Comparative In Vitro Pharmacodynamics of Imipenem and Meropenem against *Pseudomonas aeruginosa*

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MICs are commonly used to assess the in vitro activities of antimicrobial agents; however, they provide minimal information on the pattern of bacterial activities. Time-kill studies with extensive sampling allow assessment of both the rate and extent of bacterial killing and regrowth. We compared imipenem and meropenem by both MIC-MBC testing and a time-kill study with *P. aeruginosa* 27853. In the time-kill study, concentration/MIC ratios ranging from 0.0625 to 32 times the MIC were studied. The kill rate, time to 99.9% kill, doubling time of regrowth, and area under the bacterial killing curve (AUKC) were evaluated. Degradation during the testing procedure was accounted for by assessing actual drug exposure as determined by the area under the concentration-time curve. Pharmacodynamic parameters were compared by using the Wilcoxon signed-rank test. The modal MIC and MBC for imipenem were 2 and 4 μg/ml, respectively, and those for meropenem were 0.25 and 0.5 μg/ml, respectively. In the time-kill study, both agents displayed concentration-dependent activity over a range of 0.25 to 4 times the MIC. Initial killing (0 to 1 h) was faster with imipenem at the same concentration/MIC ratios (*P* = 0.0506). The time to 99.9% kill was approximately 5 h for both agents. When regrowth occurred, the doubling rate for imipenem, which was the same as that for the growth control, was twice as rapid as that for meropenem. At the same concentrations, the AUKCs over 24 h were lower for meropenem than for imipenem (*P* = 0.0280); however, when normalized by MIC, imipenem resulted in smaller AUKCs. Comparison of plots of area under the concentration-time curve versus AUKC, which accounted for drug degradation and actual drug exposure, revealed that meropenem was three times more active than imipenem, rather than the eightfold difference suggested by MICs. Time-kill curves with extensive sampling and measurement of actual drug exposure, rather than traditional MIC testing, may more accurately assess differences in the in vitro activities of antimicrobial agents.

Since in vitro susceptibility test results have been correlated with clinical outcome (11), these tests are utilized to assess the potential efficacy of an antimicrobial agent and as a method to distinguish between agents with similar antimicrobial spectra. MICs are commonly used to assess the in vitro activity of an antimicrobial agent against a microorganism; however, sole reliance on the MIC may be misleading, since it gives no information regarding the pattern of killing over the time period of the test. Time-kill curves allow one to determine the pattern of bacterial killing and regrowth and allow the calculation of pharmacodynamic parameters such as the rate and extent of killing. A calculated parameter, the area under the bacterial killing curve (AUKC), measures the in vitro effect of an antimicrobial agent for the duration of drug exposure; however, this technique requires frequent sampling and is currently limited to the research setting.

Meropenem, a carbapenem currently in phase III investigational trials, is very similar to imipenem. The two agents have similar pharmacokinetic profiles (1), postantibiotic effects (8, 12), and spectra of activity (9, 20). They display activity against a wide range of aerobic gram-positive, aerobic gram-negative, and anaerobic bacteria. Imipenem appears to be more potent against gram-positive organisms; however, meropenem appears to be more potent against gram-negative organisms, especially *Pseudomonas aeruginosa*. Affinity for penicillin-binding proteins (PBPs) differs for these agents. Generally, imipenem has high affinity for PBPs 1A and 2. Meropenem also attaches to PBP 2, although its affinity for PBP 3 is much higher than that of imipenem. Attachment of PBP 2 results in the formation of spheroplasts, whereas affinity for PBP 3 results in the development of filamentous forms (19). These differences in affinity for PBPs may explain the differences in the relative potencies of these agents and may affect their pharmacodynamic profiles. Other time-kill studies of imipenem and meropenem with *P. aeruginosa* have demonstrated concentration-dependent killing over a range of 0.5 to 8 times the MIC (5, 21). However, the number of sampling points and range of concentrations studied precluded an accurate assessment of the AUKC or early killing rates as well as the determination of the absolute minimal and maximal effects of these agents. Furthermore, previous studies have not accounted for differences in drug degradation during the testing procedure. Since we have documented the degradation of these agents under in vitro testing conditions (7), we compared imipenem and meropenem in a time-kill study with extensive sampling over a wide concentration range and accounted for drug degradation in the analysis.

**MATERIALS AND METHODS**

Antibiotics, bacterial strain, and media. Standard laboratory powders of meropenem (ICI Pharmaceuticals, Wilmington, Del.; lot W6275 [expiration date, 2/92]) and imipenem (Merck, Sharp, and Dohme, West Point, Pa.; lot 8064T [expiration date, 2/92]) were used in this study. All studies were performed with *P. aeruginosa* ATCC 27853. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.; lot 0757-01) was prepared immediately prior to use and supplemented with CaCl2 (25 μg/ml) and MgSO4 (12.5 μg/ml), according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (14). An-
RESULTS

MIC-MBC testing. By traditional twofold dilutions, the MICS of imipenem and meropenem were 2 and 0.25 μg/ml and the MBCs were 4 and 0.5 μg/ml, respectively. By intermediate dilution, the MICS were 3 and 0.375 μg/ml and the MBCs were 3 and 0.75 μg/ml for imipenem and meropenem, respectively.

Time-kill plots. For clarity, only data from every other drug concentration at 0, 2, 4, 8, 12, 18, and 24 h are graphically displayed. Both agents demonstrated killing of approximately 3 to 4 log units (Fig. 1 and 2). It appeared that bacterial killing was multiphasic for both imipenem and meropenem; however, the number of data points precluded an accurate assessment of these kill rates. Regrowth occurred with both agents at concentrations at and below the MIC and reached a plateau at approximately 10^2 CFU/ml. Regrowth was slower for meropenem than for imipenem, with median doubling times of 0.38 and 0.73 h for imipenem and meropenem, respectively. The doubling rate for the growth control was 0.39 h. At concentrations above the MIC, killing was maintained throughout the 24-h study period, with the exception of meropenem at 0.5 μg/ml (2 times the MIC).

Over the concentration ranges for both imipenem and meropenem, the pH was 7.2 at the time of maximal bacterial killing and increased to 7.4 when regrowth reached a plateau. Following exponential growth in the growth control, the pH increased to a maximum of 7.9.

Concentration-dependent activity over approximately the same range of concentrations of imipenem and meropenem as shown with the colony counts was demonstrated with absorbance values (data not shown). However, prior to 3 h, drug effects were undetectable, and the spectrophotometric data represented early morphologic changes or regrowth after early bacterial killing.

Pharmacodynamic parameter analysis. As seen in Tables 1 and 2, the median time to reach 99.9% killing of the initial inoculum was approximately 5 h and was similar for the two
agents whether they were evaluated at the same absolute concentration or the same concentration/MIC ratio. There was no difference in the kill rate from 0 to 1 h between imipenem and meropenem when they were evaluated at the same absolute concentrations (Table 1); however, as shown in Table 2, at the same concentration/MIC ratio, imipenem was more rapidly bactericidal ($P \leq 0.0506$). The $\text{AUKC}_{0–1}$ was smaller (indicating greater bactericidal activity) for imipenem at the same concentration/MIC ratio ($P \leq 0.0069$). In the $\text{AUKC}_{0–24}$ analysis, which may incorporate regrowth of the organism, the median $\text{AUKC}$ was larger for imipenem at the same concentration ($P \leq 0.0280$). However, at the same concentration/MIC ratio, the $\text{AUKC}$ was smaller for imipenem. It should be noted that although samples below the theoretical limit of detection were included in the $\text{AUKC}_{0–24}$ analysis, their contribution to the $\text{AUKC}$ was negligible.

Concentration-dependent bactericidal activity was seen with both imipenem and meropenem (Fig. 3 and 4). Both agents achieved maximal bactericidal activity at the same $\text{AUKC}_{0–24}$; however, meropenem had maximal activity at a three- to fourfold lower AUC (Fig. 3). When normalized for the traditional twofold dilution MIC (AUC/MIC), imipenem displayed a steeper concentration-effect curve, was slightly more active (lower $\text{AUKC}$), and reached its maximum effect at an $\text{AUC}_{0–24}$/MIC ratio of approximately 30. Meropenem displayed concentration-dependent activity over a wider range and reached the maximum effect at an $\text{AUKC}_{0–24}$/MIC ratio of approximately 80 (Fig. 4). The use of intermediate-dilution MICs would produce the same relative differences in these relationships for imipenem and meropenem.

**DISCUSSION**

$\beta$-Lactams are not considered to display concentration-dependent activity; however, this usually refers to activity at concentrations above the MIC. This study verifies the findings of others which demonstrated concentration-dependent activity of imipenem and meropenem over a range of concentrations (5, 21). For both drugs, concentrations of $\geq 4$ times the MIC resulted in killing of 3 log units that was maintained for 24 h. In contrast, Flukiger et al. (6) observed no concentration-dependent killing by imipenem of *P. aeruginosa* when comparing concentrations of 2 and 10 times the MIC. We noted substantial regrowth at the MIC; however, at two times the MIC, regrowth was observed only with meropenem. When regrowth occurred, it occurred earlier and was more rapid with imipenem. The doubling time of imipenem-exposed *P. aeruginosa* was virtually identical to that of the growth control. These concentration-dependent effects on regrowth were verified by the spectrophotometric analysis. Other investigators have demonstrated effects of carbapenems at concentrations below the MIC (15, 21). Our findings were similar, with demonstrable

**TABLE 1. Comparison of pharmacodynamic parameters evaluated at the same drug concentrations**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Time to 99.9% kill (h)$^a$</th>
<th>Kill rate from 0 to 1 h (h$^{-1}$)$^b$</th>
<th>$\text{AUKC}_{0–1}$ (CFU · h/ml, 10$^5$)$^c$</th>
<th>$\text{AUKC}_{0–24}$ (CFU · h/ml, 10$^6$)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>5.5 (2–6.0)</td>
<td>3.5 (0.8–5.5)</td>
<td>1.37 (0.64–1.80)</td>
<td>58,400 (1.94–302,000)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4.5 (4.5–5.0)</td>
<td>3.6 (1.9–5.7)</td>
<td>2.14 (1.01–2.68)</td>
<td>3.38 (1.98–43,200)</td>
</tr>
</tbody>
</table>

$^a$ All data presented as medians with ranges in parentheses.

$^b$ $n = 3$ (2 to 8 µg/ml).

$^c$ $n = 6$ (0.25 to 8 µg/ml).

$^d$ $n = 7$ (0.125 to 8 µg/ml).

$^e$ $P = 0.0280$. 

FIG. 2. Time-kill plots for imipenem and meropenem at and below the MIC. ○, imipenem at the MIC (2 µg/ml); ●, meropenem at the MIC (0.25 µg/ml); □, imipenem at 1/4 of the MIC (0.5 µg/ml); ■, meropenem at 1/4 of the MIC (0.0625 µg/ml); □, imipenem at 1/16 of the MIC (0.125 µg/ml); ▲, meropenem at 1/16 of the MIC (0.0156 µg/ml); ×, growth control. The dotted line indicates the limit of detection.

FIG. 3. AUC$_{0–24}$ for imipenem (○) and meropenem (●) versus effect ($\text{AUKC}_{0–24}$).
Traditionally, the MIC and/or MBC has been used to assess the in vitro activities of antimicrobial agents. However, these measurements are taken at a fixed time (usually 18 h) after the initiation of drug exposure and fail to assess the effects of initial bacterial killing rates or varying rates of regrowth. Time-kill curves allow one to assess both the AUKC as a measure of overall drug exposure effects and killing rates early after the initiation of drug exposure. Several studies have correlated the rate of in vitro bactericidal killing to outcome in animal models of infection. Drake and coworkers (3) found that the in vitro activities of antimicrobial agents. However, these differences may be explained by the relative affinities for PBPs 2 and 3 and the development of filamentous forms with meropenem (19).

Animal and human studies have shown that the length of time that the concentration of a β-lactam in serum remains above the MIC is the pharmacodynamic parameter that is the best predictor of clinical outcome (10, 18). This parameter is difficult to assess in in vitro tests such as MIC and time-kill tests that involve fixed concentrations of antimicrobial agents. Although it is usually assumed that this initial drug concentration is maintained throughout the test, we have documented degradation of imipenem and meropenem during time-kill testing (7). This decline in drug concentration during the test allows one to calculate the length of time that the drug concentration remains above the MIC; however, the twofold dilutions utilized in this study limited this analysis. Since the MIC of imipenem was 2 μg/ml, initial imipenem concentrations of ≥2 μg/ml remained at or below the MIC for the duration of the time-kill test. Since the half-life of degradation of imipenem was approximately 14 h, all initial imipenem concentrations above 8 μg/ml remained above the MIC for the duration of the test (24 h). Therefore, only the initial concentration of 4 μg/ml, for which the time that the drug concentration remained above the MIC was 14 h, revealed any information concerning the effect of the time that the concentration remained above the MIC. With this concentration, the AUC was only slightly higher than that seen with initial concentrations of ≥8 μg/ml. Because of the slower rate of degradation with meropenem (half-life of approximately 38 h), initial concentrations of ≥0.5 μg/ml remained above the MIC, whereas concentrations of ≥0.25 μg/ml were at or below the MIC for the entire 24-h period. Thus, effects of time that the drug concentration remained above the MIC with meropenem could not be assessed.

The AUC allows one to measure drug exposure during an in vitro test. If drug concentrations are constant throughout the test, the AUC would not provide any information different from the initial drug concentration, since it would simply result from multiplying the concentration by the length of time of the test. However, since drug concentrations declined during the test, the AUC would assess drug exposure more accurately than the initial drug concentration. When the AUCs of imipenem and meropenem were plotted against the AUKCs, meropenem was only three times more active than imipenem.
however, when correction for degradation is not done, it appears that meropenem is at least four times more active than imipenem. These plots of AUC versus AUKC represent the effects of actual drug exposure over time and are likely indicative of the true differences in potency between these two agents as opposed to simply comparing MBCs. Moreover, if one used MIC results to compare these agents, meropenem would appear to be eight times more active than imipenem.

In summary, meropenem and imipenem both display concentration-dependent activity over a wide range of concentrations below the MIC and up to fourfold above the MIC. Imipenem kills at a faster initial rate than meropenem; however, both maintain killing of ≥3 log units at concentrations of ≥4 times the MIC. With correction for the differences in drug degradation and measurement of the effects of drug exposure with the AUKC, meropenem appears to be approximately three times more potent than imipenem against this strain of *P. aeruginosa*, rather than the eightfold difference suggested by MIC testing.

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**REFERENCES**


