Structure-activity Relationships for Interactions between Carbapenems and \(\beta\)-Lactamases

Sir:

Carbapenems are one of the novel class of \(\beta\)-lactams recently developed. They have excellent antibacterial activity and a wide spectrum against both Gram-positive and Gram-negative bacteria\(^1\)-\(^4\). This high activity is due to good diffusion through the outer membrane in Gram-negative organisms\(^5\), high affinities for penicillin-binding proteins (PBPs)\(^6\)-\(^7\) and high stability and inhibitory activity against \(\beta\)-lactamases\(^8\). However, carbapenems were hydrolyzed by dehydropeptidase-I (DHP-I) from several animals. In previous paper, we revealed that \(\beta\)-methyl moiety on meropenem had the important role of prevention from hydrolysis by DHP-I\(^9\), furthermore, that \(\beta\)-methyl group on carbapenems affected the activity against \(\beta\)-lactamases\(^10\). Since, we have interested that the role of \(\beta\)-methyl moiety on several biological activities of carbapenems.

In the present study, therefore, we examined the interactions between carbapenems and various \(\beta\)-lactamases concerning structure-activity relationships, especially \(\beta\)-methyl moiety.

Carbapenem compounds, shown in Fig. 1, were prepared in Sumitomo Pharmaceuticals Research Center, Osaka, Japan, according to the reported procedures\(^11\)-\(^13\). \(\beta\)-Lactamase-producing bacterial strains were reference organisms stored in our laboratory\(^8\). Several \(\beta\)-lactamases were purified as described previously\(^14\)-\(^17\), with some modifications. We select four types of representative \(\beta\)-lactamases in terms of substrate specificities, which were TEM-1 penicillinase, cephalosporinases from Enterobacter cloaca (CSase) and from Proteus vulgaris with hydrolyzing activity against oxyimino-cephalosporins (CXase) and carbapenem-hydrolyzing enzyme \(\beta\)-lactamase from Xanthomonas maltophilia, according to the classification of Mitsushashi\(^18\). \(\beta\)-Lactamase activity was determined in 50 mm phosphate buffer (pH 7.0) using a spectrophotometer (UV-2100: Shimadzu Corporation, Japan) controlled at 30°C\(^19\). The \(K_m\) and \(V_{max}\) values of enzymes were determined from a Lineweaver-Burk plot. The \(K_i\) values were determined from hydrolytic rates at various concentrations of the substrate, PADAC (7-(thienyl-2-acetamide)-3-[2-(4-N,N-diethyl-aminophenylazo)-pyridinium methyl]-3-cephem-4-carboxylic acid: Hoechst AG, FRG), a chromogenic cephalosporin, using a Dixon plot. One unit enzyme activity was defined as the amount of enzyme which hydrolyzed 1 \(\mu\)mol of a substrate per minute at 30°C. These determinations were performed in duplicate.

As shown in Table 1, three types of carbapenems had good inhibitory activity, and with or without \(\beta\)-methyl moiety they showed resembled profiles each other against TEM-1, CSase and CXase. It is suggested that introduction of \(\beta\)-methyl group into carbapenem skeleton did not affect drastic changes in interactions between carbapenems and these \(\beta\)-lactamases. In addition, the introduction of benzoyl group into C-6 hydroxyethyl side chain on compound 2a (meropenem) showed a little effect on the inhibitory activity of compound against these \(\beta\)-lactamases. Of the interest of these compounds, compound 4a was hydrolyzed by TEM-1 \(\beta\)-lactamase, whereas 2a was not. Conversely, no hydrolysis of 4a by CSase was observed, as was 2a.

All carbapenems tested in this study were hydrolyzed by L-1 \(\beta\)-lactamase from X. maltophilia except compound 4a. \(\beta\)-Methyl moiety affected the affinity of these carbapenems for this enzyme. Compounds 2a and 2b having \(\beta\)-methyl group had higher affinity for L-1 enzyme than corresponding desmethyl compounds, whereas 2c showed opposite effect compared with 1c. Therefore, the effect of \(\beta\)-methyl moiety varied in C-2 side chains. Considering from \(V_{max}/K_m\) ratio, carbapenems in three series showed similar properties of hydrolysis by this enzyme whether compounds had \(\beta\)-methyl group or not. Moreover, it is also interested that no hydrolysis of 4a by this enzyme was observed in this experimental condition. With the result of interactions between 4a and TEM-1, it was conceivable that

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*Fig. 1. Chemical structures of carbapenems used in this study.*
Table 1. Kinetic parameters of carbapenem compounds for β-lactamases.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1β-methyl</th>
<th>Ki (μm)</th>
<th>L-1 β-lactamaseb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TEM-1</td>
<td>CSase</td>
</tr>
<tr>
<td>1a</td>
<td>+</td>
<td>48</td>
<td>4.6</td>
</tr>
<tr>
<td>2a</td>
<td>−</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>1b</td>
<td>+</td>
<td>53</td>
<td>8.5</td>
</tr>
<tr>
<td>2b</td>
<td>−</td>
<td>34</td>
<td>5.8</td>
</tr>
<tr>
<td>1c</td>
<td>+</td>
<td>8.6</td>
<td>1.5</td>
</tr>
<tr>
<td>2c</td>
<td>−</td>
<td>21</td>
<td>2.3</td>
</tr>
<tr>
<td>3a</td>
<td>+</td>
<td>48</td>
<td>62</td>
</tr>
<tr>
<td>4a</td>
<td>+</td>
<td>9.4d</td>
<td>15</td>
</tr>
</tbody>
</table>

a TEM-1, CSase and CXase were from E. coli harboring TEM-1 plasmid, E. cloacae and P. vulgaris, respectively.
b L-1 enzyme was from X. maltophilia. Km and Vmax are expressed as millimolar and micromoles per minute per enzyme unit, respectively.
c Not determined.
d Km value.
e Ki value.

hydroxyethyl moiety on C-6 position involved in the interactions between carbapenems and some type of β-lactamases. It was previously reported that cis-carbapenems was easily hydrolyzed by β-lactamase20 and that β-lactamase resistance owes the trans-configuration of C-6 side chain21. Our result had good correspondence to the suggestion in case of TEM-1 β-lactamase. However, 6-nor compound (4a) was not hydrolyzed by CSase and L-1 β-lactamase. These results indicated that trans-hydroxyethyl group on C-6 had nothing to do with the inhibition against CSase. On the contrary, this moiety may closely interact with L-1 enzyme. It is necessary to compare the interactions of cis-, trans- and nor-carbapenems with same side chain and these β-lactams. Further studies are under planning.

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