg) was given to the six volunteers who received the same dose in the fasted state. The concentration in plasma-time profiles of DU-6681 was very fast and was almost completed up to 4 h after oral dosing. The cumulative urinary excretion of DU-6681 (collection period, 0 to 24 h) amounted to 34.9% ± 13.2%, 44.9% ± 7.5%, 37.5% ± 6.8%, 38.9% ± 9.5%, and 31.9% ± 5.1% of the dose after oral administration of single doses of 25, 50, 100, 200, and 400 mg, respectively (Fig. 4). ClR ranged from 402 to 510 ml/min and remained almost constant as the dose increased.

The urinary excretion of DU-6681 was very fast and was delayed, resulting in a prolonged Tmax. The cumulative urinary excretion of DU-6681 tended to be lower after administration of the 400-mg dose. This suggests that a reduction in the drug absorption rate and extent occurred with the 400-mg dose.

In the present study DZ-2640 was safe and well tolerated when it was administered as single oral doses ranging from 25 to 400 mg. No clinically relevant changes in vital signs, electrocardiograms, findings from physical examinations, or laboratory study values were seen. Only a few adverse events were reported, and all were mild in intensity; moderate diarrhea, however, occurred after the administration of the lowest dose (25 mg).

DZ-2640 as the capsule formulation was found to be rapidly absorbed from the empty gastrointestinal tract, as indicated by the Tmax, which were achieved 1.00 to 1.42 h after oral administration (Fig. 2). AUC and Cmax increased almost linearly over the dose range of 25 to 200 mg (Fig. 3). The AUC and Cmax increased less than proportionally after administration of the 400-mg dose, suggesting a reduction in the level of drug absorption. The urinary excretion of DU-6681 was bound to plasma proteins. Thus, the ClR results were 402 to 510 ml/min, and remained almost constant as the dose increased.

DISCUSSION

In the present study DZ-2640 was safe and well tolerated when it was administered as single oral doses ranging from 25 to 400 mg. No clinically relevant changes in vital signs, electrocardiograms, findings from physical examinations, or laboratory study values were seen. Only a few adverse events were reported, and all were mild in intensity; moderate diarrhea, however, occurred after the administration of the lowest dose (25 mg).

DZ-2640 as the capsule formulation was found to be rapidly absorbed from the empty gastrointestinal tract, as indicated by the Tmax, which were achieved 1.00 to 1.42 h after oral administration (Fig. 2). AUC and Cmax increased almost linearly over the dose range of 25 to 200 mg (Fig. 3). The AUC and Cmax increased less than proportionally after administration of the 400-mg dose, suggesting a reduction in the level of drug absorption.

The correlation of 0.979 and 0.996, respectively. These data indicate an absence of major active metabolites.

![Fig. 2. Mean concentration in plasma-time profiles for DU-6681 in fasted healthy male volunteers following administration of single ascending doses of DZ-2640 ranging from 25 to 400 mg (HPLC data). Error bars indicate SDs (n = 6).](http://aac.asm.org/)

### TABLE 1. Pharmacokinetic parameters of DU-6681 after oral administration of single doses of DZ-2640 to fasted healthy male volunteers

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Cmax (μg/ml)</th>
<th>Tmax (h)</th>
<th>t1/2 (h)</th>
<th>AUCC (μg·h/ml)</th>
<th>MRT (h)</th>
<th>CL/F (ml/min)</th>
<th>Xmax (%</th>
<th>Cmax (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.263 ± 0.132</td>
<td>1.00 ± 0.27</td>
<td>0.47 ± 0.11</td>
<td>0.302 ± 0.120</td>
<td>1.43 ± 0.38</td>
<td>1,604 ± 729</td>
<td>34.9 ± 13.2</td>
<td>481 ± 110</td>
</tr>
<tr>
<td>50</td>
<td>0.679 ± 0.236</td>
<td>1.08 ± 0.20</td>
<td>0.59 ± 0.17</td>
<td>0.779 ± 0.233</td>
<td>1.61 ± 0.26</td>
<td>1,168 ± 409</td>
<td>44.9 ± 7.3</td>
<td>510 ± 140</td>
</tr>
<tr>
<td>100</td>
<td>0.999 ± 0.375</td>
<td>1.38 ± 0.31</td>
<td>0.69 ± 0.19</td>
<td>1.534 ± 0.430</td>
<td>1.83 ± 0.33</td>
<td>1,145 ± 238</td>
<td>37.5 ± 6.8</td>
<td>450 ± 81</td>
</tr>
<tr>
<td>200</td>
<td>2.023 ± 0.622</td>
<td>1.13 ± 0.44</td>
<td>0.89 ± 0.35</td>
<td>3.218 ± 0.697</td>
<td>1.64 ± 0.24</td>
<td>1,077 ± 229</td>
<td>38.9 ± 9.5</td>
<td>402 ± 68</td>
</tr>
<tr>
<td>400</td>
<td>2.489 ± 0.602</td>
<td>1.42 ± 0.38</td>
<td>0.71 ± 0.17</td>
<td>5.093 ± 1.156</td>
<td>1.93 ± 0.18</td>
<td>1,579 ± 378</td>
<td>31.9 ± 5.1</td>
<td>427 ± 64</td>
</tr>
</tbody>
</table>

* Data represent means ± SDs (n = 6).
of free DU-6681 was much greater than the mean glomerular filtration rate (ca. 120 ml/min). These data indicate that active processes take place in the urinary excretion of DU-6681. Non-renal clearance accounted for about two-thirds of the CL/F. The quantitative contribution of biliary excretion and/or metabolic reactions to this process has not yet been fully investigated.

The effect of food intake on the pharmacokinetics of DU-6681 was investigated by comparing the pharmacokinetic parameters obtained after administration of the single 100-mg dose in the fasted and fed states (Fig. 5). Food intake slightly but statistically significantly decreased the AUC; however, there was no significant difference in the cumulative urinary excretion of DU-6681 over 24 h. Other pharmacokinetic parameters showed no statistically significant difference between the fasted and the fed states (Table 2). The influence of food on the pharmacokinetics of DU-6681 was not considered clinically significant.

In this study the bioassay and HPLC data showed an excellent correlation, indicating that no significant concentrations of metabolites with noticeable antimicrobial activity seem to be present in plasma and urine over the time interval after single-dose administration investigated. Thus, it was proven that the concentrations measured by bioassay and HPLC are adequate for assessment of the pharmacokinetics of DU-6681 in humans with respect to pharmacological (antimicrobial) activity. However, HPLC was superior because of its higher sensitivity and better selectivity with respect to metabolites.

In conclusion, DZ-2640 was well tolerated after the administration of single oral doses up to 400 mg. DZ-2640 was rapidly absorbed from a capsule formulation and was hydrolyzed rapidly to the active parent drug, DU-6681. DU-6681 was
rapidly eliminated from the body, with elimination $t_{1/2}$s of 0.47 to 0.89 h.

REFERENCES

### TABLE 2. Effect of food on pharmacokinetics of DU-6681 after oral administration of single 100-mg doses of DZ-2640 to healthy male volunteers

<table>
<thead>
<tr>
<th>State</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$t_{1/2}$ (h)</th>
<th>$\text{AUC}_{0-24}$ (µg·h/ml)</th>
<th>$X_{\text{ne-24}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>1.094 ± 0.331</td>
<td>1.05 ± 0.27</td>
<td>0.72 ± 0.24</td>
<td>1.713 ± 0.434</td>
<td>38.4 ± 7.2</td>
</tr>
<tr>
<td>Fed</td>
<td>0.928 ± 0.252</td>
<td>1.50 ± 0.00</td>
<td>0.73 ± 0.18</td>
<td>1.565 ± 0.473</td>
<td>40.5 ± 3.2</td>
</tr>
</tbody>
</table>

$P$ value NS$^b$

$^a$ Data represent means ± SDs (n = 5).

$^b$ NS, not significant.