Stability of New Carbapenem DA-1131 to Renal Dipeptidase (Dehydropeptidase I)

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The stability of DA-1131 to renal dipeptidase (RDPase) (EC 3.4.13.19) was compared with that of imipenem and meropenem by $V_{\text{max}}/K_m$ ratios as an index of the enzyme’s preference for substrates. Our results showed a decreasing order of imipenem (6.24), meropenem (2.41), and DA-1131 (1.39). The biochemical evaluation of DA-1131 as the least preferred substrate of RDPase suggests its potential use as a novel $\beta$-lactam antibiotic which may be usable without coadministration of RDPase inhibitors once its clinical suitability is proven.

Imipenem (N-formimidoylthienamycin), developed by Merck Sharp & Dohme, West Point, Pa., was highly effective against bacterial species resistant to most $\beta$-lactam antibiotics with unusually high potency against gram-positive as well as gram-negative bacteria (8, 21, 23). It is degraded, though, in the kidneys of various animals, resulting in a reduced antibacterial activity. The enzyme responsible for this metabolism was shown to be renal dipeptidase (RDPase, also called renal dehydropeptidase I) (EC 3.4.13.19) located in the brush-border membrane of renal proximal tubules (8, 9, 20). Cilastatin, Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butoenic acid, is a specific competitive inhibitor of RDPase and is well matched in its pharmacokinetic properties for coadministration with imipenem (8, 22). Therefore, imipenem is designed to be coadministered with cilastatin to suppress RDPase for its clinical use, and they are now manufactured in a 1:1 combination.

Meropenem, (−)-(4R,5S,6S)-3-[(3S,5S)-5-(dimethyl-carbamoyl)-3-pyrrolidinylthio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid, which was introduced in the late 1980s, though relatively less active than imipenem (1, 4, 6, 7), is unusual for its unusually high potency against gram-positive as well as gram-negative bacteria (10, 12, 13, 16, 17, 18, 19), in the renal excretion mechanism (14, 15), and in nephrotoxicity-related studies (11).

We measured the kinetic parameters of imipenem, meropenem, and DA-1131 in relation to human RDPase and the RDPases of various animals to examine the stability of DA-1131 in reference to the two well-established $\beta$-lactam antibiotics, imipenem and meropenem.

DA-1131 and meropenem were supplied by Dong-A Research Laboratory. Imipenem and cilastatin were obtained from Merck Sharp & Dohme. Human RDPase was purified to homogeneity with a 2,029-fold purification, and RDPases from animal sources were purified or partially purified according to the method previously described (24).

RDPase-catalyzed hydrolysis of imipenem, meropenem, and DA-1131 was measured in the presence and absence of cilastatin (0.15 $\mu$M) according to the method described by Kim and Campbell (9) by measuring the decrease in absorbance at 298 nm and 37°C as a function of time for 2.5 min. Substrate concentration was varied over a range of 1.25 to 3.3 mM in 3-(N-morpholino)propanesulfonic acid, pH 7.1. One microgram of purified RDPase was employed in a 250-μl reaction mixture, and 2-mm light path quartz cuvettes were used. The initial velocities are expressed as enzyme units (U) (micro- moles of substrate hydrolyzed per minute) per milligram of protein with molar extinction coefficients at 298 nm for DA-1131, imipenem, and meropenem of 9.7314 × 10⁻³, 9.3222 × 10⁻³, and 1.0167 × 10⁻² M⁻¹ cm⁻¹, respectively. Protein concentrations were determined according to the Bradford method (2) with bovine serum albumin as the standard protein.

The Lineweaver-Burk plots of imipenem (Fig. 1A), meropenem (Fig. 1B), and DA-1131 (Fig. 1C) identified cilastatin as

**Table 1.** Kinetic parameters of imipenem, meropenem, and DA-1131 in human RDPase

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_m$ (mM)$^a$</th>
<th>$V_{\text{max}}$ (U/mg)$^a$</th>
<th>$V_{\text{max}}/K_m$</th>
<th>$K_v$ (μM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>2.78 ± 0.21</td>
<td>17.35 ± 2.70</td>
<td>6.24</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Meropenem</td>
<td>3.56 ± 0.15</td>
<td>8.58 ± 0.21</td>
<td>2.41</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>DA-1131</td>
<td>5.55 ± 0.19</td>
<td>7.72 ± 0.19</td>
<td>1.39</td>
<td>0.35 ± 0.01</td>
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</table>

$^a$ Values are means ± standard deviations.

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a competitive inhibitor of RDPase-catalyzed hydrolysis in all plots shown by the crossings at the $y$ axes.

The kinetic parameters and $V_{\text{max}}/K_m$ ratios are summarized in Table 1. The $V_{\text{max}}/K_m$ ratio for each substrate has been used as an index of the enzyme’s preference for substrates (3, 5). The $V_{\text{max}}/K_m$ ratio decreased in the order of imipenem (6.24), meropenem (2.41), and DA-1131 (1.39), thus identifying DA-1131 as the least preferred substrate among the three, i.e., the most stable compound against RDPase. The relative percent ratio for DA-1131 was only 22.3% for imipenem and 57.7% for meropenem. The $K_i$ values are 0.07 $\mu$M and 0.21 $\mu$M for imipenem and meropenem, and 0.35 $\mu$M for DA-1131, respectively. Comparable $K_i$ values in such a close range indicate that RDPase acts upon these carbapenems within the same catalytic site (9).

The kinetic parameters of these carbapenems in relation to the RDPases of various animals were also determined, and the $V_{\text{max}}/K_m$ ratios are summarized in Table 2. The $V_{\text{max}}/K_m$ ratio for imipenem was lower than that for meropenem in mouse and rabbit RDPase but higher in rat, dog, and porcine RDPase. Thus, imipenem and meropenem exhibited large variations in resistance to RDPase depending on the enzyme source but the $V_{\text{max}}/K_m$ ratio for DA-1131 was the lowest in all the tested animals. The $V_{\text{max}}/K_m$ ratios for meropenem (0.95) and DA-1131 (0.74) in the porcine enzyme are significantly lower than for imipenem (19.75), thus suggesting far more stability in the presence of RDPase than imipenem. The relative ratios of DA-1131 to imipenem or meropenem were less than 1.0 in all the tested animals, thus showing higher resistance of DA-1131 to RDPase than to either antibiotic. Judging from the $V_{\text{max}}/K_m$ ratio, the susceptibility of carbapenems to RDPase varied depending on the species (Tables 1 and 2). With imipenem, the $V_{\text{max}}/K_m$ ratio for human RDPase (6.24) was the lowest while that of dog RDPase (39.02) was the highest. In the case of meropenem, the porcine RDPase (0.95) exhibited the lowest ratio and the rabbit RDPase (23.38) exhibited the highest, although the ratio for mouse RDPase (21.14) was similarly high. However, DA-1131 demonstrated the lowest ratio with porcine RDPase (0.74) and the highest ratio with dog RDPase (12.56), which was still significantly lower than that of either imipenem (39.02) or meropenem (16.46).

Meropenem has been going through worldwide clinical trials since 1989, and it is now on the market as an effective $\beta$-lactam.

![FIG. 1. Lineweaver-Burk plots of imipenem (A), meropenem (B), and DA-1131 (C). $[I]$ represents the inhibitor concentration employed. Each data point represents the average of triplicate assays (mean ± standard deviation), and the lines were drawn using the least-squares fit method.](image_url)

<table>
<thead>
<tr>
<th>Animal</th>
<th>$V_{\text{max}}/K_m$ ratio for $a$:</th>
<th>Ratio of $V_{\text{max}}/K_m$ for DA-1131 to that for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imipenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td>Mouse</td>
<td>13.67 (6.29/0.46)</td>
<td>21.14 (17.97/0.85)</td>
</tr>
<tr>
<td>Rat</td>
<td>22.48 (64.06/2.85)</td>
<td>13.50 (14.71/0.89)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>8.15 (4.89/0.60)</td>
<td>23.38 (25.48/1.09)</td>
</tr>
<tr>
<td>Dog</td>
<td>39.02 (79.21/2.03)</td>
<td>16.46 (6.75/0.41)</td>
</tr>
<tr>
<td>Pig</td>
<td>19.75 (12.05/0.61)</td>
<td>0.95 (1.99/0.88)</td>
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$^a$ Values in parentheses are $V_{\text{max}}/K_m$. 
antibiotic. One of the most prominent fortes of meropenem is in its relative stability to human RDPane; thus, it can be used alone, without coadministration of any RDPane inhibitor. DA-1131 is even more stable against RDPane than meropenem in its relative stability to human RDPane; thus, it can be used as a new antibiotic. One of the most prominent fortes of meropenem is now being evaluated in preclinical studies.

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REFERENCES