Antimicrobial Effects of Varied Combinations of Meropenem, Sulbactam, and Colistin on a Multidrug-Resistant *Acinetobacter baumannii* Isolate That Caused Meningitis and Bacteremia

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Meropenem (MEM; 2 g/8 hr; minimum inhibitory concentration [MIC] = 256 mg/L) plus sulbactam (SUL; 1 g/8 hr; MIC = 128 mg/L) (two-drug–therapy period), and subsequent additional intravenous colistin (COL; 2.5 mg/kg/12 hr) and intraventricular (COL, 5 mg/day; MIC = 1 mg/L) (three-drug–therapy period) were sequentially used in a patient with postneurosurgery bacteremic meningitis due to a multidrug-resistant *Acinetobacter baumannii* (MDRAB) isolate (AB₁). We detected 4- to 32-fold increases in peak or trough cerebrospinal fluid bactericidal titer and serum bactericidal titer in three-drug–therapy period when comparing to those in two-drug–therapy period. The time-kill study with MEM, SUL, and COL alone or varied combinations (all at 1 × MIC) against AB₁ and another genetically nonrelated MDRAB isolate (AB₁₃₄ [MICs of MEM = 64 mg/L, SUL = 16 mg/L, and COL = 1 mg/L]) was performed. The two-drug combinations (MEM + SUL, MEM + COL, and SUL + COL) each elicited different inhibitory effect on AB₁ and AB₁₃₄ at 6 hr. Bacterial regrowth at 24 hr was observed in the experiments in which the MDRAB isolate was inhibited earlier by COL alone (AB₁ and AB₁₃₄), by MEM plus SUL (AB₁), and by MEM plus COL (AB₁₃₄), but not in SUL plus COL, and MEM + SUL + COL. Combined use of COL with MEM and/or SUL may provide good therapeutic options, even though MEM and SUL are in vitro resistance to the MDRAB.

**Introduction**

The mortality rate of central nervous system (CNS) infection due to multidrug-resistant *Acinetobacter baumannii* (MDRAB) was reported to range from 30% to 50%.⁷ Combination of meropenem (MEM) and sulbactam (SUL) was both in vitro and in vivo effective against MDRAB; however, experience with these combined antibiotics in the treatment of CNS infection caused by MDRAB has been very limited.⁵,⁷ In spite of poorly penetrating into CNS, colistin (COL) has a good activity against MDRAB,⁷ and is one of a few alternatives for CNS infection caused by MDRAB, especially when the MDRAB is nonsusceptible to MEM and/or SUL. The objective of this study was to better understand the in vitro activities of COL in combination with MEM and SUL against MDRAB, and the therapeutic role of COL in combination with MEM and SUL in the treatment of CNS infection due to MDRAB.

**Materials and Methods**

**Case report**

A 78-year-old man with subarachnoid hemorrhage and obstructive hydrocephalus received insertion of an external ventricular drain (EVD) on June 11, 2006, in a local hospital.

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The EVD was removed 6 days later. The tip of draining catheter and cerebrospinal fluid (CSF) both grew MDRAB (AB2), which was resistant to the all tested antimicrobials except COL. Intravenous MEM (1 g/8 hr) was used on June 21, 2006, and the patient was transferred to our institute the following day. Intravenous MEM (2 g/8 hr) and SUL (1 g/8 hr) were continued (two-drug-therapy period) after sampling blood and CSF upon arrival at our hospital; MDRAB isolates subsequently grew from culture of his blood (AB2) and CSF were continued (two-drug-therapy period) after sampling AB1–5 were resistant to amikacin, ceftazidime, cefepime, and susceptibility testing was performed. COL was added intravenously (2.5 mg/kg/12 hr) and intraventricularly (5 mg/day) (three-drug-therapy period). Blood and CSF further sampled on June 28 were sterile. Unfortunately, the patient died of hypoxemia secondary to respiratory distress syndrome on June 30. Antibiotic administration, culture results, CSF profiles, and bactericidal activities of antibiotics in serum and CSF of this patient were summarized in Table 1.

Bacterial isolate identification, clonality analysis, and susceptibility testing

All A. baumannii isolates were identified by standard methods. AB1, AB2, AB3, AB4, and AB5 (AB1-5) were proved to be of the same clone by genotyping using pulsed-field gel electrophoresis, as previously reported. AB1 was randomly selected from AB1-5 for time-kill study, as described below. The re-growing bacteria (AB1) from the initially inhibited status by COL in the time-kill experiments were subjected to repeated susceptibility testing to determine the subsequent minimum inhibitory concentration (MIC) of the tested COL. Susceptibility testing using disc diffusion method disclosed that AB1-5 were resistant to amikacin, ceftazidime, cefepime, ciprofloxacin, piperacillin-tazobactam, MEM, and SUL, but were susceptible to COL. We further identified MICs of MEM and SUL using E-test (AB Biodisk, Solna, Sweden), and MIC of COL using microdilution method. Susceptible breakpoints were ≤16 mg/L for MEM, ≤8 mg/L for SUL, and ≤2 mg/L for COL.

Serum/CSF bactericidal activities

Clinical bactericidal activities of the antibiotics being used were assessed by identifying the peak and trough serum bactericidal titer (SBT) and CSF bactericidal titer (CBT) in the patient. SBT and CBT were the maximal bactericidal dilution titers of serum and CSF, respectively. The peak SBT/CBT referred to the maximal bactericidal dilution titer of serum/CSF that was sampled 30 min after an intravenous and intraventricular administration of antibiotics, while the trough SBT/CBT referred to the maximal bactericidal dilution titer of serum/CSF that was sampled immediately before the maintenance dosing of antibiotics. SBT and CBT were determined using microtiter broth dilution method, as described elsewhere. Briefly, the patient’s serum/CSF was adjusted with normal saline to provide 100 μL serum/CSF dilutions ranging from 1:1 to 1:64. One hundred microliter of Mueller–Hinton broth with inoculation of AB1 harvested from its early log phase was added to the above-mentioned diluted serum/CSF to produce 1:2 to 1:128 diluted serum/CSF specimens each with a bacterial density of approximately 10^8 cfu/mL. Colonies were counted after an 18- to 24-hr incubation. The SBT and CBT were defined as the highest dilution that killed more than 99.9% of the AB1 inocula.

Time-kill study

Time-kill study with AB1 and another genetically different clinical A. baumannii isolate (AB134) that was used as control was performed. The tested antibiotics included standard powders of MEM (Sumitomo, Tokyo, Japan), SUL, and COL (both from TTY Biopharm, Taipei, Taiwan). Each of these antibiotics was either used alone or in varied combinations in vitro.

Table 1. Clinical Course of a Patient with Postneurosurgery Meningitis and Bacteremia Caused by Multidrug-Resistant Acinetobacter baumannii

<table>
<thead>
<tr>
<th>Date/day/month (2006)</th>
<th>Antibiotic therapy</th>
<th>Blood culture</th>
<th>WBCs/mm³ (PMN%)</th>
<th>Protein (mg/dL)</th>
<th>Glucose (mg/dL)</th>
<th>Colony count (cfu/mL)</th>
<th>SBT/CBT (peak)</th>
<th>SBT/CBT (trough)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/6</td>
<td>None</td>
<td>ND</td>
<td>2500 (94)</td>
<td>987 (&lt;5)</td>
<td>AB1 (ND)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>21/6</td>
<td>MEM (IV)</td>
<td>AB2</td>
<td>1700 (97)</td>
<td>857 (&lt;5)</td>
<td>AB2 (&gt;50,000)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22/6</td>
<td>MEM (IV) SUL (IV)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>26/6</td>
<td>MEM (IV) SUL (IV)</td>
<td>AB4</td>
<td>860 (83)</td>
<td>779 (70)</td>
<td>AB5 (10,000)</td>
<td>2/&lt;2 (two-drug therapy)</td>
<td>&lt;2/&lt;2 (two-drug therapy)</td>
<td>64/64 (three-drug therapy)</td>
</tr>
<tr>
<td>28/6</td>
<td>MEM (IV) SUL (IV)</td>
<td>Negative</td>
<td>150 (62)</td>
<td>394</td>
<td>183</td>
<td>0</td>
<td>64/64 (three-drug therapy)</td>
<td>16/8 (three-drug therapy)</td>
</tr>
</tbody>
</table>

*See text for details.

AB1, AB2, AB3, AB4, and AB5 were proven to be of the same A. baumannii strain by pulsed-field gel electrophoresis.

WBC, white blood cells; MEM, meropenem; SUL, sulbactam; COL, colistin; IV, intravenously; IA, intraventricularly; ND, not done; PMN, polymorphonuclear cell; CSF, cerebrospinal fluid; SBT, serum bactericidal titer; CBT, CSF bactericidal titer.
FIG. 1. (A) Time-kill curves of *Acinetobacter baumannii* (AB1) incubated with meropenem (1× MIC [256 mg/L]), sulbactam (1× MIC [128 mg/L]), or colistin (1× MIC [MIC = 1 mg/L]) and varied combinations of these antibiotics. The lower limitation of viable count was set at 20 cfu/ml. (B) Time-kill curves of *A. baumannii* (AB134) when incubated with meropenem (1× MIC [64 mg/L]), sulbactam (1× MIC [16 mg/L]), or colistin (1× MIC [1 mg/L]) and varied combinations of these antibiotics. The lower limitation of viable count was set at 20 cfu/ml.
time-kill experiments. Bacteria were diluted to $10^6$ cfu/ml in trypticase soy broth in which concentration of each antibiotic was adjusted to $1 \times \text{MIC}$ (used either alone or in combination), as described elsewhere.\(^9\) Bacterial colony counts were measured at 0, 2, 4, 6, and 24 hr by enumerating the number of colonies plated onto trypticase soy agar. The plates were then incubated overnight at 35°C. Synergism for the combined antibiotics was defined as $\geq 2 \log_{10}$ reduction in cfu/ml when compared with that by the most active constituent of the combined antibiotics.\(^9\) Each time-kill experiment was performed at least twice to ensure the reproducibility.

**Results**

$E$-test or microdilution method disclosed that MIC (mg/L) against $AB_{1.5}$ for MEM was 256, for SUL 128, and for COL 1, while MIC (mg/L) against $AB_{134}$ for MEM was 64, for SUL 16, and for COL 1. MIC of COL against $AB_1$ before time-kill study and $AB_1$ regrew from the once inhibited status by COL in the time-kill experiments was unchanged. Fourfold to 32-fold increases in peak or trough SBT and CBT were found in three-drug-therapy period when comparing to those in the two-drug-therapy period (Table 1).

The antimicrobial effects of each of MEM, SUL, and COL alone and varied combinations on $AB_1$ (Fig. 1A) and $AB_{134}$ (Fig. 1B) in time-kill study are shown in Figure 1. COL in combination with MEM and SUL (three-drug combination) demonstrated a rapid inhibitory effect on the $AB_1$ and $AB_{134}$. The two-drug combinations (MEM+SUL, MEM+COL, and SUL+COL) each elicited different degrees of inhibitory effect on $AB_1$ and $AB_{134}$ at 6 hr. Of note, re-growth at 24 hr was observed in the experiments in which the tested MDRAB was inhibited earlier by COL alone ($AB_1$ and $AB_{134}$), by MEM plus SUL ($AB_1$) and by MEM plus COL ($AB_{134}$). The COL-containing regimens (MEM+SUL+COL, COL+MEM, and COL+SUL) each were effective, and their constituents act synergistically against $AB_1$, while MEM+SUL+COL, COL+SUL, and MEM+SUL exerted similar effects on $AB_{134}$. Of note, MEM+SUL was not effective against $AB_1$, not to mention synergism.

**Discussion**

Synergistic effect by $0.5 \times \text{MIC}$ (8 mg/L) of MEM and $1 \times \text{MIC}$ (8 mg/L) of SUL was previously reported in an MDRAB isolate against which the MICs of MEM and SUL were remarkably lower.\(^9\) Our study suggests that when against an MDRAB isolate with moderately higher MICs (e.g., $AB_{134}$ with MIC = 64 mg/L for MEM, and MIC = 16 mg/L for SUL), $1 \times \text{MIC}$ of MEM, and $1 \times \text{MIC}$ of SUL remain act synergistically; however, there was no synergism by $1 \times \text{MIC}$ of MEM and $1 \times \text{MIC}$ of SUL against an MDRAB with further higher MICs (e.g., $AB_1$ with MIC = 256 mg/L for MEM, and MIC = 128 mg/L for SUL). This synergistic activity might be related to the concentration of bacterial penicillin-binding protein.\(^2\) Our data implicate that when it comes to antimicrobial treatment of infection caused by MDRAB, the MICs of MEM and SUL should be clearly identified to ensure that they act synergistically. Further study is warranted to determine at what MIC cutoffs for MEM and SUL, synergism of these antibiotics against an MDRAB isolate vanishes.

In time-kill study, $AB_1$ and $AB_{134}$ were initially inhibited by COL ($1 \times \text{MIC}$ alone), and began to regrow 6 hr later; the regrowth level at 24 hr was as high as those in cases $AB_1$ and $AB_{134}$ being incubated with either MEM ($1 \times \text{MIC}$) or SUL ($1 \times \text{MIC}$). Similar observations were previously reported.\(^8\) Judging from the unchanged MIC of COL against $AB_1$ before time-kill study and after $AB_1$ that regrew at 24 hr from the once inhibited status by COL in time-kill study, inactivation of COL best explains this phenomenon. COL was introduced into clinical use over 5 decades ago, and it was therefore never subject to detailed trials on pharmacology or pharmacokinetics. COL is an old drug that has put to a new use for multidrug-resistant bugs.\(^6\) Further study is deserved to determine the optimal dosing and optimal dosing time interval for COL to overcome the time-dependent inactivation of COL.

Were the comorbidity and immunocompromise absent in the patient in this report, the resolution of his meningitis might yet have occurred with continued MEM-SUL therapy or with COL alone, although our data demonstrated that COL with MEM and/or SUL may provide better therapeutic options for meningitis due to MDRAB by giving a deeper insight into the favorable microbiologic responses to COL-containing antibiotics in the time-kill study and to the COL-containing regimen in the treatment of MDRAB CNS infection. Our data also highlight the importance of COL in combination with MEM and/or SUL even though MEM and SUL are in vitro resistance to the culprit MDRAB. Further study is needed to consolidate the role of COL in the treatment infections caused by MDRAB, especially CNS infection due to this pathogen.

**Author Disclosure Statement**

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**References**


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