Interaction of Cefpirome and a Cephalosporinase from Citrobacter freundii GN7391

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Cefpirome (HR810) is a derivative of aminothiazolyl oxygenocephalosporin and has a broad antimicrobial spectrum (1, 5, 7). Its activity against cephalosporinase-producing bacteria, i.e., Enterobacter cloacae and Citrobacter freundii, is less affected by the production of the enzymes than those of cefotaxime and cefazidime (2, 6, 10). This high activity has been explained mainly by the low affinity of the enzymes for the agent (6, 10). In our studies on bacterial resistance to newer cephalosporins by cephalosporinases, we found that the hydrolytic rate at a low drug concentration is an important factor that determines the resistance level and that a low-affinity agent, cefepime (BMY-28142), is highly stable when exposed to the enzymes at low concentration (3, 4). To confirm whether this is also true of cefpirome, we studied the interaction of cefpirome and a cephalosporinase from C. freundii GN7391 in comparison with those of cefotaxime, cefotiam, and cefazidime.

Antibiotics were obtained from Hoechst Japan (cefpirome and cefotaxime), Shinnihon Jitsugyo (cefazidime), Takeda (cefotiam), and Shionogi (cephalothin). C. freundii GN7391 produces high levels of cephalosporinase (9), and the MICs of the cephalosporins were determined by an agar dilution method with an inoculum of 10⁴ CFU per spot to be 6.25 μg/ml for cefpirome and 100, >100, >100, and >400 μg/ml for cefotaxime, cefazidime, cefotiam, and cephalethin, respectively. A cephalosporinase of C. freundii GN7391 was purified as described previously (9) and had more than 95% purity. Hydrolysis of the cephalosporins was determined by a spectrophotometric method at 30°C in 50 mM phosphate buffer (pH 7.0) as described previously (8). Although cefpirome, cefotaxime, and cefazidime are poor substrates for the cephalosporinases, hydrolysis of these cephalosporins could be observed (Fig. 1); the kinetic parameters are shown in Table 1.

Cefpirome was hydrolyzed more rapidly at a higher substrate concentration and had a much greater V_{max} than did cefotaxime and cefazidime. Its V_{max} was about 5-fold greater than that of cefazidime and 20-fold greater than that of cefotaxime. The K_{m} value of cefpirome was 10-fold greater than that of cefazidime and 60-fold greater than that of cefotiam. This basal affinity of the enzyme for cefpirome indicates that the low affinity of the enzyme cefprome. Cefazidime showed biphasic hydrolysis: a short period of rapid hydrolysis, followed within 1 min by slow hydrolysis (Fig. 1). The later phase corresponds to the deacylation of its acyl enzyme intermediate reported previously (3), and the former phase may be the reaction before attainment of equilibrium. This biphasic hydrolysis was not only characteristic of cefazidime but was found with cefotaxime at a 1 to 3 μM concentration of the substance within a very short period (<0.3 min).

Inhibition of the cephalosporinase by these cephalosporins was also studied spectrophotometrically with cephalothin as the substrate. The inhibitory constants (K) of the cephalosporins were determined with and without preincubation at 30°C for 5 min (Table 2). Cefpirome did not show...
TABLE 1. Hydrolytic parameters of cephalosporins for a cephalosporinase from C. freundii GN7391a

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (μmol/min per mg of protein)</th>
<th>Hydrolytic rate at 0.1 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalothin</td>
<td>16</td>
<td>104</td>
<td>100</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>140</td>
<td>0.95</td>
<td>0.11</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&lt;25</td>
<td>0.0045</td>
<td>0.65</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>12d</td>
<td>0.17d</td>
<td>0.21</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>—c</td>
<td>0.0030c</td>
<td>—</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>2.3</td>
<td>6.7</td>
<td>44</td>
</tr>
</tbody>
</table>

a The concentrations of the enzyme in the reaction mixtures were 0.83 to 3.2 U/ml for cefpirome, ceftazidime, and cefotaxime, 0.016 U/ml for cefotaxim, and 0.0033 U/ml for cephalothin. See the legend to Fig. 1 for the definition of a unit of enzyme activity.

b Relative to cephalothin hydrolysis (as 100).

c —, Could not be obtained because of far smaller values than the substrate concentration used.

d Obtained from an early phase of hydrolysis, within 1 min.

e Obtained from a later phase of hydrolysis, after 3 min of incubation.

The hydrolytic rates of the cephalosporins at 0.1 μM were calculated from the $V_{max}$ and $K_m$ (or $K_i$ for cefotaxime) by use of the Michaelis-Menten equation (Table 1). At a low

substrate concentration, cefotaxime was hydrolyzed more rapidly than cefpirome. The rate of hydrolysis of cefotaxime at 0.1 μM was sixfold greater than that of cefpirome. Although the hydrolytic rate of ceftazidime at 0.1 μM was only twofold greater than that of cefpirome, the low permeability of ceftazidime may contribute to the reduction of bacterial susceptibility by hydrolysis, as discussed previously (3). Cefotam, which also inhibits cephalosporinases and has low activity against producers of high cephalosporinase levels, was hydrolyzed much more rapidly than the other three cephalosporins at 0.1 μM.

Thus, the hydrolytic rate of cefpirome at a low concentration was lower than those of cefotaxime and ceftazidime, owing to the low affinity of the cephalosporinase for cefpirome. The difference in the hydrolytic rates at a low concentration could explain the difference in antimicrobial activities against cephalosporinase-producing bacteria between cefpirome and other cephalosporins.

LITERATURE CITED


