Meropenem-induced alteration of the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the bactericidal activity of human polymorphonuclear leucocytes

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The analysis of the interaction between antimicrobials and phagocytosis *in vitro* provides information additional to standard MIC and MBC estimations in predicting in-vivo efficacy. This study investigated the effects of meropenem, a newly available carbapenem, on the activities of human neutrophils against *Escherichia coli* and *Staphylococcus aureus*. The results were compared with those obtained with imipenem. Pretreatment of *E. coli* and *S. aureus* with meropenem and imipenem sensitized the bacteria to leucocytic killing. In the presence of antibiotics, opsonophagocytic killing of *E. coli*, but not *S. aureus*, was synergistically enhanced.

Materials and methods

**Bacterial strains**

*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were studied. They were susceptible to the antibiotics and resistant to normal human serum (NHS). Bacteria were maintained on blood agar plates and subcultured each 2–3 weeks.

**Antibiotics and susceptibility testing**

Stock solutions of meropenem (Zeneca, Madrid, Spain) and imipenem (Merck, Sharp & Dohme, Madrid, Spain) were prepared from the pure substance as described by the manufacturers, and then stored at $-80^\circ$C. Determination of minimal inhibitory concentrations (MICs) for bacteria in the logarithmic growth phase was carried out by a tube dilution method.

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Preparation of polymorphonuclear leucocytes

PMNs were obtained by the method of Eggleton, Gargan & Fisher. Blood was drawn from healthy volunteers who were not taking medication and placed into K$_2$-EDTA tubes. Four millilitres of the blood were then added to 16 mL ice-cold NH$_4$Cl (0.83%) and NaHCO$_3$ (0.08%) solutions. After incubation at 4°C for 15 min, the suspension was centrifuged at 55g for 10 min, and the pellet was resuspended in 10 mL of the same solution. This procedure was repeated. The final buffy coat was resuspended in Hank’s balanced salt solution (HBSS) without calcium or magnesium and adjusted to a concentration of 5.5 × 10$^6$ PMNLs/mL. Seventy to eighty per cent of the cells were PMNLs. Viability of white blood cells was determined by Trypan Blue examination, and only preparations containing >95% viable white blood cells were used.

Serum

Normal human serum was obtained from ten healthy donors and pooled. The sera was stored at −20°C until the day of use.

Experimental protocol

Antibacterial activity of PMNLs in the post-antibiotic phase

In this work, a modification to McDonald, Wetherall & Pruul’s method was used. The bacteria were grown overnight in Mueller–Hinton broth and the logarithmic phase of growth was obtained by dilution in fresh medium. Bacterial cultures adjusted to 2 × 10$^7$ cfu/mL, in the logarithmic phase of growth, were exposed for 10 min to 4 × MIC of the drugs in a shaking water bath at 37°C. Controls without antibiotics were included. At the end of the treatment period, the bacteria were centrifuged at 2000g for 10 min to remove the antibiotics. Bacteria were resuspended in HBSS plus 0.1% gelatine (GHBS). Aliquots of the bacteria were added to NHS (20% v/v), or a mixture of PMNLs and NHS in GHBS. The ratio of bacteria to PMNLs was adjusted to 10:1. All mixtures then followed the same process as the mixtures with pretreated bacteria. All experiments were repeated four times using PMNLs from four different healthy donors.

Statistical analysis

The results are shown as arithmetic means. The significance of the differences between test and control results was compared by an analysis of variance (ANOVA), followed by Student’s two-tailed t-test.

Results

MICs and susceptibility testing

MIC of meropenem and imipenem for E. coli were 0.06 and 0.25 mg/L respectively. MIC of meropenem and imipenem for S. aureus were 0.25 and 0.03 mg/L respectively.

Effect of antibiotic pretreatment of E. coli and S. aureus upon PMNL antibacterial activity

The effects of pre-exposure of E. coli and S. aureus to meropenem and imipenem on susceptibility to the bactericidal activity of PMNLs are shown in the Figure. Escherichia...
Meropenem and leucocyte activity

coli pre-exposed to meropenem had increased susceptibility to opsonophagocytic killing. After exposure to meropenem 0.06 mg/L for 10 min, more bacteria were phagocytosed than in controls containing untreated bacteria. However, normal growth resumed within 2 h. Similar increased killing was observed after exposure to imipenem (0.25 mg/L, 10 min). The results are given in Table I. The data are expressed as the percentage of phagocytic killing calculated as reduction in cfu/mL (in the absence of PMNLs) - reduction in cfu/mL (in the presence of PMNLs) to identify any effect on opsonophagocytic killing.

Pretreatment of S. aureus for 10 min with 4 MIC of both carbapenems also enhanced phagocytic killing over the entire period of incubation (Table II).

Antibacterial activity of PMNLs in the presence of antibiotics

In order to differentiate between the effects of antibiotics on bacteria from the effects of PMNLs, the PMNL-associated killing and bactericidal activities of meropenem and imipenem were assayed following exposure of bacteria individually to PMNLs and antibiotics.

In the absence of PMNLs, the antibacterial activity of both carbapenems was related to the antimicrobial concentration. There was a mean (±S.D.) reduction of log_{10} 1.63 ± 0.06 in the colony count per mL of E. coli in the presence of 4 × MIC of meropenem alone, and log_{10} 1.91 ± 0.10/mL in the presence of 4 × MIC of imipenem, after 3 h of exposure.

To determine the combined antibacterial activity of PMNLs and carbapenems, bacteria were added to two concentrations (0.5 × MIC and 4 × MIC) of these antibiotics and bacterial viability was determined every hour for 3 h. Table III shows that when E. coli was incubated in the presence of both PMNLs and sub-inhibitory or supra-inhibitory concentrations of meropenem, the susceptibility of E. coli to phagocytosis was enhanced. Optimum combined bactericidal activity against E. coli occurred in the presence of 4 × MIC of meropenem. The synergistic effect was also observed when imipenem was present at 4 × MIC of imipenem there was a slight increase in phagocytic killing.

Similar experiments were performed with S. aureus. In the absence of PMNLs, there was a reduction of log_{10} 2.74 ± 0.13 in cfu/mL after 3 h incubation with 4 × MIC of meropenem and log_{10} 2.57 ± 0.09 cfu/mL in the presence of 4 × MIC of imipenem. Both antibiotics failed to enhance PMNL-bactericidal activity. The combined effect of these antibiotics (0.5 × MIC) and PMNLs was additive against S. aureus. However, experiments carried out at supra-inhibitory concentrations (4 × MIC) showed a higher number of surviving bacteria in the presence of PMNLs compared with controls without PMNLs (Tables III and IV).

**Table I.** Effects on phagocytic killing following pre-exposure of E. coli to meropenem and imipenem. Bacteria were exposed to antibiotics for 10 min before incubation with PMNLs

<table>
<thead>
<tr>
<th>Period of incubation (h)</th>
<th>control</th>
<th>% Phagocytic killing of E. coli (mean ± S.D.)</th>
<th>meropenem</th>
<th>imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.12 ± 1.8</td>
<td>79.60 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.87 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65.33 ± 0.7</td>
<td>77.01 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.88 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>71.81 ± 0.9</td>
<td>70.60 ± 2.3</td>
<td>66.11 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of phagocytic killing, at several intervals of incubation, calculated as reduction in cfu/mL in the absence of PMNLs minus reduction in cfu/mL in the presence of PMNLs. Results are expressed as the mean (±S.D.) of four independent determinations.

<sup>a</sup> Differences between pretreated and control bacteria were regarded as significant when P < 0.05.

**Table II.** Effects on phagocytic killing following pre-exposure of S. aureus to meropenem and imipenem. Bacteria were exposed to antibiotics for 10 min before incubation with PMNLs

<table>
<thead>
<tr>
<th>Period of incubation (h)</th>
<th>control</th>
<th>% Phagocytic killing of S. aureus (mean ± S.D.)</th>
<th>meropenem</th>
<th>imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.30 ± 3.5</td>
<td>67.64 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.31 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24.26 ± 0.8</td>
<td>67.63 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.92 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27.63 ± 1.8</td>
<td>57.34 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.11 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of phagocytic killing, at several intervals of incubation, calculated as reduction in cfu/mL in the absence of PMNLs minus reduction in cfu/mL in the presence of PMNLs. Results are expressed as the mean (±S.D.) of four independent determinations.

<sup>a</sup> Differences between pretreated and control bacteria were regarded as significant when P < 0.05.
**Discussion**

Many studies of the effects of antibiotics on host defence mechanisms have demonstrated that antibiotics are able to modulate these mechanisms in different ways.\textsuperscript{8,9,10} Antibiotics can directly affect phagocytic cell function. Some antibiotics are concentrated in the phagocytes where they increase intracellular killing, and some may significantly alter bacterial morphology, growth rate, metabolism and structure, such bacterial alterations being capable of modifying the interaction with phagocytes.

This study was designed to investigate the effects of meropenem on the activities of human PMNLs against E. coli and S. aureus, and to compare them with imipenem. A brief exposure to meropenem and imipenem resulted in enhanced phagocytic killing of E. coli and S. aureus, compared with the controls. Antibiotic concentrations assayed (4 $\times$ MIC) simulated in-vivo levels. $C_{\text{max}}$ of meropenem after doses of 0.25, 0.5 and 1.0 g are about 12.1, 25.6 and 55.4 mg/L respectively.\textsuperscript{11} Mean peak plasma concentrations at 20 min for imipenem/cilastatin following IV infusion of 500 mg are 35 and 42 mg/L respectively.\textsuperscript{12}

The mechanisms by which antibiotics modify bacterial susceptibility to the phagocytic and bactericidal activities of PMNLs are not well understood. A nontoxic pretreatment may alter bacterial surface properties\textsuperscript{13,14} including changes in cell surface antigens, hydrophobicity, excretion of toxins and enzymes, release of lipopolysaccharide, and changes in cell wall thickness and adherence properties.\textsuperscript{15} Pre-exposure of bacteria to antimicrobial agents could cause changes in the antibody-binding site or complement-activation sites,\textsuperscript{16} and an increase in phagocytic activities could therefore be due to a better opsonization of bacteria.\textsuperscript{17-20} Mandell & A fnan\textsuperscript{21} reported enhanced killing of E. coli following pre-exposure of the bacterium to sub-inhibitory concentrations of imipenem, independent of the ingestion by phagocytes. Other investigators\textsuperscript{22} have shown that pre-exposure of staphylococci to a bactericidal concentration of imipenem causes the bacteria to be more efficiently phagocytosed and killed and have proposed that this enhancement could be related to the post-antibiotic effect (PAE) of imipenem.\textsuperscript{23} A PAE has been also shown with meropenem on E. coli and S. aureus\textsuperscript{24} and a similar mechanism to that of imipenem is likely to be involved in this increase of bacterial susceptibility to PMNLs.

Clearly, further studies are required to determine if the increased susceptibility to phagocytic killing of meropenem- and imipenem-exposed bacteria is due solely to an increase in efficiency of opsonization or whether intracellular antibacterial mechanisms are more active against altered bacteria.

The combination of neutrophils and supra-inhibitory and, to a lesser extent, sub-inhibitory concentrations of

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**Table III.** Effect of combined activity of PMNLs and meropenem against E. coli and S. aureus. Reduction of viable bacteria ($\log_{10}$ cfu/mL) relative to control bacteria in absence of PMNLs after 3 h incubation. Results are expressed as the mean ($\pm$ s.d.) of four independent determinations

<table>
<thead>
<tr>
<th>Strain</th>
<th>PMNLs alone (A)</th>
<th>Meropenem alone (B) 4 $\times$ MIC</th>
<th>A + B* 4 $\times$ MIC</th>
<th>Meropenem with PMNLs 4 $\times$ MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.55 $\pm$ 0.08</td>
<td>1.17 $\pm$ 0.05</td>
<td>3.00 $\pm$ 0.18</td>
<td>1.93 $\pm$ 0.12 $^{a}$</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.14 $\pm$ 0.02</td>
<td>0.72 $\pm$ 0.05</td>
<td>3.62 $\pm$ 0.06</td>
<td>1.01 $\pm$ 0.11 $^{a}$</td>
</tr>
</tbody>
</table>

*a A + B: The reduction in the number of viable bacteria incubated with PMNLs alone relative to that of the control (A) added to the reduction in the number of viable bacteria incubated with antibiotic only relative to that of the control (B).

*b Differences between (meropenem with PMNLs) and (A + B) were regarded as significant when $P < 0.05$.

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**Table IV.** Effect of combined activity of PMNLs and imipenem against E. coli and S. aureus. Reduction of viable bacteria ($\log_{10}$ cfu/mL) relative to control bacteria in absence of PMNLs after 3 h incubation. Results are expressed as the mean ($\pm$ s.d.) of four independent determinations

<table>
<thead>
<tr>
<th>Strain</th>
<th>PMNLs alone (A)</th>
<th>Imipenem alone (B) 4 $\times$ MIC</th>
<th>A + B* 4 $\times$ MIC</th>
<th>Imipenem with PMNLs 4 $\times$ MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.55 $\pm$ 0.08</td>
<td>1.49 $\pm$ 0.07</td>
<td>3.48 $\pm$ 0.09</td>
<td>2.21 $\pm$ 0.13 $^{a}$</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.14 $\pm$ 0.02</td>
<td>0.73 $\pm$ 0.05</td>
<td>3.45 $\pm$ 0.10</td>
<td>0.92 $\pm$ 0.12 $^{a}$</td>
</tr>
</tbody>
</table>

*a A + B: The reduction in the number of viable bacteria incubated with PMNLs alone relative to that of the control (A) added to the reduction in the number of viable bacteria incubated with antibiotic only relative to that of the control (B).

*b Differences between (imipenem with PMNLs) and (A + B) were regarded as significant when $P < 0.05$. 

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