Postantibiotic Leukocyte Enhancement of Meropenem against Gram-Positive and Gram-Negative Strains

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The postantibiotic leukocyte enhancement (PALE) of meropenem in vitro in comparison with that of imipenem was evaluated with 24 recently isolated gram-positive and gram-negative strains. In general, pre-exposure to carbapenems (at four times the MIC for 2 h) led to increased polymorphonuclear cell phagocytic killing. The PALE of imipenem was generally significantly less than that observed with meropenem.

The pharmacodynamic aspects of antimicrobial drugs, such as the postantibiotic effect (PAE), have been extensively studied in the last 15 years with gram-positive and gram-negative bacteria in order to analyze their possible influence on antibiotic dosage regimens (3). Bacterial susceptibility to leukocyte phagocytosis and killing is usually enhanced when organisms are in the PAE phase (11, 12, 19, 20). The occurrence of significant postantibiotic leukocyte enhancement (PALE) in vitro due to different antibiotics such as quinolones or aminoglycosides has been fully established, while fewer data are available for β-lactams (12, 19, 20). Usually, these antibiotics produce a PAE only against gram-positive strains and have a short or no PAE on gram-negative rods (22). On the contrary, different authors have demonstrated that carbapenems induce a consistent PAE both in vitro and in vivo against both gram-positive and gram-negative strains, including Pseudomonas aeruginosa (1, 3, 6, 7, 15, 18).

Meropenem is a new carbapenem which differs from imipenem in having a dimethylcarbamoylpyrrolidinethio side chain at C-2 which assures it a broad spectrum of activity against aerobes and anaerobes (9, 17). This carbapenem derivative has a pharmacokinetic profile very similar to that of imipenem (3, 7, 15, 18).

Therefore, the aim of this study was to investigate the occurrence of a PALE of meropenem against gram-positive and gram-negative pathogens in comparison to imipenem, which may be considered the reference drug among carbapenems.

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Meropenem was supplied by Zeneca S.p.A., Milan, Italy. Imipenem was kindly provided by Merck Sharp & Dohme International. Both antibiotics were obtained as reference powders with known potencies. The two carbapenems were solubilized in sterile distilled water. Fresh dilutions were prepared on the day of use.

Twenty-four recent clinical isolates of methicillin-susceptible Staphylococcus aureus (MSSA), Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli, Enterobacter cloacae, and P. aeruginosa (four strains of each group) were selected. Only one isolate from each patient was tested to avoid multiple copies of the same strain.

MICs were determined in accordance with National Committee for Clinical Laboratory Standards guidelines (16). PAE was determined as previously described (19). Briefly, bacteria were exposed to meropenem or imipenem at four times the MIC for 2 h with continuous shaking. The antibiotic was removed by filtration. Bacterial cells were washed three times and diluted into fresh prewarmed medium. Culture turbidity was monitored spectrophotometrically at 620 nm, and bacteria were centrifuged and resuspended at a concentration of 5 × 10^7 organisms/ml. Polymorphonuclear cells (PMNs) were prepared from heparinized blood from healthy volunteers by sedimentation on 2.5% dextran T500 (Pharmacia) in 0.9% NaCl at 37°C for 30 min. The supernatant was layered onto Ficoll-Hypaque (Pharmacia) and centrifuged at 800 × g for 20 min. The neutrophil pellet was washed twice in sterile heparinized saline, and erythrocytes were removed by hypotonic lysis in water. PMNs were resuspended in Hanks balanced salt solution (Gibco, Ltd.) with 0.1% gelatin (pH 7.4) to a final concentration of 2 × 10^6 cells/ml. Viability was evaluated by 0.2% trypan blue exclusion and was greater than 95%. PMNs (10^7 cells in 0.5 ml) were added to tubes containing bacterial cells (approximately 2 × 10^7 organisms) and 10% pooled human serum to give a ratio of bacteria to PMNs of 2:1 and incubated at 37°C under rotation. Controls without PMNs were included. Killing of antibiotic-treated and untreated bacteria by PMNs was determined during incubation at 0, 2, and 4 h by adding 0.1-ml aliquots from tubes to 9.9 ml of cold sterile water to disrupt PMNs. Bacterial counts were done by plating serial dilutions on Mueller-Hinton agar. S. pneumoniae and H. influenzae strains were plated on tryptone soy agar (Difco, Ltd.) with the addition of 5% sheep blood. Results were expressed as log_{10} differences (mean ± standard deviation) between the total counts of viable bacteria at 0 and 2 or 4 h after exposure to PMNs in at least four experiments for each strain tested (12, 19, 20). Data were analyzed using NCSS (version 5.03) statistical analysis software. Differences in PAE between the two carbapenems were determined by analysis of variance. Individual differences within groups were analyzed with Tukey’s biweight test.

MICs of meropenem and imipenem for the chosen strains are shown in Table 1. Meropenem was generally 2- to 32-fold more active than imipenem on gram-negative rods (with the exclusion of H. influenzae strains).
Table 2 shows the data regarding potentiation of the bactericidal activity of PMNs determined by pre-exposure to meropenem and imipenem. Pre-exposure to meropenem led to a 3- to 6-fold increase in phagocytic killing of MSSA strains in comparison with the control organisms, while with S. pneumoniae strains, the potentiation ranged from approximately 8- to 22-fold. The PALE of meropenem was significantly greater than that of imipenem. The latter antibiotic did not modify or had a limited effect on the susceptibility of these gram-positive strains to PMN killing.

In general, pre-exposure of gram-negative strains to carbapenems led to increased PMN phagocytic killing, although imipenem pretreatment of P. aeruginosa strains failed to increase their susceptibility to the bactericidal activity of PMNs.

Exposure to meropenem produced a consistent PALE with increases in phagocytic killing of approximately 7- to 10-fold for P. aeruginosa, 30- to 57-fold for enterobacteria, and 40- to 100-fold for H. influenzae. The imipenem PALE (with the exclusion of E. coli at 2 h) was always significantly less than that of meropenem (Table 2).

Our data on the in vitro activities (MICs) of meropenem and imipenem against selected gram-positive and gram-negative strains show that the antimicrobial activity of meropenem is superior to that of imipenem against gram-negative rods, confirming the results of a previous study (17).

Our results indicate that carbapenems, after 2 h of exposure to concentrations four times the MICs, render the chosen bacterial strains more susceptible to the bactericidal activity of PMNs (with the exclusion of P. aeruginosa strains pretreated with imipenem), thus demonstrating PALE.

Pre-exposure of gram-positive and gram-negative strains to meropenem caused a dramatic enhancement of their susceptibility to phagocytic killing which was significantly greater than that caused by imipenem. For example, the results obtained with E. coli strains show that while 15 to 35% of untreated (control) bacteria remained viable 2 or 4 h after exposure to PMNs and serum, only 0.5 to 1.0% of the residual bacteria pretreated with meropenem were capable of evading leukocyte killing.

Pruul and McDonald showed that even after a shorter exposure (10 to 20 min) to high doses of antibiotics (i.e., quinolones and chloramphenicol), E. coli strains become more susceptible to the killing activity of phagocytes (20). Moreover,

<table>
<thead>
<tr>
<th>Organism</th>
<th>Geometric mean MIC (mg/liter)</th>
<th>Imipenem</th>
<th>Meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>0.03</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>0.15</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>H. influenzae</td>
<td>0.25</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>0.5</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>E. cloacae</td>
<td>1.0</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>3.25</td>
<td>1.25</td>
<td></td>
</tr>
</tbody>
</table>

* Each MIC was determined by broth dilution test with an inoculum of $5 \times 10^5$ bacteria. Four isolates in each group were tested.

TABLE 2. PALE on gram-positive and gram-negative strains after bacterial exposure to imipenem and meropenem

<table>
<thead>
<tr>
<th>Organism</th>
<th>Change in log CFU/ml for control</th>
<th>$P$ value for control vs imipenem</th>
<th>Change in log CFU/ml for imipenem</th>
<th>$P$ value for imipenem vs meropenem</th>
<th>Change in log CFU/ml for meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>7.33 ± 0.31</td>
<td>&lt;0.05</td>
<td>7.66 ± 0.32</td>
<td>&lt;0.05</td>
<td>7.28 ± 0.22</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>7.29 ± 0.33</td>
<td>&lt;0.01</td>
<td>7.29 ± 0.23</td>
<td>&lt;0.01</td>
<td>7.22 ± 0.39</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>7.19 ± 0.29</td>
<td>&lt;0.01</td>
<td>7.19 ± 0.32</td>
<td>&lt;0.01</td>
<td>7.16 ± 0.23</td>
</tr>
<tr>
<td>E. coli</td>
<td>7.33 ± 0.15</td>
<td>&lt;0.01</td>
<td>7.55 ± 0.37</td>
<td>&lt;0.05</td>
<td>7.24 ± 0.13</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>7.37 ± 0.31</td>
<td>&lt;0.01</td>
<td>7.37 ± 0.35</td>
<td>&lt;0.05</td>
<td>7.27 ± 0.27</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>7.17 ± 0.17</td>
<td>&lt;0.01</td>
<td>7.12 ± 0.38</td>
<td>&lt;0.05</td>
<td>7.23 ± 0.25</td>
</tr>
</tbody>
</table>

* Bacteria were exposed to each carbapenem at four times the MIC for 2 h. Results are expressed as the mean ± the standard deviation of data from at least 16 experiments for each species (four experiments for each strain).

b Determined by analysis of variance.

c NS, no statistically significant difference.
these pharmacodynamic phenomena.

One possible explanation for a greater PAE in vivo than in vitro may be partly the interaction of the antibiotic with the host defenses or modification of the bacterial cell surface during pre-exposure, which renders the microorganisms more susceptible to phagocytosis and intracellular killing, thus supporting the occurrence of PALE (3, 4).

Carbapenems possess relatively consistent PAEs, both in vitro and in vivo, even on gram-negative rods, including *P. aeruginosa* (2, 6, 7, 13, 14, 18). Carbapenems preferentially inhibit penicillin-binding protein 2 (PBP-2), inducing spheroplast formation, and the observed PAE may represent the time required for the de novo synthesis of PBPs and to synthesize a new cell wall (10, 21). Gottfredsson and coworkers have observed enhanced DNA synthesis in gram-negative rods (*E. coli* and *P. aeruginosa*) after exposure to imipenem, which is related to either continued DNA replication in the presence of inhibited bacterial division and multiplication or positive feedback on DNA synthesis during PBP interference, while Hostacka demonstrated a decrease in the surface hydrophobicity of *Acinetobacter baumannii* after its exposure to subinhibitory concentrations of meropenem, which suggests the existence of multiple mechanisms of PAE activity (5, 8). Therefore, the PAE induced by carbapenems is probably due to the production or promotion of phenotypic modifications with changes on the bacterial cell surface, represented by metabolic alterations in the bacterial wall structure, making microorganisms more susceptible to phagocytosis and killing (5, 8, 12, 19, 20).

There are no final explanations for the greater effect of meropenem than imipenem. Nevertheless, if we assume that the occurrence of PALE activity is related to phenotypic modifications of the bacterial cell surface and spheroplast formation, these differences may be related, at least in part, to the high affinity of meropenem for both PBP-2 and -3 in *E. coli* and *P. aeruginosa*, differentiating it somewhat from imipenem, and partly to its enhanced antimicrobial activity against gram-negative rods due to the side chain attached at the C-2 position partly to its enhanced antimicrobial activity against gram-negative rods due to the side chain attached at the C-2 position of meropenem than imipenem. Nevertheless, if we assume that the occurrence of PAE and PALE activities, studies have been conducted with the purpose of investigating the possible mechanisms underlying PAE and PALE activities, additional studies are necessary to elucidate the nature of these pharmacodynamic phenomena.

**REFERENCES**