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*Article*

# Meropenem Alone and in Combination with Vancomycin in Experimental Meningitis Caused by a Penicillin-Resistant Pneumococcal Strain

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**Abstract** In a rabbit model of meningitis caused by a pneumococcus highly resistant to penicillin (MIC, 4 μg/ml), meropenem, a broad-spectrum carbapenem, was bactericidal (–0.48 ± 0.14 $\Delta\log_{10}$ cfu/ml·h) and slightly superior to ceftriaxone (–0.34 ± 0.23 $\Delta\log_{10}$ cfu/ml·h) and vancomycin (–0.39 ± 0.19 $\Delta\log_{10}$ cfu/ml·h). Although the combination of vancomycin with ceftriaxone was significantly more active than ceftriaxone alone (–0.55 ± 0.19 $\Delta\log_{10}$ cfu/ml·h), only an insignificant gain was observed by the addition of vancomycin to meropenem (–0.55 ± 0.28 $\Delta\log_{10}$ cfu/ml·h).

## Introduction

Before the emergence of penicillin-resistant pneumococci, penicillin was usually the first-line antibiotic used in the treatment of pneumococcal infections. The increasing spread of resistant strains has changed this situation [1]. In serious infections, particularly in meningitis, penicillin is ineffective even against strains with intermediate resistance, and penicillin resistance is often associated with resistance to other β-lactam agents. Indeed, because treatment failures in meningitis have been observed with cephalosporin monotherapy [2, 3], a combination of vancomycin and an extended-spectrum cephalosporin (ceftriaxone or cefotaxime) is usually recommended for treatment of meningitis caused by resistant strains [4].

The availability of reliably active monotherapy would represent a significant advantage to the empiric therapy of meningitis. Meropenem is a broad-spectrum carbapenem that has good activity against a majority of human pathogens, including penicillin-resistant pneumococci, and achieves good penetration into inflamed cerebrospinal fluid (CSF) [5, 6]. In the present study, we tested meropenem alone and in combination with vancomycin in the rabbit meningitis model and in vitro. The comparison regimen consisted of ceftriaxone alone and in combination with vancomycin.

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## Materials and Methods

*In Vivo Experiments.* The meningitis model, originally described by Dacey and Sande [7], was used in a slightly modified way. Briefly, young New Zealand white rabbits weighing 2–2.5 kg were anesthetized by intramuscular injections of ketamine (30 mg/kg) and xylazine (15 mg/kg) and were immobilized in stereotactic frames for induction of meningitis and CSF sampling. An inoculum containing approximately $1 \times 10^5$ cfu of penicillin-resistant pneumococci serotype 6 was injected directly into the cisterna magna. The minimal inhibition concentrations (MICs) of the antibiotics for this strain were as follows: penicillin G, 4 μg/ml; ceftriaxone, 0.5 μg/ml; meropenem, 0.5 μg/ml; and vancomycin, 0.25 μg/ml.

After a long-acting anesthetic (ethylcarbamate [urethane], 3.5 g/rabbit) was injected subcutaneously, the animals were returned to their cages. Fourteen hours later the cisterna magna was punctured again for periodic CSF sampling before and 2, 4, 6, and 8 h after initiation of therapy. Antibiotics were administered through a peripheral ear vein as bolus injections at the following concentrations: meropenem, 125 mg/kg; ceftriaxone, 125 mg/kg; and vancomycin, 20 mg/kg. Ceftriaxone was injected once at hour 0 and meropenem and vancomycin at hours 0 and 4 according to

the method of Friedland et al. [8]. Untreated controls received saline.

Bacterial titers were measured by tenfold serial dilution of CSF samples, plated on blood agar plates containing 5% sheep blood, and incubated overnight at 37 °C. In parallel, 20 μl undiluted CSF samples were plated (limit of detectability, 50 cfu/ml). Comparison between the different dilutions of CSF was used to exclude significant carryover effects of antibiotics. The antimicrobial activity of the regimens during the 8 h treatment was calculated by linear regression analysis and expressed as decrease of $\log_{10}$ cfu per milliliter per hour ($\Delta\log_{10}$ cfu/ml·h). A value of 1.7 ($\log_{10}$ of the limit of detectability) was assigned to the first sterile CSF sample and a value of 0 to subsequent sterile samples. Results were expressed as the mean ± standard deviation (SD). Statistical significance was determined by the Newman-Keuls test.

CSF was sampled at approximately 90 min and 4 h after each intravenous antibiotic dose in order to measure the peak and trough antibiotic concentrations, respectively. Meropenem, ceftriaxone, and vancomycin concentrations in the CSF were determined by an agar diffusion method. Control curves were calculated for saline that contained 5% rabbit serum in order to mimic CSF protein concentration during meningitis [9]. *Bacillus subtilis* (ATCC 6633) was used as the test strain for meropenem and vancomycin and *Escherichia coli* (ATCC 29522) was used for ceftriaxone [10, 11]. The intra- and interday variability of this method was less than 10%. The limit of detection was 0.3 μg/ml for meropenem and 0.5 μg/ml for vancomycin and ceftriaxone.

*In Vitro Experiments.* The pneumococcal strain was grown in C+Y medium [12] to optical density 0.3 at 590 nm and then diluted 40-fold to $10^6$ cfu/ml, corresponding to the CSF bacterial titer in rabbits before therapy was initiated. Meropenem was added in concentrations ranging from 0.5 to 5 μg/ml, corresponding to 1×, 2×, 5×, and 10× the MIC. Combinations of vancomycin with meropenem were also tested in concentrations corresponding to 0.5× and 1× the MIC. Bacterial titers were determined at hours 0, 2, 4, and 6 by serial dilution of samples plated on agar plates containing 5% sheep blood and incubated at 37 °C for 24 h. Experiments were performed in triplicate and results were expressed as the mean ± SD. Synergism was defined as a bactericidal effect of a drug combination significantly exceeding the sum of the bactericidal effect of each agent alone.

## Results

The CSF antibiotic peak and trough levels are presented in Table 1. The meropenem concentration in the CSF approximately 90 min after injection was 3.75 μg/ml, declining to 0.69 μg/ml 4 h after the injection, when the second dose was administered (Table 1). Ceftriaxone levels ranged between 6 μg/ml (peak level) and 4 μg/ml (trough level); vancomycin levels ranged between 4.1 μg/ml and 1.57 μg/ml. The peak concentration/MIC ratio was 7.4 μg/ml for meropenem, 16 μg/ml for vancomycin, and 12 μg/ml for ceftriaxone.

The killing rates of the different antibiotics, calculated by linear regression analysis, are shown in Table 2. In untreated controls, bacterial titers showed minimal growth rates (0.06 ± 0.10 $\Delta\log_{10}$ cfu/ml·h) during the 8 h period. Vancomycin and ceftriaxone produced comparable bactericidal activity (–0.39 ± 0.19 and –0.31 ± 0.20 $\Delta\log_{10}$ cfu/ml·h), whereas meropenem showed slightly higher activity (–0.48 ± 0.14 $\Delta\log_{10}$ cfu/ml·h, not significantly different compared to ceftriaxone or vancomycin).

**Table 1** Mean peak and trough antibiotic levels in CSF of rabbits with pneumococcal meningitis

| Antibiotic | CSF concentration (μg/ml) | |
|---|---|---|
| | Peak level | Trough level |
| Meropenem | 3.76 ± 4.48 | 0.69 ± 0.76 |
| Vancomycin | 4.04 ± 1.74 | 1.57 ± 1.58 |
| Ceftriaxone | 6.0 ± 3.0 | 4.1 ± 2.4 |

The combination of vancomycin (20 mg/kg at 0 and 4 h) with either meropenem or ceftriaxone showed similar bactericidal activity (–0.55 ± 0.19 $\Delta\log_{10}$ cfu/ml·h and –0.55 ± 0.28 $\Delta\log_{10}$ cfu/ml·h, respectively, for the two combination regimens). Compared to ceftriaxone alone, the combination with vancomycin was more active ($P < 0.05$), whereas the combination of meropenem with vancomycin did not significantly improve the activity of either drug alone.

In time-killing experiments, meropenem showed dose-related bactericidal activity in concentrations above the MIC (Figure 1). Combination therapies were also tested in vitro. In the first set of experiments, antibiotic concentrations equal to the MIC were chosen, with each monotherapy showing only a marginal killing effect (Figure 2). The addition of vancomycin produced a synergistic effect on the bactericidal activity of meropenem monotherapy. In similar experiments, concentrations below the MIC were also tested (Figure 3). As expected, monotherapies at concentrations of one-half

**Table 2** Bactericidal activity of meropenem versus ceftriaxone alone and in combination with vancomycin in experimental meningitis due to *Streptococcus pneumoniae* resistant to penicillin

| Antibiotic | No. of rabbits | Mean value ± SD | |
|---|---|---|---|
| | | Initial titer (cfu/ml) | Δlog (cfu/ml)/h |
| Untreated control | 5 | 6.37 ± 0.71 | 0.06 ± 0.10* |
| Vancomycin | 9 | 6.53 ± 1.25 | –0.39 ± 0.19 |
| Ceftriaxone | 7 | 6.31 ± 0.20 | –0.31 ± 0.20** |
| Meropenem | 7 | 6.55 ± 1.08 | –0.48 ± 0.14 |
| Ceftriaxone + vancomycin | 8 | 6.33 ± 0.25 | –0.55 ± 0.19** |
| Meropenem + vancomycin | 12 | 6.19 ± 0.70 | –0.55 ± 0.28 |

*$P < 0.05$ versus all other groups using the Newman-Keuls multiple comparison test

**$P < 0.05$ ceftriaxone versus ceftriaxone plus vancomycin

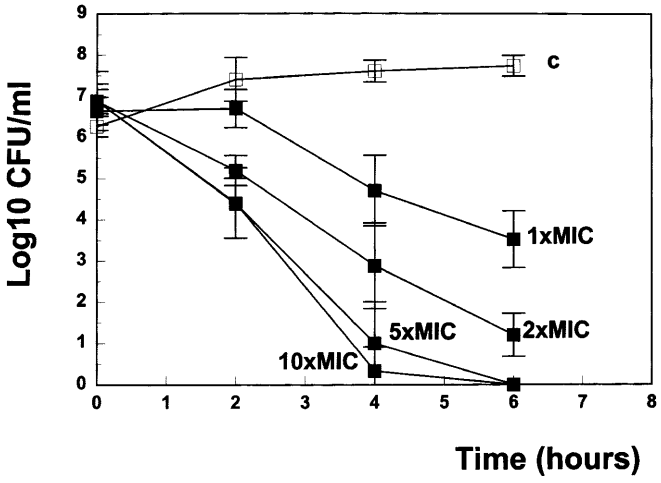


**Figure 1** In vitro killing rates of meropenem at concentrations ranging from 1× the MIC to 10× the MIC. Experiments were performed in triplicate and killing rates were expressed as mean ± SD

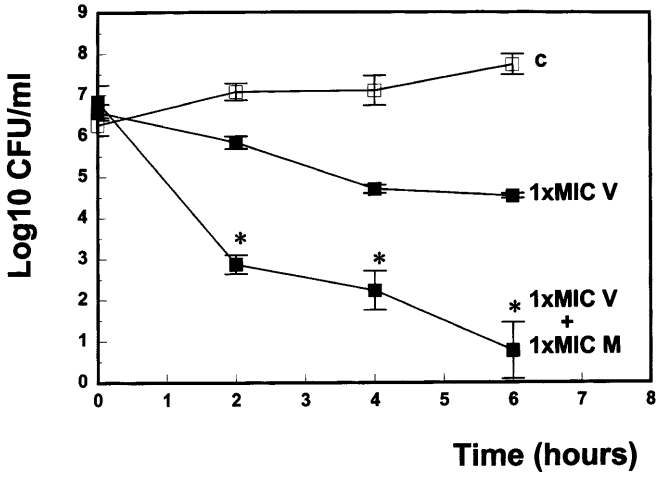


**Figure 2** In vitro killing rates of meropenem (M), vancomycin (V), and meropenem combined with vancomycin (M+V) at concentrations equal to the MIC. Experiments were performed in triplicate and killing rates were expressed as mean ± SD. $P < 0.05$

the MIC were comparable to untreated controls. The combination of both antibiotics at these low concentrations produced significant bactericidal activity.

## Discussion

In general, infections due to penicillin-resistant strains of pneumococci can be treated successfully with high-dose $\beta$-lactam antibiotics, providing that the penetration of the drugs into infected tissues (i.e., lung) is sufficient [13]. The therapy of meningitis caused by strains highly resistant to penicillin and cephalosporins (MIC, >1 μg/ml) is difficult due to the limited penetration of

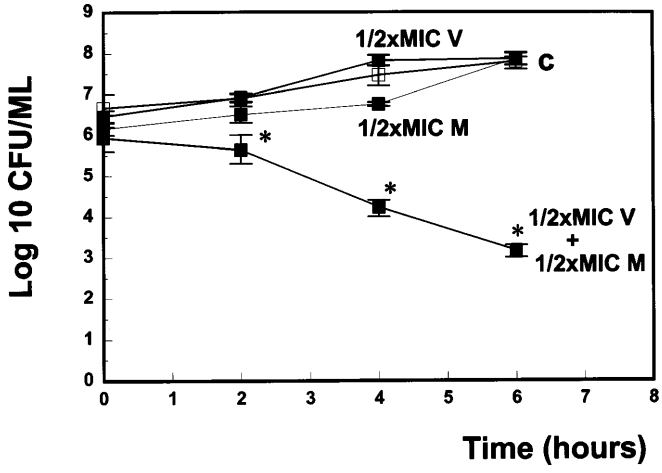


**Figure 3** In vitro killing rates of meropenem (M), vancomycin (V), and the combination of vancomycin and meropenem (V+M) at concentrations corresponding to one-half the MIC. Experiments were performed in triplicate and killing rates were expressed as mean ± SD. $P < 0.05$

antibiotics into the subarachnoid space. Although recent animal studies show efficacy of newer quinolones in meningitis caused by penicillin-resistant pneumococci [14, 15], the standard therapy for these infections continues to be a combination of a cephalosporin (cefotaxime or ceftriaxone) with vancomycin.

The carbapenems possess the broadest antibacterial spectrum of any class of antibiotics [16]. They are usually active against most clinically relevant pathogens with the exception of methicillin-resistant staphylococci, *Enterococcus faecium*, and *Stenotrophomonas maltophilia* [17]. Meropenem is highly effective against the major pathogens that cause meningitis, including *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. Furthermore, meropenem is active in vitro against penicillin-resistant pneumococci [4, 18]. In contrast to imipenem, meropenem rarely induces seizures in animal models or in clinical studies in children and adults [19, 20]. Limited data exist concerning the effectiveness of meropenem against resistant pneumococci in meningitis [21, 22]. In the rabbit model of experimental meningitis, we compared meropenem alone and in combination with vancomycin to the standard therapy for meningitis caused by penicillin-resistant pneumococci, i.e., ceftriaxone combined with vancomycin.

Meropenem penetrated well into inflamed meninges. In previous experimental studies in rabbits, 125 mg/kg i.v. produced CSF peak concentrations of about 2 μg/ml [8]. In children, doses of 20 mg/kg and 40 mg/kg resulted in mean peak CSF concentrations of 1.14 and 3.28 μg/ml, respectively [23]. The higher dose in rabbits was used in order to compensate for the shorter half-life in animals due to hydrolysis by dihydropeptidases [24].

The concentrations measured in our experimental meningitis model (Table 1) correlated closely with those obtained in humans receiving 40 mg/kg i.v. [23]. The doses of vancomycin ($2 \times 20$ mg/kg) and ceftriaxone ($1 \times 25$ mg/kg) were standard doses used in previous studies in the same experimental model [8]; these doses have been shown to produce in this model serum and CSF concentrations corresponding to high-dose therapy in humans [15, 25, 26].

The ratios of CSF peak concentration/MIC may have suggested an advantage for ceftriaxone and vancomycin (CSF peak concentration/MIC of ceftriaxone, 12; of vancomycin, 16; of meropenem, 7.4), although this parameter is not crucial for the bactericidal activity of $\beta$-lactam antibiotics. Meropenem monotherapy was at least as active as the two comparative regimens.

Interestingly, the addition of vancomycin increased the killing potential of meropenem in our animal model only marginally as compared to the improved efficacy of ceftriaxone observed when vancomycin was added ($P < 0.05$). It remains unclear whether the addition of vancomycin to meropenem contributed to a clinically significant therapeutic benefit in this animal model. On the other hand, Hughes et al. (G.D. Hughes et al., 36th IDSA Annual Meeting, 1998, Abstract no. 232) described synergism between meropenem and vancomycin in a guinea-pig model in which lower doses of both antibiotics were used. These contrasting results might be explained by the different antibiotic concentrations used.

Nevertheless, based on our in vitro data, the addition of vancomycin could result in some therapeutic benefit by, for example, maintaining the bactericidal activity of meropenem if the drug concentration in the CSF fell below the MIC.

The good bactericidal activity of meropenem against penicillin-resistant pneumococci in vitro and the satisfying concentrations of meropenem achieved in inflamed meninges qualify meropenem alone or in combination with vancomycin as a potential candidate for the treatment of pneumococcal meningitis caused by strains highly resistant to penicillin. This new combination could be particularly useful in cases in which a broad spectrum of activity is required in the initial empiric treatment of meningitis and when resistant pneumococcal strains are suspected. Meropenem has already been successfully used in the treatment of bacterial meningitis due to penicillin-susceptible strains in adults and in children [27]. Our results suggest that a similarly good activity might be expected in cases in which pneumococci are resistant to penicillin.

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