The Effect of 1β-Methyl Substituent and the Basicity in C-2 Side Chain in Carbapenem Antibiotics on the Activity against *Pseudomonas aeruginosa oprD2* and *nalB* Mutants

YOSHIHIRO SUMITA, HIROSHI YAMAGA and MAKOTO SUNAGAWA

Sumitomo Pharmaceuticals Research Center, 3-1-98, Kasugade-naka, Konohana, Osaka 554, Japan

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Carbapenems have potent antibacterial activity and a wide spectrum of action against Gram-positive and Gram-negative bacteria[1~4]. They also show high *in vitro* and *in vivo* antibacterial activity against *Pseudomonas aeruginosa*, which is a clinically important organism. *P. aeruginosa*, a common soil and water bacterium, is associated with acute and chronic infections of humans[5]. *P. aeruginosa* is highly resistant to many antibiotics[6], mainly because of the permeation barrier to drugs through the outer membrane[7]. The high activity of carbapenems against this organism is due to their good diffusion through the outer membrane[8,9], high affinity for penicillin-binding proteins (PBPs)[10] and high stability and inhibitory activity against β-lactamases[11]. Recently, carbapenem-resistant *P. aeruginosa* strains have been isolated clinically[12,13]. Most of them lack outer membrane protein D2 (OprD2) which forms a specific porin channel for basic amino acids and carbapenem antibiotics[9,14]. Investigation of imipenem-resistant *P. aeruginosa* strains revealed that meropenem still showed good activity against these strains. This result was in agreement with that from a previous report[15]. We isolated two genetically distinct meropenem-resistant mutants, designated *mpmA* and *mpmB* mutants. The *mpmA* and *mpmB* were thought to be alleles of *oprD2*[16] and *nalB*[17], respectively, both related to diffusion of meropenem through the outer membrane. These mutants showed different susceptibility profiles to carbapenems (data not shown). In this report, we examined the antipseudomonal activities of carbapenems affected by *oprD2* and *nalB* mutations, with regard to structure-activity relationships, especially 1β-methyl group and the basicity in C-2 side chain.

Carbapenem compounds, shown in Fig. 1, were prepared in Sumitomo Pharmaceuticals Research Center, Osaka, Japan, according to the reported procedures[18~24]. *P. aeruginosa* PAO2152 and TL2666 were laboratory and clinically isolated strains, respectively, being susceptible to carbapenems. SF04 and SF20 were spontaneous meropenem-resistant mutants of PAO2152 selected at a concentration of 1.56 μg/ml. SF41 and SF44 were isolated from TL2666 at the same concentration of meropenem. SF04 and SF20 were thought to be *nalB* and *oprD2* mutants, respectively, genetically confirmed by using transductional analysis. SF41 and SF44 were also conceived *nalB* and *oprD2* mutants, respectively, from the profiles of antibiotic susceptibility and patterns of outer membrane proteins. The MICs were determined by the two-fold serial agar dilution method using Muellar-Hinton Medium (Difco Laboratories, Detroit, Mich). Overnight cultures of test organisms were diluted with phosphate-buffered saline containing gelatin, and then dilutions were plated onto agar surface to give a final inoculum of 10^5 cfu per spot. Plates were incubated at 37°C for 20 hours. The MIC was defined as the lowest concentration of antibiotic that completely inhibited development of visible growth.

Antibacterial activities of the carbapenem compounds tested are summarized in Tables 1 and 2.

As shown in Table 1, the antipseudomonal activity of most of various carbapenems was affected by *oprD2* mutation, lacking OprD2 protein. Considering well known carbapenems, the activity of **1a** (meropenem) was also influenced by *nalB* mutation, whereas **2b** (thienamycin), **2c** (imipenem) and **2d** (panipenem) were not. The marked difference in the structure between **1a** and the other carbapenems is that **1a** has a 1β-methyl group on the carbapenem skeleton. First, the structural effect of 1β-methyl moiety was investigated in several carbapenem series. With introduction of a 1β-methyl group onto carbapenem skeleton, the activities of these compounds were affected by not only *oprD2* but also *nalB* mutations compared with the corresponding desmethyl derivatives. Despite having a 1β-methyl moiety, however, **1f** (biapenem) showed the same activity against *nalB* and parent strains. On the contrary, *nalB* also influenced the activity of **2a** (desmethyl meropenem) without 1β-methyl group.

Therefore, it is likely that permeability in *P. aeruginosa*

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

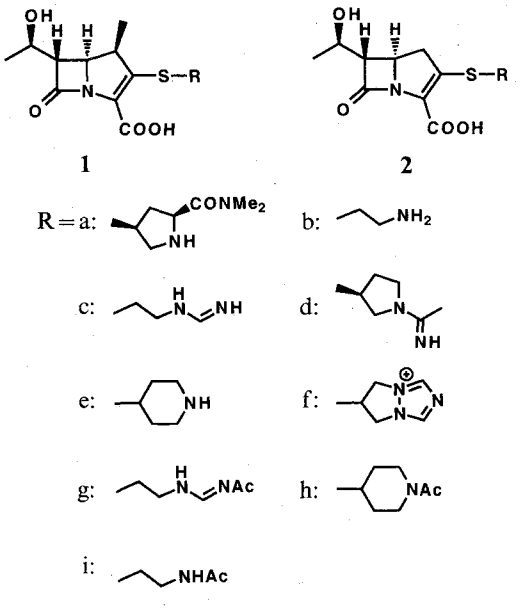

Table 1. Effect of 1β-methyl substituent on the activity of carbapenems against *P. aeruginosa* parents and their mutant strains.

Compound	MIC (μg/ml)*					
	PAO2152 (Wild)	SF20 (*oprD2*)	SF04 (*nalB*)	TL2666 (Wild)	SF44 (*oprD2*)	SF41 (*nalB*)
1a	0.78	3.13 (4)	3.13 (4)	0.78	3.13 (4)	3.13 (4)
2a	3.13	25 (8)	6.25 (2)	6.25	25 (4)	12.5 (2)
1b	1.56	3.13 (2)	3.13 (2)	3.13	6.25 (2)	6.25 (2)
2b	3.13	25 (8)	3.13 (1)	6.25	12.5 (2)	6.25 (1)
1c	1.56	6.25 (4)	3.13 (2)	3.13	12.5 (4)	6.25 (2)
2c	0.78	12.5 (16)	0.78 (1)	1.56	12.5 (8)	1.56 (1)
1d	6.25	12.5 (2)	12.5 (2)	12.5	12.5 (1)	12.5 (1)
2d	6.25	12.5 (2)	6.25 (1)	6.25	12.5 (2)	6.25 (1)
1e	3.13	6.25 (2)	6.25 (2)	6.25	6.25 (1)	6.25 (1)
2e	6.25	12.5 (2)	6.25 (1)	6.25	12.5 (2)	6.25 (1)
1f	0.39	3.13 (8)	0.39 (1)	0.78	12.5 (16)	0.78 (1)

* The values in parentheses are expressed as multiple of the MIC to parent strain.

Table 2. Effect of basicity in C-2 side chain on the activity of carbapenems against *P. aeruginosa* parents and their mutant strains.

Compound	MIC (μg/ml)*					
	PAO2152 (Wild)	SF20 (*oprD2*)	SF04 (*nalB*)	TL2666 (Wild)	SF44 (*oprD2*)	SF41 (*nalB*)
1g	50	50 (1)	100 (2)	50	50 (1)	100 (2)
1h	100	100 (1)	400 (4)	100	100 (1)	400 (4)
2i	100	100 (1)	200 (2)	100	100 (1)	200 (2)
2h	100	100 (1)	200 (2)	100	100 (1)	200 (2)

* The values in parentheses are expressed as multiple of the MIC to parent strain.

may also depend on their physico-chemical properties, such as the basicity in C-2 side chain of carbapenem compounds. The carbapenems tested have a basic moiety on the C-2 side chain, while the basicity of **1a** and **2a** are very weak. Consequently, effect of the basicity in C-2 side chain was examined (Table 2). Masking of basic functional groups by *N*-acetylation considerably reduced and altered the antipseudomonal activity of carbapenems, with or without 1β-methyl moiety. The activities of *N*-acetylated carbapenems were only influenced by *nalB* mutation and showed the same activity against parent and *oprD2* mutant. Thus, the basicity of carbapenems was another factor involved in the activity against *nalB* mutants. In addition, the poor activity of carbapenems without any basic groups in C-2 side chain revealed that basicity was necessary to maintain high antipseudomonal activity depending upon the OprD2 pathway, as suggested previously[25].

The activity of compound **1f** was only affected by *oprD2* mutation despite having a 1β-methyl group. This seemed to be due to the quaternary heteroaromatic group in C-2 side chain. The same results were obtained in the other quaternarized 1β-methyl carbapenems which were synthesized in our Research Center[26]. Thus, the 1β-methyl moiety seems to partially affect the activity of carbapenems against *nalB*-type mutants of *P. aeruginosa* and the cationic moiety in C-2 side chain prevents the carbapenem permeation through *nalB*-dependent pathway. Against OprD2-defective mutant, therefore, the activity of carbapenems which contained a cationic moiety such as imipenem, panipenem and biapenem was considerably diminished because of the increased relative dependency upon the OprD2 pathway.

In conclusion, it is demonstrated that the 1β-methyl moiety on carbapenems not only affects its affinity for PBPs and consequent antipseudomonal activity as described in our previous study[27], but also partially affects its permeation through the outer membrane by altering the *nalB*-depended pathway in *P. aeruginosa*. The cationic character in C-2 side chain in carbapenem is strongly correlated with the permeability through *nalB*-depended pathway.

Antipseudomonal activities of carbapenems, such as **1a**, **2a**, **1b** and **1c**, were affected by both *oprD2* and *nalB* mutations. This suggests that these carbapenems can pass through the outer membrane of *P. aeruginosa via* two independent routes, *oprD2* and *nalB* pathways. It would be of interest to investigate the dependent ratio on *oprD2* and *nalB* pathways in these carbapenems. This is in progress.

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