Effects of a β-Lactamase Inhibitor, Sulbactam, on the Activity of Carbapenems against *Pseudomonas aeruginosa*

NOBUYOSHI HAZUMI*, YOSHIAKI KATO, TERUTAKA HASHIZUME and KOUJI MATSUDA

Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba Techno-Park Oho, Okubo 3, Tsukuba 300-33, Japan

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Imipenem, one of the most powerful antibiotic agents, is widely used in clinical practice, including the treatment of nosocomial infections caused by *Pseudomonas aeruginosa*. This expanded-spectrum carbapenem[1,2] is resistant to hydrolysis by most β-lactamases, possesses potent β-lactamase inhibitory activity, and exhibits equal activity against β-lactamase-inducible and -derepressed *P. aeruginosa*[3]. In spite of its potent activity against *P. aeruginosa*, mutants resistant to imipenem have been isolated from both clinical sources and laboratory[4,5]. It has been reported that the mechanism of resistance is due to both D2 porin loss and slow but significant hydrolysis by chromosomally-mediated β-lactamase[6,7]. Thus, β-lactamase resistance to carbapenems has been identified. We previously found that differences in activity between four carbapenems, BO-2727, meropenem, biapenem, and panipenem, against imipenem-resistant *P. aeruginosa* depended mainly on the β-lactamase sta-

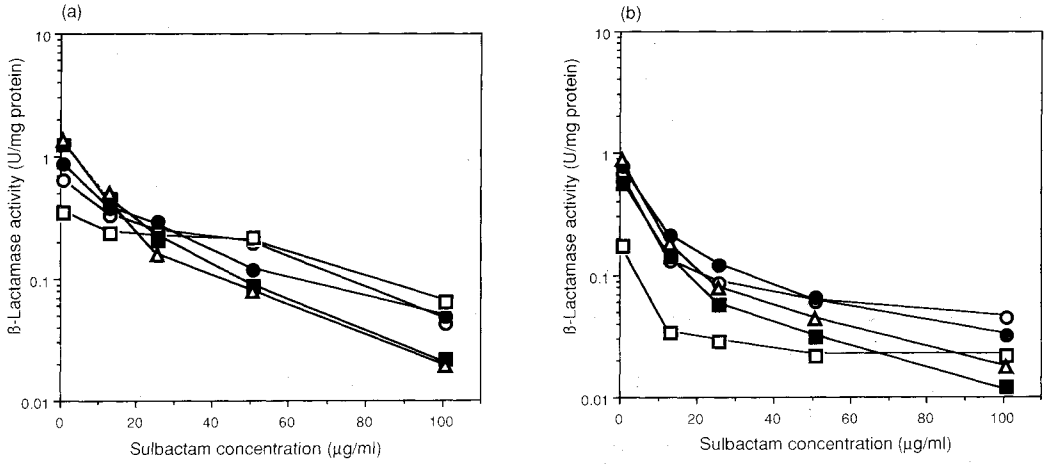
Table 1. Effect of sulbactam on the susceptibility of *P. aeruginosa* PAO derivatives to carbapenems.

Organism	Sulbactam conc. (μg/ml)	MIC (μg/ml)[a] BO-2727	Biapenem	Meropenem	Panipenem	Imipenem
	0	0.78	0.39	0.78	6.25	1.56
	12.5	0.39	0.39	0.78	1.56	0.78
F2M	25	0.39	0.39	0.78	0.78	0.39
(D2+/Bla+)[b]	50	0.39	0.2	0.78	0.39	0.39
	100	0.39	0.1	0.39	0.39	0.2
	0	1.56	6.25	3.13	12.5	6.25
	12.5	1.56	6.25	3.13	1.56	3.13
A1M	25	0.78	3.13	3.13	0.78	3.13
(D2-/Bla+)	50	0.78	3.13	3.13	0.78	1.56
	100	0.78	1.56	3.13	0.39	0.78
	0	0.39	0.2	0.39	0.39	0.39
	12.5	0.39	0.2	0.39	0.39	0.39
PAO4089	25	0.39	0.1	0.39	0.2	0.2
(D2+/Bla-)	50	0.2	0.1	0.39	0.2	0.2
	100	0.2	0.1	0.2	0.2	0.2
	0	0.78	0.78	3.13	0.78	0.78
	12.5	0.78	0.78	3.13	0.78	0.78
8M2	25	0.78	0.78	3.13	0.39	0.78
(D2-/Bla-)	50	0.39	0.39	3.13	0.39	0.39
	100	0.39	0.39	3.13	0.39	0.39

[a]Broth dilution method using Mueller-Hinotn broth (Difco) and inoculum size of about 10^4 cells/ml.

[b]D2, protein D2; Bla, ß-lactamase.

Fig. 1. Levels of β-lactamase activity induced by panipenem (■), imipenem (△), biapenem (●), meropenem (□), and BO-2727 (○) in *P. aeruginosa* F2M (a) and A1M (b) in the presence of various concentrations of sulbactam.



The β-lactamase activity (U/mg protein) of strains F2M and A1M without carbapenem and sulbactam was 0.0076 and 0.0084, respectively.

bility of the drugs[7].

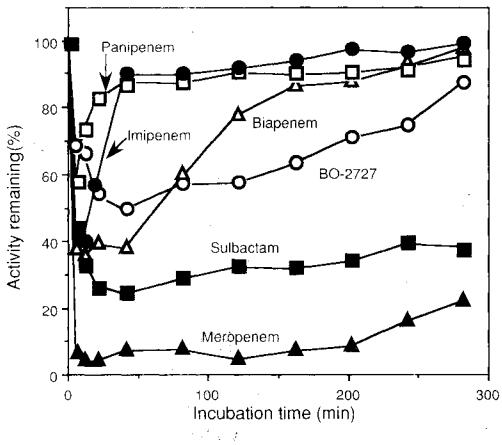
In this paper, we determined the effects of a β-lactamase-inhibitor, sulbactam, on the activity of carbapenems against *P. aeruginosa* and confirmed the effects of chromosomally-mediated β-lactamase on carbapenem susceptibility. Sulbactam potentiated the activity of panipenem, imipenem and biapenem to a greater extent than that of BO-2727 and meropenem. The increased levels of susceptibility to panipenem, imipenem and biapenem were attributed to a permanent inactivation of β-lactamase by sulbactam.

BO-2727, meropenem, biapenem and sulbactam were synthesized in the authors' laboratories. The following antibiotics were obtained commercially: imipenem and colistin (Banyu Pharmaceutical Co., Ltd., Tokyo), cephaloridine (Shionogi & Co., Japan), panipenem (Sankyo Co., Ltd., Tokyo), ceftazidime (Tanabe Pharmaceutical Co., Ltd., Osaka) and norfloxacin (Kyorin Pharmaceutical Co., Ltd., Tokyo).

The *P. aeruginosa* PAO derivatives used were as follows: PAO4089 (D2-producing and β-lactamase-deficient strain), F2M (D2- and β-lactamase-producing strain), 8M2 (D2- and β-lactamase-deficient strain) and A1M (D2-deficient and β-lactamase-producing strain). All of these microorganisms have been described previously[7]. Clinical isolates of *P. aeruginosa* that have been collected from clinical sources in Japan since 1983 were used.

MICs were determined by the agar or broth dilution method using Mueller-Hinton agar (Difco Laboratories, Detroit, U.S.A.) or broth inoculated with about 10^4 cells/spot or 10^4 cells/ml, respectively. The MIC was defined as the lowest concentration that inhibited visible growth after incubation at 37°C for 18 hours.

Fig. 2. Inactivation of purified β-lactamase from *P. aeruginosa* F2M by sulbactam or carbapenems.



Enzyme (1 U) was incubated at 30°C with a 1-μM concentration of each test antibiotic or MOPS buffer as a control. Enzyme activity during incubation with the antibiotic was determined with cephaloridine as the substrate and was expressed as a percent of the corresponding control.

β-Lactamase was prepared as described previously[7] and β-lactamase activity was determined by a spectrophotometric method[8] measuring the decrease in absorbance at 260 nm of 100 μM cephaloridine in a temperature-controlled spectrophotometer (Hitachi U3210)

at 30°C. One unit (U) of β-lactamase was defined as the amount of enzyme that hydrolyzed 1 μmol cephaloridine per minute at 30°C. The β-lactamase induced by each carbapenem was also assessed in the presence of various concentrations of sulbactam. Induction was performed for 90 minutes, after which cell extracts were prepared and evaluated for β-lactamase activity. The ability of sulbactam and the carbapenems to inactivate purified β-lactamase from *P. aeruginosa* F2M was determined as follows: the enzyme (1 U) was preincubated with 1 μM of test antibiotic in 1 ml of 10 mM 3-(N-morpholino)-propane sulfonate buffer (MOPS, pH 7.0) at 30°C and the remaining enzyme activity was determined from the initial hydrolysis rate of cephaloridine after 100-fold dilution with 100 μM cephaloridine at various time intervals. The dilution was expected to remove competitive inhibition in the enzyme activity assay by lowering the concentration of inhibitor in the assay reaction mixture below the level at which such inhibition is detected[7].

The effects of sulbactam on the MICs of BO-2727, biapenem, meropenem, panipenem or imipenem against four isogenic *P. aeruginosa* PAO strains were examined. The results shown in Table 1 demonstrate that the MICs of panipenem, imipenem, and biapenem against the chromosomally-inducible β-lactamase strains F2M and A1M decreased with an increase in sulbactam concentration. This effect was not observed when another β-lactamase inhibitor, clavulanic acid[9], or a β-lactamase synthesis inhibitor, clindamycin[10], was used. In contrast, the MICs of BO-2727 and meropenem were little changed, and the effects of sulbactam on the MICs of all of the carbapenems tested against the β-lactamase-deficient strains, PAO4089 and 8M2, were negligible. Furthermore, the MIC of sulbactam alone was higher than 800 μg/ml for strains F2M, A1M, PAO4089 and 8M2. Therefore, it was thought that the inhibition of the

Table 2. Change of susceptibility of clinical isolates of *P. aeruginosa* to various antibiotics with or without sulbactam.

Organism (No. of isolates)	Antibiotic	MIC (μg/ml)[a]			
		- Sulbactam		+ Sulbactam (100 μg/ml)	
		Range	Geometric mean MIC	Range	Geometric mean MIC
	BO-2727	0.05 - 3.13	0.36	0.05 - 3.13	0.2 (1.8)[b]
	Biapenem	0.1 - 6.25	0.80	0.1 - 1.56	0.35 (2.3)
	Meropenem	0.1 - 12.5	0.67	0.05 - 12.5	0.34 (2.0)
Imipenem-	Panipenem	0.2 - 50	6.40	0.1 - 12.5	0.90 (7.1)
susceptible	Imipenem	0.39 - 6.25	1.60	0.2 - 3.13	0.41 (3.9)
strains	Ceftazidime	0.39 - >200	7.52	0.39 - 200	2.48 (3.0)
(30)	Aztreonam	1.56 - 200	7.01	0.78 - 100	4.22 (1.7)
	Piperacillin	0.78 - 200	14.36	0.78 - 25	4.32 (3.3)
	Norfloxacin	0.39 - 50	1.88	0.39 - 50	1.84 (1.0)
	Colistin	0.39 - 1.56	0.70	0.2 - 3.13	0.78 (0.9)
	BO-2727	0.78 - 25	2.46	0.2 - 25	0.99 (2.5)
	Biapenem	6.25 - 100	14.10	0.78 - 100	3.53 (4.0)
	Meropenem	0.78 - 100	4.62	0.1 - 100	2.53 (1.8)
Imipenem-	Panipenem	12.5 - 100	29.10	0.78 - 100	2.61 (11.1)
resistant	Imipenem	12.5 - 50	18.50	0.78 - 50	2.86 (6.5)
strains	Ceftazidime	0.78 - >200	5.22	0.78 - >200	2.61 (2)
(23)	Aztreonam	1.56 - 25	5.54	0.39 - 25	3.42 (1.6)
	Piperacillin	0.78 - 100	8.45	1.56 - 25	4.77 (1.8)
	Norfloxacin	0.39 - 12.5	1.16	0.2 - 6.25	1.34 (0.9)
	Colistin	0.39 - 0.78	0.76	0.78 - 1.56	0.83 (0.9)

[a]Agar dilution method using Mueller-Hinton agar (Difco) and an inoculum size of about 10^4 cells/spot.

[b]Values in parentheses indicate the fold-reduction in geometric mean MIC afforded by the addition of sulbactam.

β-lactamase present in the *P. aeruginosa* strains was responsible for the decreased MICs. It should also be noted that an increase in sulbactam concentration reduced the levels of β-lactamase activity induced by each carbapenem by 1/2×MIC in the two β-lactamase-producing *P. aeruginosa* strains, F2M and A1M (Fig. 1). Thus, a correlation was found to exist between the extent of β-lactamase inhibition by sulbactam and the susceptibility of the *P. aeruginosa* strains to relatively β-lactamase-susceptible carbapenems in the presence of sulbactam. However, a high concentration of sulbactam was required to afford a significant degree of protection, although sulbactam inactivated β-lactamase to a greater extent than did the carbapenems except meropenem (Fig. 2). This finding may be due to the poor outer membrane permeability of sulbactam. Importantly, sulbactam, unlike panipenem, imipenem and biapenem, functioned as an irreversible β-lactamase inhibitor under these conditions (Fig. 2). The above results indicate that panipenem, imipenem and biapenem avoided β-lactamase hydrolysis, probably because of inactivation of inducible β-lactamase by sulbactam, and that BO-2727 and meropenem did not require sulbactam because of high β-lactamase stability as described previously[7].

Table 2 shows the antibacterial activity of five carbapenems, ceftazidime, aztreonam, piperacillin, norfloxacin and colistin, against 53 clinical isolates of *P. aeruginosa* in the presence and absence of 100 μg/ml of sulbactam. Sulbactam at a concentration of 100 μg/ml did not show an inhibitory effect against any of the bacteria tested. The geometric mean MIC (G-MIC) of panipenem against imipenem-sensitive *P. aeruginosa* was decreased about 7-fold by the addition of sulbactam. The decrease in G-MIC for imipenem was about 4-fold, while that for BO-2727, biapenem and meropenem was only about 2-fold. The decrease in G-MIC against imipenem-resistant *P. aeruginosa* was about 11-, 6- and 4-fold for panipenem, imipenem and biapenem, respectively. The presence of sulbactam caused a decrease of only about 2-fold in the G-MICs of BO-2727 and meropenem. The activity of BO-2727 was about 2- to 5-fold greater than that of the other carbapenems even in combination with sulbactam. Retention of the activity of BO-2727 against imipenem-resistant *P. aeruginosa* is attributed to the relatively high β-lactamase stability and better penetration of the drug, as described previously[7]. In contrast, the G-MICs of ceftazidime, aztreonam, and piperacillin against imipenem-sensitive/resistant *P. aeruginosa* were decreased about 2- to 4-fold, and combined effects of sulbactam with norfloxacin or colistin were not observed.

This study has demonstrated that carbapenem resistance to *P. aeruginosa* involves inactivation of carbapenem by the chromosomally-mediated β-lactamase of *P. aeruginosa*, as well as by the loss of D2 porin, to which resistance is widely ascribed.

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