Postantibiotic Effect of Carbapenems against *Pseudomonas aeruginosa*

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Recently, nontraditional β-lactam antibiotics, carbapenems, have been synthesized vigorously, and several analogs are under development. With respect to the antimicrobial activity of carbapenems, the postantibiotic effect (PAE) is a prominent feature. Carbapenems have been shown to produce PAEs not only against Gram-positive bacteria but also against Gram-negative bacteria by several investigators[1~5]. The aim of the present study was to simultaneously compare the PAE of newer carbapenems (such as meropenem and biapenem) with that of imipenem against strain NU12 of *Pseudomonas aeruginosa*.

Biapenem and meropenem were synthesized at Chemical and Formulation Laboratories of Lederle (Japan), Ltd. All other antibiotics were obtained from commercial sources.

MICs were determined by the two-fold agar dilution method.

Culture was grown in Sensitivity Test Broth (STB; Nissui Pharmaceutical, Japan) or on Sensitivity Disk Agar-N (SDA; Nissui).

The *in vitro* PAE of each antibiotic was studied using *P. aeruginosa* NU12 in the logarithmic phase of growth (10^6 cfu/ml), which had been exposed to 4× MIC of the respective antibiotic at 37°C for 2 hours. The antibiotic was removed by a 10^{-2} dilution into fresh STB medium, and then the culture was reincubated at 37°C in a shaking water bath. Thereafter, viable count was determined every 1 hour by inoculating a series of dilutions of a bacterial suspension onto the SDA plates.

The *in vitro* PAE was calculated according to the method of GERBER and CRAIG[6].

For the determination of *in vivo* PAE, a thigh infection model in neutropenic mice (female ICR mice, 7 weeks, 22~28 g) caused by cyclophosphamide (250 mg/kg, ip) was used. The inoculum of *P. aeruginosa* NU12 was prepared from a logarithmic phase culture. About 10^4 cfu in a volume of 0.1 ml were intramuscularly injected into bilateral thighs of each mouse. After allowing the bacteria to grow for 2 hours, each drug was administered subcutaneously at a dose of 50 mg/kg. Two mice were sacrificed at 1, 2, 4, 6, 8, 10, 12 and 22 hours after the injection to prepare thigh homogenates. Viable count was determined by plating serially 10-fold diluted samples of homogenate on the SDA plates. The duration of *in vivo* PAE was calculated as described by VOGELMAN *et al.*[7].

Plasma concentrations of the parent compound in mice given a dose of 50 mg/kg were determined by HPLC assay, and the optimal fitting curve of time courses of the concentrations was obtained based upon a one-compartment model to calculate the "time above MIC" value.

As shown in Table 1, the MICs of imipenem/cilastatin, meropenem, biapenem and ceftazidime for *P. aeruginosa* NU12 used in this study were 3.13, 3.13, 1.56 and 3.13 μg/ml, respectively. The time-above-MIC (hour) values determined by pharmacokinetic studies are also shown in Table 1. It is well-known that the PAE values obtained are very variable possibly because of dependence upon methods used to quantify the effect. Therefore, we made a simultaneous comparison among the PAE values of various carbapenems using a single method. The *in vitro* PAE values of imipenem/cilastatin, meropenem, biapenem and ceftazidime obtained from the standard viable counting method were 1.8, 1.5, 1.6 and −1.0 hours, respectively (Fig. 1, Table 1: Table 1 shows the *in vitro* PAE calculated from Fig. 1). The *in vivo* PAE evaluated in the neutropenic mouse thigh model gave results as follows: imipenem/cilastatin 0.9, meropenem 1.5, biapenem 1.8 and ceftazidime −0.5 hours (Fig. 2, Table 1: Table 1 shows the *in vivo* PAE calculated from Fig. 2). PAE values mentioned above represent mean values from two experiments. Both *in vitro* and *in vivo* PAE values obtained in this study were very similar to the results reported by other investigators[5,8,9]. As shown

Table 1. Postantibiotic effect of carbapenems against *Pseudomonas aeruginosa* NU12 *in vitro* and *in vivo*.

Drug	*In Vitro*			*In Vivo*		
	MIC (μg/ml)	4×MIC (μg/ml)	PAE* (hour)	Dose (mg/kg)	Time above MIC (hour)	PAE* (hour)
Imipenem/Cilastatin	3.13	12.5	1.8	50	1.0	0.9
Meropenem	3.13	12.5	1.5	50	0.86	1.5
Biapenem	1.56	6.25	1.6	50	1.5	1.8
Ceftazidime	3.13	12.5	-1.0	50	2.0	-0.5

*PAE, postantibiotic effect.

Fig. 1. *In vitro* postantibiotic effect of imipenem/cilastatin, meropenem, biapenem and ceftazidime against *Pseudomonas aeruginosa* NU12.

○ Imipenem/cilastatin, ▽ meropenem, □ biapenem, △ ceftazidime, ● control.

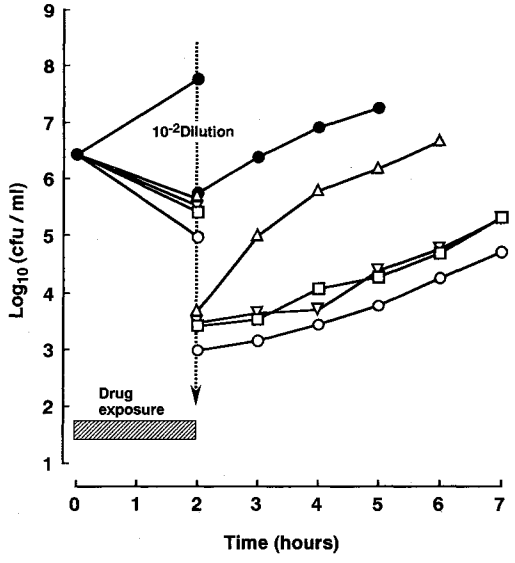
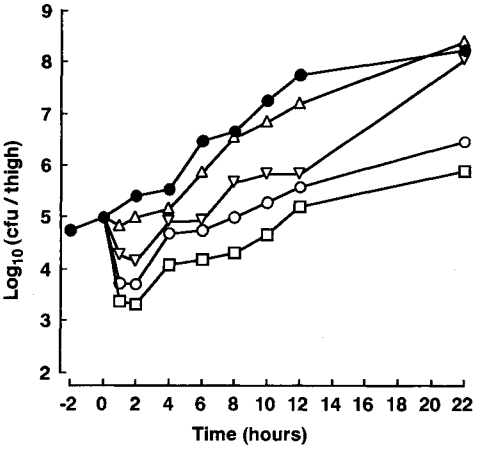


Fig. 2. Postantibiotic effect of imipenem/cilastatin, meropenem, biapenem and ceftazidime against *Pseudomonas aeruginosa* NU12 in the thigh infection model in neutropenic mice.

○ Imipenem/cilastatin, ▽ meropenem, □ biapenem, △ ceftazidime, ● control.



in Table 1, the time-above-MIC values for antibiotics tested ranged from 0.86 to 2.0 hours. With respect to the biochemical basis of the PAE, GOULD *et al.*[2] expressed the notion that affinity for PBP2 relates to induction of PAE. Several other theories have been proposed, but not been conclusively elucidated[1,9]. Thus, this study showed that all carbapenems tested induced a PAE against *P. aeruginosa* NU12, whereas ceftazidime caused no PAE. The fact that carbapenems, unusually for a β-lactam antibiotic, produce a PAE against a strain NU12 of *P. aeruginosa* will spur further research and development of new carbapenem derivatives.

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