Structure-activity Relationships for Interactions between Carbapenems and β-Lactamases

Sir:

Carbapenems are one of the novel class of β-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 β-lactamases[8]. However, carbapenems were hydrolyzed by dehydropeptidase-I (DHP-I) from several animals. In previous paper, we revealed that 1β-methyl moiety on meropenem had the important role of prevention from hydrolysis by DHP-I[9], furthermore, that 1β-methyl group on carbapenems affected the activity against *Pseudomonas aeruginosa*[10]. Since, we have interested that the role of 1β-methyl moiety on several biological activities of carbapenems. In the present study, therefore, we examined the interactions between carbapenems and various β-lactamases concerning structure-activity relationships, especially 1β-methyl moiety.

Carbapenem compounds, shown in Fig. 1, were prepared in Sumitomo Pharmaceuticals Research Center, Osaka, Japan, according to the reported procedures[11~13]. β-Lactamase-producing bacterial strains were reference organisms stored in our laboratory[8]. Several β-lactamases were purified as described previously[14~17], with some modifications. We select four types of representative β-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 L-1 β-lactamase from *Xanthomonas maltophilia*, according to the classification of MITSUHASHI[18]. β-Lactamase activity was determined in 50 mM phosphate buffer (pH 7.0) except L-1 enzyme in 50 mM MOPS buffer (pH 7.0) using a spectrophotometer (UV-2100: Shimadzu Corporation, Japan) controlled at 30°C[19]. The *Km* and V_{max} values of enzymes were determined from a Lineweaver-Burk plot. The *Ki* 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 μmol 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 1β-methyl moiety they showed resembled profiles each other against TEM-1, CSase and CXase. It is suggested that introduction of 1β-methyl group into carbapenem skeleton did not affect drastic changes in interactions between carbapenems and these β-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 β-lactamases. Of the interest of these compounds, compound **4a** was hydrolyzed by TEM-1 β-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 β-lactamase from *X. maltophilia* except compound **4a**. 1β-Methyl moiety affected the affinity of these carbapenems for this enzyme. Compounds **2a** and **2b** having 1β-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 1β-methyl moiety varied in C-2 side chains. Considering from V_{max}/Km ratio, carbapenems in three series showed similar properties of hydrolysis by this enzyme whether compounds had 1β-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

Fig. 1. Chemical structures of carbapenems used in this study.

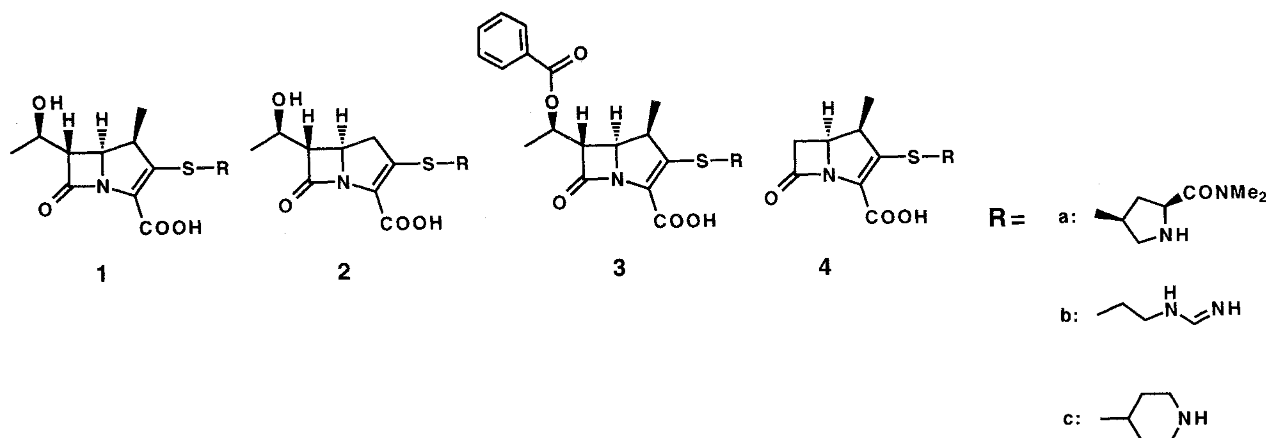

Table 1. Kinetic parameters of carbapenem compounds for β-lactamases.

Compound	1β-methyl	Ki (μM)[a]			L-1 β-lactamase[b]		
		TEM-1	CSase	CXase	Km	V_{max}	V_{max}/Km
1a	+	48	4.6	3.3	45	0.049	1.1
2a	−	15	14	0.23	9.3	0.0095	1.0
1b	+	53	8.5	1.1	390	0.21	0.54
2b	−	34	5.8	7.7	31	0.022	0.71
1c	+	8.6	1.5	0.33	0.61	0.060	98
2c	−	21	2.3	0.40	3.1	0.23	74
3a	+	48	62	2.4	N.D.[c]	N.D.	N.D.
4a	+	9.4[d]	15	N.D.	0.28[e]	—	—

[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 V_{max} 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-lactamase[20] and that β-lactamase resistance owes the *trans*-configuration of C-6 side chain[21]. 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. It is possible that the precise interactions between carbapenems and these β-lactamases will be revealed when crystallization of these enzymes is performed.

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