In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant Acinetobacter baumannii strain

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Objectives: The potential therapeutic role of meropenem combined with sulbactam against a clinical endemic isolate of multidrug-resistant Acinetobacter baumannii, Ab-153, was investigated.

Methods: The antimicrobial susceptibility of Ab-153 to various drugs was studied by the agar dilution method and Etest strips. The antibacterial activity of meropenem and sulbactam were investigated by a time–kill study in vitro and further examined for therapeutic efficacy in vivo in a murine model.

Results: In the time–kill study, at a concentration of 0.5 × MIC (4 mg/L) of meropenem, 1 × MIC (8 mg/L) of sulbactam and both in combination, only the combination demonstrated bactericidal effects and there was at least a 5 log10 reduction in bacterial colony counts after 48 h, compared with either drug alone. BALB/c mice infected with 2.1–2.6 × 10⁷ cfu of Ab-153 were treated with 20 mg/kg meropenem every 8 h, 40 mg/kg sulbactam every 8 h or both in combination. The survival rate of mice in the combination group was significantly higher than that in the meropenem-treated or sulbactam-treated group (87% versus 35%, P = 0.0004; 87% versus 30%, P = 0.0002).

Conclusions: Meropenem in conjunction with sulbactam can exhibit more potent antimicrobial activity against Ab-153 than meropenem or sulbactam alone.

Keywords: synergy, combination therapy, time–kill curves

Introduction

Recently, Acinetobacter baumannii, a previously uncommon non-enteric Gram-negative bacillus, has become a common nosocomial pathogen, especially in intensive care units.1 A. baumannii can colonize multiple body sites of hospitalized patients,2 and survive for a long time on inanimate surfaces.3 Both characteristics contribute, at least in part, to the prominent role of A. baumannii in nosocomial infections, including bloodstream infections, ventilator-associated pneumonia, surgical site infections and urinary tract infections.4 The options for treatment of A. baumannii infections are limited due to the upsurge in antimicrobial resistance. Although the therapeutic efficacy of monotherapy with intravenous ampicillin–sulbactam5 or colistin6 has been promising, the antibacterial activity of the former was due merely to the intrinsic bacteriostatic activity of sulbactam7 and may be inadequate for life-threatening infections, and the latter can be neuro- or nephrotoxic. Other antimicrobial options are required urgently.

We studied the in vitro and in vivo antibacterial activity of meropenem combined with sulbactam against a multidrug-resistant isolate of the endemic clone in the Tainan area of southern Taiwan.

Materials and methods

Bacterial strain

Nosocomial infections caused by A. baumannii susceptible or intermittently resistant to ampicillin–sulbactam or carbapenems only, have been recognized increasingly since 2001 in the Tainan area of Taiwan. Clinical isolates with this resistant phenotype are genetically related, as revealed by PFGE (data not shown). Such a bacteraemic isolate, Ab-153, was selected and stored at –70°C in Protect Bacterial Preservers (Technical Service Consultants Limited, Heywood, Lancashire, England) before...
being cultured on Luria–Bertani agar (Difco Laboratories, Detroit, MI, USA).

**MICs determined by Etest and the agar dilution method**

MIC values of meropenem and sulbactam were measured by the agar dilution method, as described previously by the NCCLS. Meropenem powder (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in pH 7.2, 0.01 M phosphate buffer, and sulbactam (USP Reference Standards, Rockville, MD, USA) in sterile water and diluted to the required concentration. *Pseudomonas aeruginosa* ATCC 27853 was used in each run as the control. The MIC values of other antibiotics, including piperacillin, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, amikacin, gentamicin and ciprofloxacin, were determined by Etest strips (AB Biodisk, Solna, Sweden).

**Inhibitory activities of meropenem, sulbactam and both in time–kill studies**

Bacteria were diluted to $4 \times 10^5$ cfu/mL in fresh Mueller–Hinton broth. The drug concentrations of meropenem and sulbactam used in the following time–kill studies were adjusted to the $1 \times$ MIC or $0.5 \times$ MIC of each antimicrobial agent as indicated. Each flask was incubated at 37°C. Bacterial counts were enumerated at 0, 2, 4, 6, 8, 12, 24, 30, 36 and 48 h, and measured by enumerating the colony number from 10-fold serially diluted specimens of $100 \mu$L aliquots plated on nutrient agar (Difco Laboratories). The lower limit of detection was 100 cfu/mL. The experiments were carried out in duplicate.

**In vivo mice study**

Female inbred BALB/c mice (Animal Center, National Science Council, Taipei, Taiwan) weighing on average 20 g (5–6 weeks old) were used throughout the study. A bacterial suspension in a volume of 0.1 mL was delivered intraperitoneally into each mouse. The marketed parental preparations of meropenem and sulbactam were used to treat *A. baumannii* infection. The dosage of meropenem for mice is 20 mg/kg every 8 h, as described previously. The daily dose of sulbactam is, as previously described, 120 mg/kg, divided into three doses. The solutions of antimicrobial agents, prepared the morning the experiment was conducted, were diluted in sterile 0.85% saline and delivered intraperitoneally in a volume of 0.1 mL in sterile disposable plastic syringes. Antimicrobial agents were given 2 h after the animal was infected, and six doses were administrated subsequently overall. The number of surviving mice was recorded at 8 h intervals for 48 h, and at 120 h. There were four experimental groups: the control group (no antimicrobial agent was given); meropenem; sulbactam; and combined meropenem–sulbactam. There were five to ten mice in each group and the experiments (conducted in compliance with the relevant national guidelines of the Republic of China and approved by the Chi Mei Foundation Medical Center) were performed three times for confirmation of results.

**Statistical methods**

The log-rank test was applied to compare the effect between different treatments. A $P$ value of $p < 0.05$ was considered statistically significant. Data analyses were performed with SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**MIC values for Ab-153**

Ab-153 was resistant to: piperacillin, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepime, amikacin or gentamicin (MIC $>256$ mg/L); ciprofloxacin ($>32$ mg/L); and aztreonam (48 mg/L). The MICs of meropenem and sulbactam determined by the agar dilution method using a standard inoculum of $10^4$ cfu, for Ab-153, were 8 and 8 mg/L, respectively.

**Time–kill studies**

When Ab-153 at an initial inoculum of $4 \times 10^5$ cfu/mL was incubated with meropenem at a concentration of $0.5 \times$ MIC ($4 \mathrm{mg/L}$), the bacterial growth was inhibited temporarily for 6 h, but then Ab-153 regrew (Figure 1). A sulbactam concentration of $0.5 \times$ MIC ($4 \mathrm{mg/L}$) was not inhibitory, although at a concentration of $1 \times$ MIC ($8 \mathrm{mg/L}$) there was transient inhibitory activity lasting for <4 h. With a combination of meropenem and sulbactam, both at a concentration of $0.5 \times$ MIC, inhibitory activity lasted for <24 h. In contrast, with meropenem at a concentration of $0.5 \times$ MIC, combined with sulbactam at a concentration of $1 \times$ MIC, there was sustained synergistic inhibitory activity lasting for more than 48 h, defined as a reduction in viable bacterial colonies by at least two orders of magnitude compared with either drug alone.

**Therapeutic efficacy of monotherapy or combination therapy in the murine model**

With an inoculum of $2.4 \times 10^6$ cfu of Ab-153 via intraperitoneal administration, all four mice survived for at least 48 h. With a higher inoculum, $2.4 \times 10^7$ cfu, by 24 h all four mice had died.

Three rounds of mice experiments were performed and the initial inoculum was $2.1 \times 10^7$, $2.3 \times 10^7$ and $2.6 \times 10^7$ cfu, respectively (Table 1). Without antimicrobial therapy, only two (9%) of 22 mice survived to 5 days after intraperitoneal infection with Ab-153. Of mice treated with meropenem (20 mg/kg every 8 h) or sulbactam (40 mg/kg every 8 h), the survival rate was significantly higher than that of mice without treatment (8/23, 35% versus 9%, $P = 0.05$; 7/23, 30% versus 9%, $P = 0.04$). Moreover, if infected mice were treated with meropenem at a concentration of 0.5 $\times$ MIC, inhibitory activity lasted for <24 h. In contrast, with meropenem at a concentration of 0.5 $\times$ MIC, combined with sulbactam at a concentration of 1 $\times$ MIC, there was sustained synergistic inhibitory activity lasting for more than 48 h, defined as a reduction in viable bacterial colonies by at least two orders of magnitude compared with either drug alone.

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Combination therapy for multidrug-resistant Acinetobacter baumannii

Table 1. Survival rates of BALB/c mice infected intraperitoneally with Ab-153 at 5 days after treatment with saline, sulbactam, meropenem or both in combination

<table>
<thead>
<tr>
<th>Initial inoculum (cfu)</th>
<th>Surviving mouse no./total mouse no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
</tr>
<tr>
<td>2.1 × 10⁷</td>
<td>1/8 (12.5)</td>
</tr>
<tr>
<td>2.3 × 10⁷</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>2.6 × 10⁷</td>
<td>1/9 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>2/22 (9.1)</td>
</tr>
</tbody>
</table>

aData versus sulbactam-treated group, P = 0.054; control versus sulbactam-treated group, P = 0.042.

Discussion

A. baumannii is well-known for its potential to be resistant to many commonly used antimicrobial agents, including penicillins, cephalosporins, monobactams, aminoglycosides and fluoroquinolones. Infections resulting from highly resistant A. baumannii isolates, for which there are limited therapeutic options, can lead to a high fatality rate. Carbapenems are effective antimicrobial agents against A. baumannii, but the emergence of strains with reduced susceptibility to carbapenems, such as our endemic strain, has often been described.5,6 Previously, rifampicin-containing combinations of antimicrobial agents have been shown to be synergistic or additive against multidrug-resistant isolates of A. baumannii in vitro.10 However, a recent clinical study indicating the emergence of rifampicin resistance during imipenem–rifampicin combination therapy when treating carbapenem-resistant A. baumannii infections, suggested its limitation in clinical utilization.11 Sulbactam has been found to be effective in treating carbapenem-resistant A. baumannii infections,12 and we studied this combination against our multi-resistant isolate.

In the time–kill study, the combination of meropenem (0.5 × MIC) and sulbactam (1 × MIC) resulted in a sustained synergistic bactericidal effect lasting for at least 48 h. In the murine experiment, the survival rate of infected mice treated with sulbactam (30%) confirmed the effect lasting for at least 48 h. In the murine experiment, the survival and sulbactam (1 × MIC) concentration significantly improved the survival rate of mice infected with Ab-153, compared with that in animals treated with either drug alone.

Meropenem plus sulbactam was effective against our multi-resistant isolate of A. baumannii both in vitro and in vivo, and this combination warrants further clinical studies to delineate its clinical significance.

Acknowledgements

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References