In vitro postantibiotic effects of tomopenem (CS-023) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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The postantibiotic effect (PAE) of tomopenem was determined after a 2 h exposure of two strains of meticillin-susceptible and meticillin-resistant *Staphylococcus aureus* (MSSA and MRSA), and imipenem-susceptible and imipenem-resistant *Pseudomonas aeruginosa*, to tenfold the respective MIC. The PAEs on MSSA and *P. aeruginosa* were approximately 1 h and they were comparable to those of meropenem. The PAE on MRSA was 1.5 to 3 h, equal to or longer than those of vancomycin. The PAEs of tomopenem not only were found for MRSA, but also were present in the imipenem-resistant *P. aeruginosa* tested.

INTRODUCTION

Tomopenem (formerly CS-023) is a novel 1 β -methyl carbapenem with a broad spectrum of activity against clinically important Gram-positive and Gram-negative bacteria, including meticillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. We have previously reported that the favourable features of tomopenem are as follows: (i) better antibacterial activity against *P. aeruginosa* than imipenem and meropenem, and in particular, potent activity against imipenem-resistant *P. aeruginosa* (Koga *et al.*, 2005); (ii) correlation between the potent antibacterial activity against MRSA and a high affinity for penicillin-binding protein 2a (Koga *et al.*, 2008); and (iii) a longer elimination half-life than imipenem and meropenem in clinical studies (Shibayama *et al.*, 2006).

The purpose of this study was to determine the *in vitro* postantibiotic effects (PAEs) of tomopenem on meticillin-susceptible *S. aureus* (MSSA), MRSA, imipenem-susceptible *P. aeruginosa*, and imipenem-resistant *P. aeruginosa* and to compare them with the PAEs of the antibiotics routinely used in clinical practice to treat infections caused by these pathogens.

METHODS

Antibiotics. Tomopenem was synthesized at Daiichi Sankyo Research Laboratories, Tokyo, Japan. Pure samples of imipenem (Banyu Pharmaceutical), meropenem (Dainippon Sumitomo Pharma) and ceftazidime (GlaxoSmithKline) were extracted at

Daiichi Sankyo from commercially supplied compounds. Vancomycin hydrochloride was purchased from Sigma-Aldrich.

Bacterial strains. MRSA 12404, MRSA 12412, and imipenem-resistant *P. aeruginosa* 12435 and 12438 (metallo- β -lactamase-negative strains) were isolated in Japanese hospitals in 1999. The reference strains used in this study were stock cultures from our laboratory.

Susceptibility tests. The MICs were determined by a standard microdilution broth method (CLSI, 2006). Mueller–Hinton broth (Becton Dickinson) containing 25 mg Ca²⁺ l⁻¹ and 12.5 mg Mg²⁺ l⁻¹ [cation-adjusted Mueller–Hinton broth (CAMHB)] was used. The MIC was defined as the lowest concentration of the compound that completely inhibited the viable growth of the organism in the microdilution wells. The MIC determination was performed in duplicate.

PAE. The PAE was determined by the viable plate count method (Craig & Gudmundsson, 1996). The organisms were freshly subcultured and incubated at 37 °C with shaking to obtain a final inoculum of approximately 5 $\times$ 10⁶ c.f.u. ml⁻¹. Bacterial suspensions of each organism were then exposed to 10 $\times$ MIC of each compound for 2 h. A culture containing no compound was included as a growth control. At the end of the exposure period, the cultures were diluted 1000-fold with CAMHB in order to remove the compounds. Viable counts were determined for all cultures at this time (time 0). The cultures were placed back in a shaker, with sampling performed every hour on heart infusion agar plates. The 10-fold serially diluted samples (diluted with CAMHB) were pipetted into a sterile Petri plate and 10 ml melted heart infusion agar was added. Three independent tests were carried out for each bacterial strain.

For calculation of the PAE (min), the PAE was defined as $T - C$, where T represents the time required for the count in the compound-exposed culture to increase by 1 log₁₀ above the count observed at time 0, and where C is the corresponding time for the control culture. Each PAE was calculated independently. The mean and its 95% confidence interval for each PAE were determined using SAS system release 8.2.

Abbreviations: MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*; PAE, postantibiotic effect.

Statistical analysis. A *t* test was performed with the hypothesis that the mean PAE of the three experiments was equal to 0 for each compound. A *P* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Tomopenem has enhanced activity against MRSA and *P. aeruginosa*, and improved pharmacokinetic properties in comparison with commercially available carbapenems such as imipenem and meropenem. Carbapenems induce a consistent PAE both *in vitro* and *in vivo* against both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* (Odenholt *et al.*, 1989). However, very few data have been reported regarding the PAE of carbapenems on

MRSA and imipenem-resistant *P. aeruginosa*. Therefore, in this study we determined the PAEs on these pathogens. Since the duration of the PAE increased until a maximum response was obtained, usually at a concentration that was approximately $10 \times \text{MIC}$ (Vogelman & Craig, 1985; Zhanel *et al.*, 1991), we chose this concentration. Table 1 shows the PAEs of tomopenem against *S. aureus* and *P. aeruginosa*. For MSSA, the PAEs of tomopenem were almost the same as those of imipenem, meropenem and ceftazidime. For MRSA 12412, the PAEs of tomopenem were longer than those of vancomycin. MacGowan *et al.* (2008) reported that tomopenem had a superior effect compared to vancomycin in an *in vitro* pharmacokinetic model of MRSA infection, possibly because of its longer PAE. For *P. aeruginosa* ATCC 27853, the PAE of imipenem in the

Table 1. PAE of drugs on *S. aureus* and *P. aeruginosa*

A *t* test was performed for the hypothesis that the mean PAE was equal to 0.

Organism and drug	MIC (mg l ⁻¹)	PAE (min)	SD	95 % confidence interval	<i>P</i> value
<i>S. aureus</i> ATCC 6538P					
Tomopenem	0.06	61	4	50–72	0.0017
Imipenem	0.008	64	3	56–72	0.0008
Meropenem	0.06	56	12	26–87	0.0156
Ceftazidime	1	54	18	10–98	0.0336
<i>S. aureus</i> ATCC 29213					
Tomopenem	0.12	55	14	21–89	0.0199
Imipenem	0.016	59	1	55–62	0.0002
Meropenem	0.12	56	4	46–66	0.0018
Ceftazidime	4	48	8	29–68	0.0086
MRSA 12404					
Tomopenem	4	101	11	74–128	0.0039
Vancomycin	0.5	89	10	63–115	0.0046
MRSA 12412					
Tomopenem	4	192	10	165–218	0.0010
Vancomycin	1	110	15	72–148	0.0063
<i>P. aeruginosa</i> ATCC 15692					
Tomopenem	0.5	66	8	47–85	0.0044
Imipenem	1	100	18	55–145	0.0108
Meropenem	1	60	12	30–90	0.0133
Ceftazidime	2	–41	7	–60– –23	0.0105
<i>P. aeruginosa</i> ATCC 27853					
Tomopenem	0.5	73	17	32–114	0.0168
Imipenem	2	96	23	39–152	0.0181
Meropenem	0.5	64	18	19–110	0.0257
Ceftazidime	2	–45	14	–81– –10	0.0320
IPM-resistant <i>P. aeruginosa</i> 12435*					
Tomopenem	4	79	7	60–97	0.0030
Meropenem	16	71	3	63–79	0.0008
IPM-resistant <i>P. aeruginosa</i> 12438*					
Tomopenem	4	45	8	26–64	0.0097
Meropenem	8	54	4	44–64	0.0018

*Imipenem-resistant *P. aeruginosa* (MIC of imipenem ≥ 16 mg l⁻¹).

current study is similar to that in another report (Odenholt *et al.*, 1989). The PAEs of tomopenem for *P. aeruginosa* were almost the same as those of meropenem, and were slightly shorter than those of imipenem. Ceftazidime showed negative PAEs against two strains of *P. aeruginosa*. Ceftazidime showed a high affinity to PBP 3 and caused a filament formation on Gram-negative bacteria, which therefore produced negative PAEs. On the contrary, Gould *et al.* (1989) expressed the notion that an affinity to PBP 2 is related to the induction of PAE. Since tomopenem binds to PBP 2 in *P. aeruginosa* with high affinity that results in bulge formations on cells (Koga *et al.*, 2009), it showed positive PAEs, as did the other carbapenems. For the imipenem-resistant *P. aeruginosa* tested, the PAEs of tomopenem were almost the same as those of meropenem, and the PAEs on imipenem-susceptible and -resistant isolates were no different. The reason for this is not clear; however, the PAEs may not be different between susceptible and resistant strains on the basis of MIC. Since there have been reports about various types of MRSA (Hanssen & Ericson Solid, 2006) and carbapenem-resistant *P. aeruginosa* (Quale *et al.*, 2006), further study is required on tomopenem against additional strains to provide more detailed information about PAEs.

Tomopenem has a longer half-life (1.7 h) in comparison with imipenem (Drusano & Standiford, 1985) and meropenem (Bax *et al.*, 1989), and the protein binding value of tomopenem in human serum is low (9.5%; Shibayama *et al.*, 2007), similar to those of imipenem and meropenem. Since we have shown that the activity of tomopenem against *S. aureus* and *P. aeruginosa* was minimally affected by twofold with the addition of horse serum (Ohya *et al.*, 2000), it is suggested that serum would have little impact on the PAEs. A single 1400 mg dose in healthy Caucasian volunteers (Shibayama *et al.*, 2006) and a 1500 mg dose in healthy Japanese volunteers (Sesoko *et al.*, 2006) yielded maximum concentrations in serum of 102 ± 15 and 122 ± 14 mg l⁻¹, respectively. At these doses, the concentrations in serum could exceed 40 mg l⁻¹ in about 2 h. Since the MIC of tomopenem against MRSA and the imipenem-resistant *P. aeruginosa* tested was 4 mg l⁻¹, tomopenem may help prevent the regrowth of these bacteria if the drug levels drop below the MIC between doses for these organisms. In addition to the PAEs, the effect of subinhibitory concentrations on bacteria previously treated (the postantibiotic subMIC effect) or not (subMIC effect) is another important parameter (Odenholt-Tornqvist *et al.*, 1991). The post-antibiotic subMIC effect represents the time interval that includes the PAE plus the additional time during which growth is suppressed by subMIC concentrations. The postantibiotic subMIC effect and subMIC effect reflect serum drug levels that may exist between antibiotic dosing intervals and the success of discontinuous dosing regimens. These parameters should also be considered in the event that an optimal dosage regimen for tomopenem can be determined.

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