Journal of Antimicrobial Chemotherapy (1989) **24**, *Suppl. A*, 233–238

The antibacterial activity of meropenem in combination with gentamicin or vancomycin

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The kinetics of bacterial killing by meropenem alone and in combination with gentamicin (for *Pseudomonas aeruginosa*) and vancomycin (for *Staphylococcus aureus*) were studied for two strains of each species. Against the two strains of *P. aeruginosa*, meropenem at concentrations up to 4 × MIC was rapidly bactericidal—but regrowth occurred by 24 h. The addition of half the MIC of gentamicin to the MIC of meropenem led to a more rapid decline of the colony count and to the prevention of regrowth of the strain which was gentamicin-susceptible. Similar results were obtained for a methicillin-susceptible strain of *S. aureus* when vancomycin was added at a concentration of half the MIC. A methicillin-resistant strain also was killed by a combination of vancomycin at half the MIC plus meropenem at the MIC. The study showed that the killing action of meropenem against *P. aeruginosa* and staphylococci is enhanced by the addition of gentamicin or vancomycin respectively.

Introduction

The in-vitro activity of the carbapenem, meropenem, encompasses a wide range of bacteria. In serious infections, in particular those caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa*, it is possible that this agent may be used in combination with other antimicrobials, either to attain a synergistic interaction or, if the susceptibilities of the pathogen are unknown, to 'cover' the possibility that the pathogen may be resistant to one of the agents. We have studied the kinetics of bactericidal action of meropenem in combination with gentamicin against strains of *P. aeruginosa* and with vancomycin against *S. aureus*.

Materials and methods

Two strains of *P. aeruginosa* and two of *S. aureus* were chosen. The MICs for these strains are shown in Table I. These values were derived in the same medium with the same initial inoculum, 10^5 cfu, as was used in the killing kinetic studies. Strain 4 was methicillin-resistant (MRSA).

Media

In all studies Iso-Sensitest broth or agar (Oxoid, Basingstoke, UK) was used.

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Table I. In-vitro MIC (mg/l) to meropenem, gentamicin and vancomycin

Strain (no.)	Meropenem	Gentamicin	Vancomycin
P. aeruginosa (1)	0·12	0·25	
P. aeruginosa (2)	1·0	8	
Staph. aureus (3)	0·25		2
Staph. aureus (4)	4		2

Colony count procedure

An overnight broth culture of the organism with a colony count of 10^9 cfu/ml (as determined by preliminary studies) was diluted 1:100 in 10 ml of broth pre-warmed to 37°C, to give an inoculum of approximately 10^7 cfu/ml. This broth was then incubated at 37°C, with shaking for 1 h in order to allow the organisms to attain logarithmic phase growth. At this time a further 1:100 dilution was made into 50 ml of pre-warmed broth to give an initial inoculum of approximately 10^5 cfu/ml. Antibiotic was then added (t = 0). The concentrations of antibiotic used were as follows: for *S. aureus*, meropenem at half the MIC, the MIC and four times the MIC and vancomycin at half the MIC, alone and in combination with meropenem at the above concentrations; for *P. aeruginosa*, gentamicin replaced vancomycin.

Two-ml aliquots of broth were removed at times 0, 0·5, 1, 2, 3, 4, 6, 8 and 24 h. The aliquots were diluted 1:100 and 1:1000 in distilled water (for staphylococci) or sterile broth (for *P. aeruginosa*) and then counted after plating with a spiral plater system (Don Whitley Scientific, Shipley, UK). The results are expressed as $\log_{10}$ count.

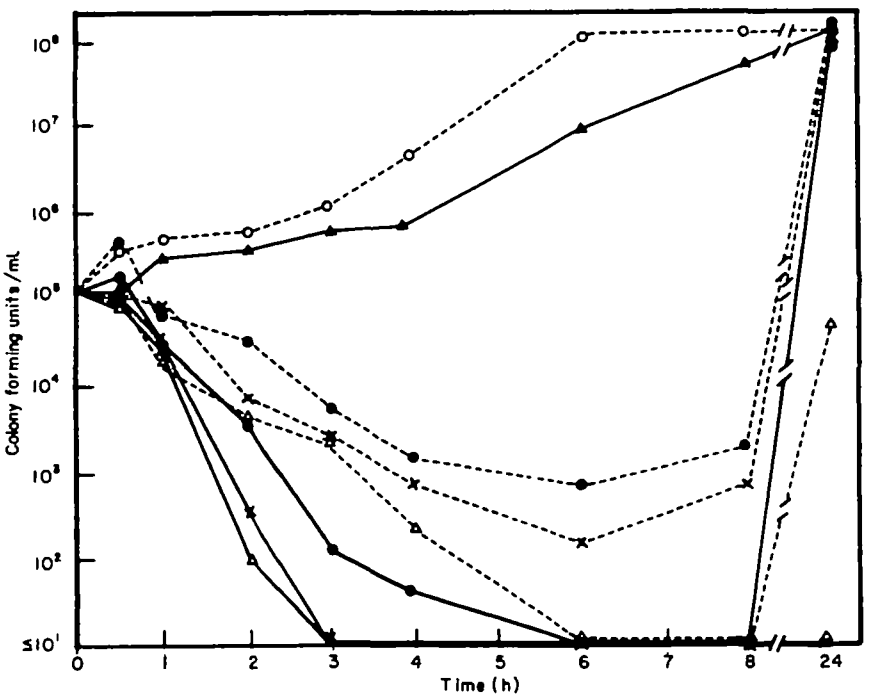

Figure 1. The effect of meropenem and gentamicin on *P. aeruginosa* (gentamicin-susceptible). ●———●, Meropenem ½ MIC, gentamicin ½ MIC; ×———×, meropenem MIC, gentamicin ½ MIC; △———△, meropenem MIC × 4, gentamicin ½ MIC; ▲———▲, gentamicin ½ MIC; ●-----●, meropenem ½ MIC; ×-----×, meropenem MIC; △-----△, meropenem MIC × 4; ○-----○, control.

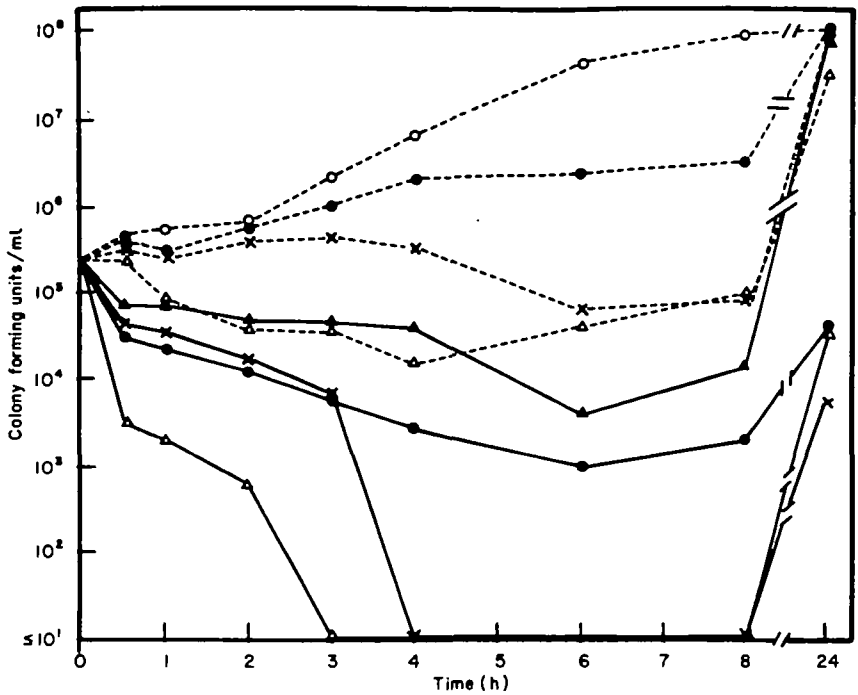

Figure 2. The effect of meropenem and gentamicin on *P. aeruginosa* (gentamicin-resistant). (Symbols as in Figure 1.)

Results

The results are shown in Figures 1 to 4. In all cases, except the MRSA (strain 4, Figure 4), the initial count of the control organism was $> 10^5$ cfu/ml. For the MRSA it was $4{\cdot}0 \times 10^4$ cfu/ml. The control count then increased and was in excess of 10^8 cfu/ml by 24 h for all strains.

Growth of a gentamicin-sensitive *P. aeruginosa* isolate (strain 1) was inhibited by concentrations of meropenem of 0·06 and 0·12 mg/l, in that the colony counts up to and including the 8 h sampling point did not increase above the time 0 h count and by 8 h the colony count was decreased by 2–3 log. By 24 h these cultures had regrown to counts similar to the control. A concentration of 0·5 mg/l (4 × MIC) allowed no detectable growth by 6 h but at 24 h there was regrowth. The addition of gentamicin at half the MIC (0.12 mg/l) to meropenem at a concentration of 0·06 mg/l allowed no detectable growth at 6 h but overnight regrowth. Increasing meropenem to 0·12 mg/l or more allowed no detectable growth or regrowth from 3 h onwards if gentamicin 0·12 mg/l was also present.

The gentamicin-resistant (MIC 8 mg/l) *P. aeruginosa* (strain 2) appeared to be less susceptible to meropenem than strain 1 in that concentrations of meropenem up to 4 × MIC only had the effect at 8 h of maintaining the colony count at the level of the initial inoculum. Meropenem at concentrations of 1 and 4 mg/l (i.e. equivalent to the MIC and 4 × MIC respectively) when combined with gentamicin at half the MIC (4 mg/l) yielded no detectable growth from the 4 h to the 8 h sampling point but regrowth occurred overnight.

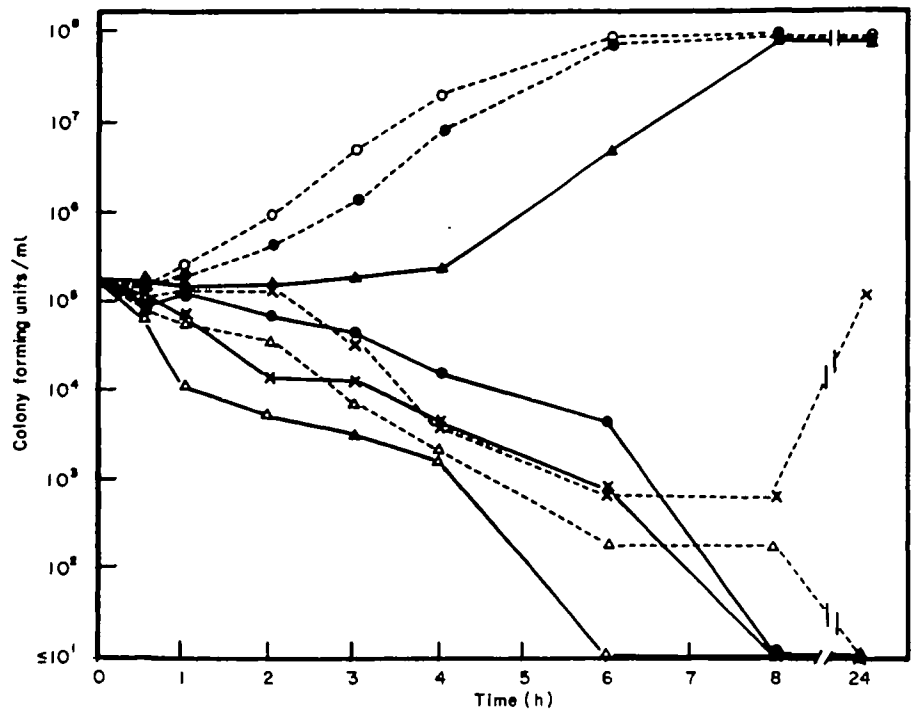

Figure 3. The effect of meropenem and vancomycin on *S. aureus* (methicillin-susceptible). ●———●, Meropenem ½ MIC, vancomycin ½ MIC; ×———×, meropenem MIC, vancomycin ½ MIC; △———△, meropenem MIC × 4, vancomycin ½ MIC; ▲———▲, vancomycin ½ MIC; ●-----●, meropenem ½ MIC; ×-----×, meropenem MIC; △-----△, meropenem MIC × 4; ○-----○, control.

A concentration of half the MIC of meropenem had little effect upon the growth of the methicillin-sensitive strain of *S. aureus* (strain 3) and a concentration equivalent to the MIC and four times the MIC inhibited growth such that the colony count in each case decreased to about 10^2 cfu/ml at 6 h and in the case of meropenem at four times MIC no detectable growth or regrowth was obtained at 24 h. Vancomycin at a concentration of half the MIC had minimal effect upon the growth of this strain. However, a combination of meropenem at half the MIC and vancomycin at half the MIC allowed no detectable growth at 8 h and no later regrowth occurred. Increasing the concentration of meropenem to 1 mg/l (4 × MIC) allowed no growth from 6 h onwards if 1 mg/l of vancomycin was present.

Meropenem at concentrations of half MIC, MIC and four times the MIC inhibited growth of the methicillin-resistant *S. aureus* (strain 4) over the first 8 h but by 24 h regrowth had occurred. Vancomycin alone at a concentration of half the MIC had little effect on growth but did when combined with half the MIC, MIC or four times MIC of meropenem there was no detectable growth at 24 h.

Discussion

The interaction of β-lactams with aminoglycosides is commonly synergistic, presumably owing to enhancement of the entry into the bacterial cell of the aminoglycoside by the action of the β-lactam on the cell wall (Moellering & Weinberg, 1971; Plotz &

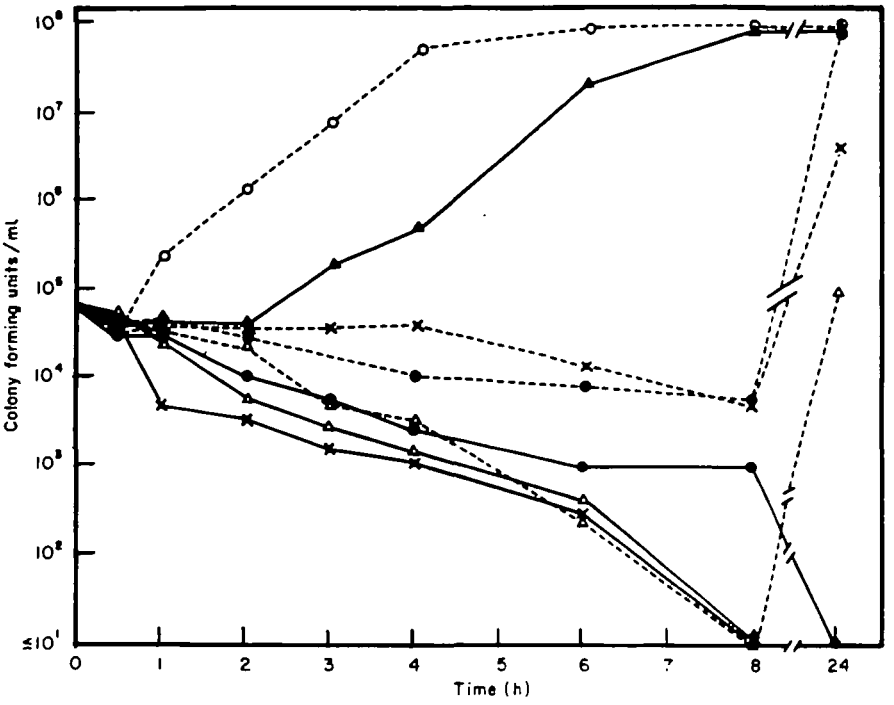

Figure 4. The effect of meropenem and vancomycin on *S. aureus* (methicillin-resistant). (Symbols as in Figure 3.)

Davis, 1962). However, vancomycin has been reported as only showing synergy with β-lactams (against Gram-negative bacteria) at concentrations of the former that would probably be toxic (Donabedian & Andriole, 1977). There is little information on the synergy of vancomycin with β-lactams against Gram-positive bacteria.

There are considerable difficulties in interpreting and quantifying antimicrobial interactions (Krogstad & Moellering, 1980). The most precise method of quantifying any interaction is to use a chequerboard procedure and estimate the fractional inhibitory concentrations. This method, however, only studies bacteriostatic interactions, whereas we wished to study both the dynamics of the killing effect of meropenem with other agents and the bactericidal effect that this might have.

The results demonstrate that, in the case of *P. aeruginosa*, there is an interaction between meropenem and gentamicin. Whereas meropenem at a concentration of four times the MIC failed to prevent regrowth of the gentamicin-susceptible strain, there was no detectable growth when 1 × MIC of meropenem was combined with half the MIC of gentamicin. In addition, killing was more rapid in the presence of gentamicin. The effect on the gentamicin-resistant strain was, as would be expected, less marked, but again the rate of decrease of colony count was more rapid in the presence of the aminoglycoside.

Similar results were experienced with the strains of *S. aureus* in that the presence of half the MIC of vancomycin yielded more rapid killing of both the MRSA and MSSA strains than meropenem alone.

Although meropenem has good activity against both staphylococci and *P. aeruginosa* there may well be clinical conditions when it may be advisable to consider a combina-

tion, for example a severe pseudomonal infection, or an infection which may be caused by a MRSA.

This study does not prove that there is a definite synergistic interaction between meropenem and either gentamicin or vancomycin, as quantification of such an interaction was not possible. It does, however, show that the presence of the other agent does enhance the activity of the meropenem by increasing both the rate and amount of bacterial killing.

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