THE EFFECT OF 1β-METHYL AND IMIDOYL SUBSTITUENTS ON THE ANTIPSEUDOMONAL ACTIVITY OF CARBAPENEMS

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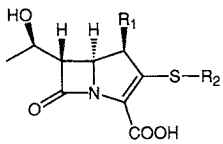
Among the many clinical pathogens *Pseudomonas aeruginosa* is understood as the bacterium that causes opportunistic infections in patients under immunosuppressed conditions or with cystic fibrosis or diffuse panbronchiolitis. It is difficult to cure *P. aeruginosa* infections because of its intrinsic resistance to antibiotics[1]. Several new β-lactam antibiotics with antipseudomonal activity have been introduced into clinical therapeutics. Among these, carbapenems are of particular interest as they have a powerful effect against *P. aeruginosa*. The discovery of thienamycin[2] created a scientific sensation as it has its potent antibacterial activity and a wide spectrum of action against Gram-positive and Gram-negative bacteria. A number of carbapenems, such as olivanic acids[3], carpetimycins[4], asparenomycins[5], and others, have been discovered. However, none are available for clinical use except imipenem, an *N*-formimidoyl derivative of thienamycin. Meropenem and panipenem are new carbapenems under pre-registration in Japan. Meropenem, which differs chemically from imipenem and panipenem, has a 1β-methyl group and no imidoyl substituent on the 2-sulfur side chain. In this study, we investigated the antipseudomonal activity of four series of carbapenems in terms of the structure-activity relationships, especially the 1β-methyl and imidoyl moieties.

Meropenem and the other carbapenems, summarized in Fig. 1, were prepared in Sumitomo Pharmaceuticals Research Center, Osaka, Japan. [^{14}C]Benzylpenicillin potassium salt was purchased from Amersham International plc, Bucks., U.K. Various *P. aeruginosa* strains were reference organisms stored in our laboratories, and recent clinical isolates were obtained from various hospitals in Japan. The MICs were measured by agar dilution on Sensitivity Test Agar (Nissui Pharmaceutical, Japan), which is modified Mueller-Hinton agar. An overnight culture of the test organism in Sensitivity Test Broth (Nissui) was diluted in buffered saline gelatin, and about 10^4 cfu/spot were inoculated onto a drug-containing agar surface. Plates

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



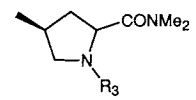
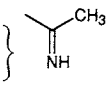
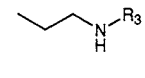
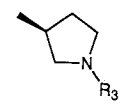
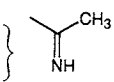
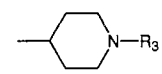
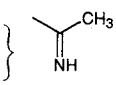
Compound	R_1	R_2	R_3
Meropenem	CH_3		H
Desmethyl meropenem	H		H
Compound A	CH_3		
Desmethyl compound A	H		
1β-Methyl thienamycin	CH_3		H
Thienamycin	H		H
1β-Methyl imipenem	CH_3		
Imipenem	H		
Compound B	CH_3		H
Desmethyl compound B	H		H
1β-Methyl panipenem	CH_3		
Panipenem	H		
Compound C	CH_3		H
Desmethyl compound C	H		H
Compound D	CH_3		
Desmethyl compound D	H		

Table 1. Antipseudomonal activity of various carbapenem series.

Compound	MIC (μg/ml)					MIC (μg/ml) of clinical isolates[a]		
	NCTC10490	T	IFO3451	PAO2152	TL2666[b]	MIC_{50}	MIC_{90}	Range
Meropenem	0.025	0.39	0.39	0.78	0.78	0.55	2.87	0.05～12.5
Desmethyl meropenem	0.10	12.5	3.13	3.13	6.25	1.92	14.9	0.20～50
Compound A	0.20	3.13	3.13	3.13	6.25	4.17	18.7	0.39～25
Desmethyl compound A	1.56	25	12.5	12.5	25	14.4	28.1	1.56～50
1β-Methyl thienamycin	0.78	3.13	1.56	1.56	3.13	1.99	5.26	0.39～12.5
Thienamycin	1.56	6.25	3.13	3.13	6.25	3.23	6.06	0.78～25
1β-Methyl imipenem	1.56	6.25	1.56	1.56	3.13	2.21	5.83	0.39～12.5
Imipenem	0.78	1.56	1.56	0.78	1.56	0.96	4.42	0.39～12.5
Compound B	0.20	1.56	1.56	1.56	1.56	1.19	4.05	0.20～ 6.25
Desmethyl compound B	1.56	3.13	1.56	1.56	3.13	1.51	4.82	0.39～25
1β-Methyl panipenem	1.56	25	6.25	12.5	12.5	13.5	23.1	0.39～50
Panipenem	1.56	12.5	3.13	3.13	6.25	4.42	11.9	0.39～25
Compound C	0.39	12.5	1.56	3.13	6.25	4.58	10.1	0.39～12.5
Desmethyl compound C	0.78	12.5	3.13	6.25	6.25	6.01	14.0	0.39～25
Compound D	0.78	25	3.13	12.5	6.25	13.5	22.1	0.20～25
Desmethyl compound D	0.78	12.5	3.13	6.25	6.25	9.32	21.0	0.20～50

[a] $n=25$.
[b] Typical clinical isolate.

were incubated at 37°C for 18 hours. The MIC was defined as the lowest concentration that completely inhibited visible growth. MIC_{50}s and MIC_{90}s were calculated from the equation describing the linear part of the curve.

P. aeruginosa cell membranes were prepared as previously described[6]. *P. aeruginosa* grown to late-exponential phase was harvested, and the cells were ruptured by sonication. The membrane fractions were pelleted by ultracentrifugation. The affinities of the carbapenems for penicillin-binding proteins (PBPs) were determined by means of a competition assay using [^{14}C]benzylpenicillin, essentially as previously described[6,7]. We used *P. aeruginosa* NCTC10490 because this strain always produces very sharp and clear PBP bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Meropenem and imipenem show the same binding profiles to PBPs in three strains of *P. aeruginosa*.[6] Therefore, this result could be generalized for all susceptible *P. aeruginosa*.

The permeability of carbapenems through the outer membrane of *P. aeruginosa* was measured using β-lactamase enclosed proteoliposome reconstitution assays[8,9]. The outer membrane was purified from *P. aeruginosa* PAO2152 by the procedure previously described[10]. The L-1 type β-lactamase was purified from *Xanthomonas maltophilia* IID1273[11] by the method of SAINO[12].

Table 1 shows the antipseudomonal activities of various carbapenems. We used 4 standard laboratory strains and 26 clinical isolates of *P. aeruginosa*. Of 16 carbapenems used in this study meropenem was most the active one against *P. aeruginosa*, followed by imipenem and compound B. In all four carbapenem series, the introduction of a 1β-methyl substituent to compounds having an imidoyl group reduced the antipseudomonal activity, whereas 1β-methyl compounds without an imidoyl moiety were more active than their corresponding desmethyl derivatives. There may be some congeniality among 1β-methyl and imidoyl substituents on antibacterial activity against *P. aeruginosa*. However, desmethyl compound A was much less active than compound A. This may be because the bulky side chains in compound A, acetimidoyl and dimethylcarbamoyl groups on the pyrrolidine moiety, interfere with its own binding to the target PBPs.

The antibacterial activity of carbapenems greatly depends on their affinity and access to targets (PBPs) of *P. aeruginosa*. We selected, therefore, meropenem and imipenem series of carbapenems as representatives and examined their affinity for PBPs and the permeation through the outer membrane using proteoliposomes containing L-1 β-lactamase. The affinities of four carbapenems are summarized in Table 2. Meropenem had higher

Table 2. Affinities of the series of meropenem and imipenem for PBPs in *Pseudomonas aeruginosa* NCTC10490[a].

Antibiotic	IC_{50} (μg/ml)[b]					
	1A	1B	2	3	4	5
Meropenem	0.96	0.84	0.13	0.060	0.022	>10
Desmethyl meropenem	0.49	2.6	0.87	0.38	0.054	>10
1β-Methyl imipenem	1.7	3.2	0.49	0.94	0.018	>10
Imipenem	0.40	0.81	0.23	0.53	0.014	4.6

[a] Membrane fractions were incubated with carbapenems at various concentrations for 10 minutes at 30°C. Subsequently, [^{14}C]benzylpenicillin was added and the incubation was continued for another 10 minutes. Reactions were terminated by adding excess non-radioactive benzylpenicillin and sarkosyl. [^{14}C]Benzylpenicillin-protein complexes were resolved by SDS-PAGE, followed by fluorography.

[b] Values indicate concentrations of antibiotics required to reduce [^{14}C]benzylpenicillin binding by 50%.

affinities than desmethyl meropenem, for all PBPs except PBP1A. Conversely, 1β-methyl imipenem had less affinity than imipenem.

The relative outer membrane permeability of meropenem, desmethyl meropenem, 1β-methyl imipenem and imipenem were 100, 110$\pm$28, 167$\pm$25 and 207$\pm$44, respectively, assays performed in triplicate. The permeation of meropenem and desmethyl meropenem through the outer membrane was lower than that of 1β-methyl imipenem and imipenem. However, meropenem was most active against *P. aeruginosa*. These results indicate that the antipseudomonal activity of these four carbapenems was directly reflected in their affinity for PBPs but not related to outer membrane permeability. With or without the 1β-methyl moiety, meropenem and imipenem series showed almost the same permeability rate through the outer membrane of *P. aeruginosa*. The permeability of carbapenem seemed to depend on the structure of their 2-side chain.

In this report, we revealed that the 1β-methyl moiety on carbapenem directly affects its affinity for PBPs and consequently its antipseudomonal activity. We also showed that there is some congeniality between the 1β-methyl and imidoyl groups in producing activity against *P. aeruginosa*.

It has been clarified that carbapenems utilize their specific channel D2 outer membrane protein in *P. aeruginosa*[13,14]. In addition, a defective D2 protein considerably affects antipseudomonal activity of carbapenems. It would be of interest to clarify the affinity of our series of carbapenems for D2 protein from the viewpoint of structure-activity relationships. This is currently under way.

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