In-vitro interactions of DX-8739, a new carbapenem, meropenem and imipenem with amikacin against multiresistant Pseudomonas aeruginosa

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In order to investigate the antimicrobial interactions against multiresistant Pseudomonas aeruginosa, thirty-seven strains resistant to antimicrobial agents of five different chemical classes were exposed in vitro to the combination of three carbapenems, DX-8739, a novel DHP-I stable analogue, meropenem and imipenem with amikacin. The tested combinations expressed an enhanced killing activity against 38–46% of strains and an additive effect against 5–13%. These effects were the same whether the applied carbapenem was DX-8739, meropenem or imipenem; they were also independent of the MIC of any antimicrobial.

Introduction

Newer dehydropeptidase-I stable carbapenems, like DX-8739 and meropenem, have proved highly active against multiresistant Pseudomonas aeruginosa strains in vitro (Giamarellos-Bourboulis, Grecka & Giamarellou, 1995). Our purpose was to investigate whether the in-vitro combination of DX-8739, meropenem or imipenem and amikacin might be active against nosocomial P. aeruginosa strains resistant to all four antimicrobial agents as well as whether in-vitro synergy depends on the level of multiresistance of these strains.

Materials and methods

Thirty-seven nosocomially-acquired P. aeruginosa strains isolated from the following sources were studied: bronchial secretions (17), pus (11), sputum (4), urine (3) and blood (2).

All carbapenems and amikacin were provided in the form of a freeze-dried amorphous powder: DX-8739 (Daichi Pharmaceuticals, Tokyo, Japan), meropenem (ICI Pharmaceuticals, Macclesfield, UK), imipenem (Merck Co., Rahway, N. Jersy, USA), amikacin (Bristol Myers Squibb, USA).

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MIC values of DX-8739, meropenem, imipenem and amikacin were determined by a microdilution technique and those of ticarcillin, piperacillin, ceftazidime, ciprofloxacin, gentamicin and tobramycin by application of ready-made microdilution plates (Sensititre Ltd, West Sussex, UK). A log-phase \textit{P. aeruginosa} inoculum appropriately diluted to $5 \times 10^5$ cfu/mL was applied with ATCC 27853 strain as a control.

The activities of the three carbapenem and amikacin combinations on \textit{P. aeruginosa} were studied by a modified time-kill assay: Eight different tubes per tested strain, containing a volume of 10 mL were used. In three of those tubes both a carbapenem concentration of 16 mg/L and an amikacin concentration of 16 mg/L were added, whereas four tubes containing either a carbapenem or amikacin and one tube without added antimicrobial served as relevant controls. The tested antimicrobial concentrations represented their mean serum level after the administration of conventional doses (Mandell & Sande, 1990; Sande & Mandell, 1990; Matsubayashi et al., 1992; Dreetz et al., 1995). All tubes contained a $5 \times 10^5$ cfu/mL log-phase \textit{P. aeruginosa} inoculum in Mueller-Hinton broth (Oxoid Ltd, London, UK). After preparation all tubes were incubated at 37°C. At standard intervals (0, 3, 5 and 24 h) the number of viable bacteria in each tube was determined by consecutive dilutions in Mueller-Hinton broth with 1 logio difference from each other to avoid antimicrobial carry-over effect. The time intervals were selected so that a 1 logio increase of the bacterial growth would have been achieved inbetween by the growth control. The whole procedure was repeated for six strains to establish its repeatability, so that a total of 344 time-kill curves were performed.

The above described procedure differs from the applied time-kill assay for synergy definition in two main points: classically the tested strains are susceptible to the used antimicrobials and they are exposed to antimicrobial concentrations equal to various multiples of the MIC (Hindler, 1992). In the present study all strains were multiresistant and the applied concentrations equal to mean serum levels so that clinical relevance could be established. According to the described differences from the classic time-kill assay the observed effect might be more accurately described as one of an enhanced killing, rather than synergy, as already proposed (Giamarellou & Petrikkos, 1987).

The criterion of establishing an enhanced carbapenem and amikacin killing effect were: (i) the combination was bactericidal, i.e. it produced $\geq 3$ logio decrease in viable cell counts at a specific time of growth compared to the growth control; and (ii) the combination provoked $\geq 2$ logio decrease in viable cell counts at a specific time of growth compared with the most active single agent (Hindler, 1992). When the latter decrease was between 1–2 logio the effect was considered as an additive one.

Statistical analysis of results was performed by the $\chi^2$ test ($P < 0.05$).

**Results**

All strains were resistant to DX-8739, meropenem, imipenem (MIC $> 4$ mg/L) and amikacin (MIC $> 16$ mg/L), whereas 24 (64.9%) strains were resistant to all 10 agents tested. MIC90–MIC90 to DX-8739, meropenem, imipenem and amikacin were 16–$\geq 256$, 16–128, 32–$\geq 256$ and 128–$\geq 256$ mg/L respectively.

The in-vitro enhanced killing effect between the three carbapenems and amikacin observed at different time intervals is presented in the Table. Single antimicrobial agents remained inactive against the strains tested. That killing effect was simultaneously
Table. In-vitro enhanced killing effect of three carbapenems and amikacin against 37 *P. aeruginosa* strains

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>DX-8739 + amikacin</th>
<th>Meropenem + amikacin</th>
<th>Imipenem + amikacin</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4 (10.8)</td>
<td>4 (10.8)</td>
<td>6 (16.2)</td>
<td>0.645 (P NS)</td>
</tr>
<tr>
<td>5</td>
<td>11 (29.7)</td>
<td>12 (32.4)</td>
<td>15 (40.5)</td>
<td>1.022 (P NS)</td>
</tr>
<tr>
<td>24</td>
<td>16 (43.2)</td>
<td>17 (45.9)</td>
<td>14 (37.8)</td>
<td>0.225 (P NS)</td>
</tr>
</tbody>
</table>

expressed by all three carbapenem and amikacin combinations in eight (21.6%) strains. The carbapenem and amikacin combination was additive in five (13.5%) strains exposed to DX-8739 and amikacin at 24 h and in two (5.4%) and three (8.1%) strains exposed at 3 h to meropenem and amikacin and imipenem and amikacin respectively. Five (13.5%) strains remained completely indifferent to all three carbapenem and amikacin combinations. The in-vitro interaction between the three carbapenems and amikacin on *P. aeruginosa* related to their MIC level is presented in the Figure. No statistically significant correlation could be established between them (P NS).

From the total number of strains where the DX-8739 and amikacin combination expressed an enhanced killing effect at 3, 5 and 24 h intervals, two, six and ten strains respectively were resistant to all ten antimicrobial agents studied. The figures for the meropenem and amikacin and for the imipenem and amikacin combinations were two, seven and eight strains and three, seven and nine strains, respectively. No statistically significant correlation could be established between the number of

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**Figure.** Correlation between the carbapenem and amikacin enhanced killing activity and the carbapenem or amikacin MIC level. □, DX-8739; ☐, meropenem; ☉, imipenem; ■, amikacin.
antimicrobial agents to which *P. aeruginosa* strains were resistant and the existence of a carbapenem and amikacin enhanced killing effect (*P* = NS).

**Discussion**

The present study revealed that the combination of a carbapenem with amikacin possesses an enhanced killing effect *in vitro* on 38–46% of multiresistant *P. aeruginosa* strains and an additive effect on 5–13% of them at antimicrobial concentrations equal to their mean serum levels. Both phenomena were not found to differ significantly whether the carbapenem in the in-vitro combinations tested was DX-8739, meropenem or imipenem. Our findings are consistent with in-vitro studies demonstrating the imipenem and amikacin combination to be superior to imipenem or amikacin alone (McGrath, Lamp & Rybak, 1993). However these studies involved two strains becoming resistant to imipenem only after their sequential exposure to it *in vitro*, and not nosocomial multiresistant strains.

Since the time-kill assay for synergy definition uses concentrations close to the MIC (Hindler, 1992), a carbapenem and amikacin enhanced killing effect would be expected for strains with an MIC of 8–32 mg/L. Contrary to that, no statistical correlation could be established between the existence of an enhanced killing effect *in vitro* and the MIC level to carbapenem or amikacin (Figure).

Various hypotheses could be proposed to explain why other *P. aeruginosa* strains behave *in vitro* as susceptible to the tested combination and others as indifferent to it. These might involve either the production of different levels of chromosomal β-lactamase by these strains, since carbapenem resistance in *P. aeruginosa* is principally determined by the susceptibility of the carbapenem molecule to chromosomal β-lactamase (Livermore, 1992), or the expression of a multidrug resistance efflux system by strains remaining indifferent to the tested combination (Masuda, Sakagawa & Ohya, 1995). Both hypotheses are based on the lack of correlation between the carbapenem and amikacin interaction and the antimicrobial MIC level.

The present study suggests that the interactions of newer carbapenems with amikacin *in vitro* result in either an enhanced or additive killing effect on multiresistant *P. aeruginosa* strains which is independent of the type of the carbapenem used; that effect remains unrelated to the MIC level of carbapenem or amikacin, as well as to the number of antimicrobials to which *P. aeruginosa* is resistant. The findings merit further verification *in vivo* and further research *in vitro* to establish the mechanism of carbapenem and amikacin interaction on multiresistant *P. aeruginosa*.

**Acknowledgement**

Presented in part at the 19th International Congress of Chemotherapy, July 1995, Montreal, Canada, Abstract 1208.

**References**

Carbapenems and amikacin against resistant Pseudomonas


(Received 31 October 1995; returned 6 December 1995; revised 26 January 1996; accepted 26 March 1996)