In Vivo Pharmacodynamic Activity of Tomopenem (formerly CS-023) against *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus aureus* in a Murine Thigh Infection Model

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Tomopenem (formerly CS-023) is a novel carbapenem with broad-spectrum activities against diverse hospital pathogens, including *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA). We examined the in vivo pharmacodynamic characteristics of tomopenem against *P. aeruginosa* and MRSA using a neutropenic murine thigh infection model with *P. aeruginosa* 12467 (MIC, 1 μg/ml) and MRSA 12372 (MIC, 2 μg/ml). The mice had 10^6 to 10^7 CFU/thigh of each strain 2 h after inoculation and were treated for 24 h with a fractionated administration of tomopenem given at intervals of 3, 6, 12, and 24 h. The serum protein binding of tomopenem was 17.4%. The efficacy of tomopenem in both infection models was enhanced by frequent dosing, which indicates that the efficacy is driven by the time above MIC (T_MIC). In a sigmoid model, the cumulative percentages of the 24-h period that the concentrations of free, unbound fractions of the drug exceeded the MIC under steady-state pharmacokinetic conditions (%T_MIC) were best correlated with efficacy when R^2 was 0.79 and 0.86 against *P. aeruginosa* and MRSA, respectively. Other pharmacokinetic and pharmacodynamic (PK-PD) indexes for the free, unbound fractions, the area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC) and the maximum concentration of the drug in serum divided by the MIC (C_max/MIC), showed poor correlation with efficacy when R^2 was ≤0.42. The %T_MIC values required for a static effect, 1-log kill, and 2-log kill against *P. aeruginosa* were 29, 39, and 51, respectively, which were similar to those for meropenem, for which the values were 24, 33, and 45, respectively. Against MRSA, the values for tomopenem were 27, 35, and 47. In conclusion, the pharmacodynamic characteristics of tomopenem were similar to those of meropenem against *P. aeruginosa*, and there was no difference between the target values for *P. aeruginosa* and MRSA required for efficacy in this study.

*Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) are well known as difficult-to-treat pathogens in nosocomial infections (5, 9, 19). Carbapenems are often used for empirical treatments, including those for *P. aeruginosa* infections. However, the activities of commercially available carbapenems against MRSA are not strong enough. Tomopenem has a long half-life, which is almost twice as long as those of other carbapenems, such as imipenem, meropenem (18, 21). In this study, to provide information for its clinical efficacy, we evaluated the in vivo pharmacodynamic activities of tomopenem against *P. aeruginosa* and MRSA. For the purpose of comparison, we also evaluated the activity of MEM against *P. aeruginosa*. (This work was presented at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 25 to 28 October 2008 [23].)

**MATERIALS AND METHODS**

Bacteria, media, and compounds. Clinical isolates of *P. aeruginosa* 12467 and MRSA 12372 were used for this study. These strains were isolated from the sputum and blood, respectively, of patients in Japan in 1999. The MICs of tomopenem and MEM were tested by the broth microdilution method (6) and were 1 and 2 μg/ml, respectively, against *P. aeruginosa* 12467. The MIC of
FIG. 1. Concentration-time profiles of the free fractions of tomoopenem (A) and meropenem (B) in plasma after single administrations at doses of 50, 100, 200, 400, and 800 mg/kg with a fixed dose of cilastatin at 40 mg/kg in a mouse infection model. Each symbol represents the mean ± the standard deviation (n = 4).

TABLE 1. Pharmacokinetic parameters for tomoopenem and meropenem after single administrations at different doses and a fixed dose of cilastatin in a mouse infection modela

<table>
<thead>
<tr>
<th>Drug and dose (mg/kg)</th>
<th>t1/2 (h)</th>
<th>fCmax (µg/ml)</th>
<th>AUC0–inf (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomopenem/cilastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50/40</td>
<td>0.24</td>
<td>44.1</td>
<td>32.4</td>
</tr>
<tr>
<td>100/40</td>
<td>0.27</td>
<td>92.5</td>
<td>73.7</td>
</tr>
<tr>
<td>200/40</td>
<td>0.39</td>
<td>140</td>
<td>134</td>
</tr>
<tr>
<td>400/40</td>
<td>0.41</td>
<td>230</td>
<td>261</td>
</tr>
<tr>
<td>800/40</td>
<td>0.43</td>
<td>346</td>
<td>484</td>
</tr>
<tr>
<td>Meropenem/cilastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50/40</td>
<td>0.24</td>
<td>42.8</td>
<td>27.6</td>
</tr>
<tr>
<td>100/40</td>
<td>0.18</td>
<td>76.8</td>
<td>51.1</td>
</tr>
<tr>
<td>200/40</td>
<td>0.30</td>
<td>175</td>
<td>138</td>
</tr>
<tr>
<td>400/40</td>
<td>0.28</td>
<td>303</td>
<td>280</td>
</tr>
<tr>
<td>800/40</td>
<td>0.50</td>
<td>456</td>
<td>551</td>
</tr>
</tbody>
</table>

a Cilastatin was given at a dosage of 40 mg/kg. All parameters are for free, unbound fractions of the drugs.

FIG. 2. Relationship between the tomoopenem dose level and its in vivo efficacy in a murine thigh infection model with P. aeruginosa (A) and MRSA (B). Each symbol represents the mean ± the standard deviation (n = 4).

centrifugation of the heparinized blood samples, the plasma was immediately separated and diluted with an equal volume of 500 mM 3-morpholinopropanesulfonic acid buffer (pH 6.0). The plasma concentrations were determined by using high-performance liquid chromatography with UV detection as reported previously (17). The range of concentrations in plasma used for quantification of both compounds was from 0.5 µg/ml up to 1,000 µg/ml. The reproducibility and accuracy of this method were evaluated by using quality control samples, which ensured the accuracy of batch analysis, spiked at concentrations of 1.5, 150, and 750 µg/ml. The reproducibilities of the interassays for tomoopenem and MEM did not exceed 6.9% and 2.9%, respectively. The accuracies of the interassays ranged from 95.6% to 111% for tomoopenem and from 98.5% to 114% for MEM. The intra-assay reproducibilities ranged from 95.6% to 100% for tomoopenem and 99.9% to 108% for MEM. The intra-assay precisions for tomoopenem and MEM did not exceed 2.2% and 0.2%, respectively. Regarding the specificity of the method, the linearity of the standard curve and the stability of both compounds in plasma from preparation to the end of the analysis were confirmed.

Pharmacodynamic studies. The mice were treated for 24 h with a fractionated administration of tomoopenem and MEM given at intervals of 3, 6, 12, and 24 h from 2 h after inoculation. Viable cell counts in the thighs were determined following a 24-h treatment period. Untreated control mice received sterile saline and were sacrificed at the initiation of therapy and after 24 h of saline treatment.

Data analysis. The concentrations of the free, unbound fractions of the drugs in the mouse plasma were calculated by using protein binding ratios of 17.4% and 33.8% for tomoopenem and MEM, respectively (22, 24). The mean value of four plasma concentrations at each time point was used for calculation of the PK parameters. The free-drug concentration–time profiles were analyzed using WinNonlin professional software (version 4.0.1; Pharsight Corp.). A one-compartment model with the various dosages was fit to the observations. The best-fit model was determined by Akaike’s information criteria. The cumulative percent-age of the 24-h period that the drug concentration exceeded the MIC under steady-state pharmacokinetic conditions (%T >MIC) was calculated using SAS System release 8.2 software (SAS Institute, Inc.), and the %MIC for the free, unbound drug (%T >MIC) was also calculated with the ratios for protein binding in mouse sera described above. The values for half-life (t1/2), Cmax, and AUC for 0 h to infinity (AUC0–∞) at various dosages were determined using a noncompart-mental model with extravascular input in WinNonlin professional software. A sigmoid maximum effect (Emax) model was used to examine the relationship between each PK–PD index, %T >MIC, fC >MIC, and (AUC/MIC) and the efficacy of the drug. For each PK–PD index, nonlinear regression analysis using the following sigmoid model was applied: viable cell count in the thighs (log10 CFU/thigh) = E0 − (Emax × D) [(ED50)n + D2], where n is the sigmoidicity factor, E0 is the minimum effect (mean of the viable cell counts in the thighs of the saline group), Emax is the maximum effect, D is the value of the PK–PD parameter, and ED50 is the PK–PD index value required to achieve the 50% effective dose, (E0 − Emax)/2. From the sigmoid Emax model, the coefficient of the determinant (R2) and the magnitudes of the PK–PD index required for a static effect, 1-log kill, and 2-log kill related to efficacy were calculated. The analysis was performed using SAS System release 8.2 software.
RESULTS

Pharmacokinetics. The concentration-time profiles of the free fractions of tomopenem and MEM in plasma administered subcutaneously at 50, 100, 200, 400, and 800 mg/kg in a murine thigh infection model by \textit{P. aeruginosa} 12467 are shown in Fig. 1. The pharmacokinetic parameters of both compounds are shown in Table 1. Over the range of the single doses tested, the AUC\textsubscript{0-inf} of the free fraction of tomopenem increased proportionally with the dose, but the \(C_{\text{max}}\) of the free drug did not and the \(t_{1/2}\) was not constant, suggesting that the pharmacokinetics of tomopenem were not linear after subcutaneous administration in the mice. The same characteristics were observed for MEM. The differences in the administration volume was thought to be one reason for these nonlinear pharmacokinetics; therefore, we used these 5 doses for multiple administrations of both compounds in the following studies. In order to investigate how to calculate the free fraction of the AUC\textsubscript{0-inf} after multiple administrations, we calculated how much greater the AUC\textsubscript{0-inf} for the free drug was after 8 administrations at 200 mg/kg than it was after a single administration of tomopenem or MEM at the same dose. As the increasing ratio was below 1%, the AUC\textsubscript{0-inf} of the free drug after multiple administrations was calculated by multiplying the AUC\textsubscript{0-inf} of the free drug for a single administration by the dose number. The \(C_{\text{max}}\) of the free drug was not changed by multiple administrations (data not shown). Extrapolated ratios of the AUC\textsubscript{0-inf} at 800 mg/kg of tomopenem and MEM were 6.9% and 8.8%, respectively, compared to the AUC\textsubscript{0-2 h}, for which the ratios were calculated by WinNonlin software using a noncompartment model. Since the \(t_{1/2}\) at a higher dose had a tendency to be longer, as shown in Table 1, the extrapolation ratio should be higher at a higher dose. However, the extrapolation at the highest dose was below 8.8%, indicating that the calculated AUC\textsubscript{0-inf} was not overextrapolated and was suitable for the determination and comparison of PK-PD parameters of both compounds.

PK-PD index determination. The relationships between the tomopenem dose level and the \textit{in vivo} efficacy in a murine thigh infection model with \textit{P. aeruginosa} 12467 and MRSA 12372 are shown in Fig. 2, and those between the MEM dose level and \textit{in vivo} efficacy are shown in Fig. 3. At the start of therapy, the mice had 6.32 and 6.85 log\textsubscript{10} CFU of \textit{P. aeruginosa} 12467 and MRSA 12372, respectively. The two strains grew to 8.74 (\textit{P. aeruginosa} 12467) and 8.24 log\textsubscript{10} (MRSA 12372) CFU after 24 h of treatment in the untreated control mice. Even though a single dose of 800 mg/kg tomopenem did not reduce the viable cell counts, every-3-h doses of 100 mg/kg (a total of 800 mg/kg) reduced them by more than 4 and 2 log\textsubscript{10} CFU against \textit{P. aeruginosa} 12467 and MRSA 12372, respectively. Overall, as in the case of MEM against \textit{P. aeruginosa} 12467, the efficacy of tomopenem against both strains was enhanced by frequent dosing, which indicates that the efficacy is driven by the \(T_{\text{MIC}}\).

The relationships between each free-drug PK-PD index, \(f\%T_{\text{MIC}}\), \(fC_{\text{max}}/\text{MIC}\), and \(f\text{AUC}/\text{MIC}\), and the efficacies of tomopenem against both strains are shown in Fig. 4 and those for MEM against \textit{P. aeruginosa} 12467 are shown in Fig. 5.

FIG. 3. Relationship between the meropenem dose level and its \textit{in vivo} efficacy in a murine thigh infection model with \textit{P. aeruginosa}. Each symbol represents the mean ± the standard deviation (\(n = 4\)).
The best correlation with the MRSA infection model. The results for MEM also showed the best relationship with the MRSA infection model and with the P. aeruginosa infection model. The results for MEM also showed the best correlation with fT/MIC, with an R² of 0.74 in the P. aeruginosa infection model.

Magnitude of PK-PD index required for efficacy. The magnitudes of fT/MIC required for the in vivo efficacies of toomopenem and MEM are shown in Table 2. The fT/MIC values of toomopenem required for a static effect, 1-log kill, and 2-log kill against P. aeruginosa 12467 were 29, 39, and 51, respectively, which were similar to those for MEM, which were 24, 33, and 45, respectively. Against MRSA 12372, the values for toomopenem were 27, 35, and 47, respectively, which were similar to those for MEM, which were 24, 33, and 45, respectively. Against MRSA 12372 and MRSA 12467 were almost equal, whereas toomopenem required a fT/MIC of 27% (2, 8, 11, 12) in the in vitro study.

FIG. 5. Relationship between the PK-PD index and the in vivo efficacy of meropenem against P. aeruginosa. The dotted lines indicate the viable cell counts at the start of therapy. NC*, not calculable (nonlinear regression using the sigmoid E_max model failed to converge).

discussion

For antimicrobial agents, PK-PD analysis using a murine infection model has become a standard method to predict clinical efficacy and is often used for the determination of optimal doses for clinical trials. This method was established by Craig et al. (2, 4, 8). However, it remains difficult to evaluate in vivo efficacy for humans in a murine model because plasma exposure in humans may be higher than that in mice. Craig and Andes and Nicolau et al. used uranyl nitrate to induce renal impairment and obtain a long half-life for beta-lactams in mice (1, 13). As the half-lives of toomopenem and MEM were also as short in mice as those reported for other carbapenems (15, 26), we used cilastatin to obtain longer half-lives by inhibiting murine dehydropeptidase I. Moreover, we used doses equal to or higher than 50 mg/kg to obtain an adequate amount of T/MIC at each administration. To evaluate the PK-PD index required for efficacy and its target value properly, these approaches are necessary in case the pharmacokinetics in mice is much shorter than in humans.

The PK-PD indexes of beta-lactams required for efficacy are known to be the T/MIC. In this study, we demonstrated that the efficacy of toomopenem is driven by the T/MIC and that the magnitude necessary for a static effect against P. aeruginosa is similar to that of MEM. In the in vitro study, the bactericidal activity and postantibiotic effect (PAE) of toomopenem against P. aeruginosa were shown to be as strong as those of MEM (16, 25). The values of magnitude required for efficacy against both P. aeruginosa and MRSA were almost the same in this study. In the in vitro study, toomopenem also showed strong bactericidal activity and PAE against MRSA, similar to that against P. aeruginosa. These in vitro activities may support the results of our study.

The exposure to toomopenem required for a static effect is similar to those for commercially available carbapenem and doripenem against P. aeruginosa and MRSA (10, 13). On the other hand, current anti-MRSA beta-lactams seem to require less exposure for a static effect against MRSA than toomopenem. The anti-MRSA cephalosporins, RWJ-54428, ceftobiprole, and ceftaroline (PPI-0903), as well as carbapenem SMP-601, were shown to achieve static effects with fT/MICs of 14 to 20%, 23 to 25%, 15 and 21%, and 23%, respectively, whereas toomopenem required a fT/MIC of 27% (2, 8, 11, 12). Although it is not clear whether this difference is significant or not, the different strains used in these experiments might be one of the reasons for the difference. It is important to directly compare these compounds and to evaluate the efficacies such as killing ratios and PAEs in depth if there are any differences.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tomopenem</th>
<th>Meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fT/MIC</td>
<td></td>
</tr>
<tr>
<td>MRSA 12372</td>
<td>27 (24–29)</td>
<td>35 (31–37)</td>
</tr>
</tbody>
</table>

a ND, not determined.
To improve the efficacies of compounds related to the $T_{50}\%$ it is clear that the length of the half-life and a low MIC are important factors. Considering the longer elimination half-life of tomopenem and its potent activity, it is expected to be more effective than other carbapenems against diverse hospital pathogens, including *P. aeruginosa* and MRSA. Although further study is needed to provide more detailed information regarding the clinical efficacy of tomopenem against various strains, it is thought to be a promising compound for nosocomial infections.

REFERENCES


