Therapeutic Efficacy of BO-3482, a Novel Dithiocarbamate Carbapenem, in Mice Infected with Methicillin-Resistant Staphylococcus aureus

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The in vivo activity of BO-3482, which has a dithiocarbamate chain at the C-2 position of 1β-methyl-carbapenem, was compared with those of vancomycin and imipenem in murine models of septicemia and thigh infection with methicillin-resistant Staphylococcus aureus (MRSA). Because BO-3482 was more susceptible than imipenem to renal dehydropeptidase I in a kinetic study of hydrolysis by this renal enzyme, the therapeutic efficacy of BO-3482 was determined during coadministration with cilastatin. In the septicemia models, which involved two homogeneous MRSA strains and one heterogeneous MRSA strain, the 50% effective doses were, respectively, 4.80, 6.06, and 0.46 mg/kg of body weight for BO-3482; 5.56, 2.15, and 1.79 mg/kg for vancomycin; and >200, >200, and 15.9 mg/kg for imipenem. BO-3482 was also as effective as vancomycin in an MRSA septicemia model with mice with cyclophosphamide-induced immunosuppression. In the thigh infection model with a homogeneous MRSA strain, the bacterial counts in tissues treated with BO-3482–cilastatin were significantly reduced in a dose-dependent manner compared with the counts in those treated with vancomycin and imipenem–cilastatin ($P < 0.001$). These results indicate that BO-3482–cilastatin is as effective as vancomycin in murine systemic infections and is more bactericidal than vancomycin in local-tissue infections. The potent in vivo activity of BO-3482–cilastatin against such MRSA infections can be ascribed to the good in vitro anti-MRSA activity and improved pharmacokinetics in mice when BO-3482 is combined with cilastatin and to the bactericidal nature of the carbapenem.

Although more than 30 years have passed since the first report of methicillin-resistant Staphylococcus aureus (MRSA) (13), MRSA still presents a serious problem as a cause of nosocomial infections worldwide (19). Vancomycin, a cyclic glycopeptide antibiotic, is extensively used in clinics to treat MRSA infections; however, this antibiotic is not an ideal antibiotic because of the slow clinical response to this agent (12) and its possible adverse effects (4). In the late 1980s, methicillin-resistant coagulase-negative staphylococci (20) and vancomycin-resistant enterococci began to appear (9, 24). The possibility that vancomycin-resistant MRSA strains are emerging is suggested by the demonstration that vancomycin resistance genes can transfer from enterococci to S. aureus and is expressed by S. aureus (16). Therefore, it is necessary to develop a new antibiotic that is clinically useful against MRSA.

Recently, various anti-MRSA β-lactams such as the carbapenems L-695,256 (3) and SM-17466 (21) and the cephems TOC-39 (6), FK-037 (23), and 2-oxacephems (22) have been described.

In our laboratory, a new 1β-methyl-carbapenem, BO-3482 sodium, (1R,5S,6S)-6-[(R)-1-hydroxyethyl]-2-[(2-hydroxyethyl)-N-methyl amino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate (Fig. 1), was discovered in the course of derivatization directed toward anti-MRSA carbapenems (1, 7). The introduction of a dithiocarbamate side chain at the C-2 position of 1β-methyl-carbapenem led to good binding to PBP 2* (or PBP 2a) of MRSA, which reflected high activity against homogeneous MRSA. In this report, we describe the in vivo antimicrobial activity of BO-3482 against MRSA strains and compare it with those of vancomycin and imipenem in murine septicemia and thigh infection models.

MATERIALS AND METHODS

Antibiotics. BO-3482 was synthesized at the Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba, Japan. Imipenem and cilastatin sodium were the products of Banyu Pharmaceutical Co., Ltd. Vancomycin was purchased from Sigma Chemical Co., St. Louis, Mo. The solutions of antimicrobial agents were freshly prepared on the day of use. In the in vitro study, vancomycin was dissolved in 50 mM phosphate buffer (pH 7.0) and BO-3482 and imipenem were dissolved in 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0). In the pharmacokinetic and therapeutic efficacy studies, the antibiotics were dissolved in saline or cilastatin-saline. Cilastatin was coadministered at a dose of 40 mg per kg of body weight per dose in treatments with imipenem or BO-3482.

Bacterial strains. Clinical isolates of MRSA, isolates BB6221, BB6226, and BB6156, which were collected from several hospitals in Japan over the past several years, and methicillin-susceptible S. aureus (MSSA) Smith were used for the murine septicemia model. MRSA BB6294 was used for the murine thigh infection model. MRSA BB6221, BB6226, and BB6294 are β-lactamase negative and homogeneously resistant, while strain BB6156 is β-lactamase positive, inducible, and heterogeneously resistant. β-Lactamase production was investigated by incubating the cell suspension with nitrocefin, a chromogenic cephalosporin (17). The inducibility was determined by the same method in the absence or presence of a sub-MIC of imipenem, an inducer. All bacteria were maintained in glycerol broth at −80°C.

Susceptibility tests. MICs were determined by the twofold agar dilution method with Mueller-Hinton medium (Difco Laboratories, Detroit, Mich.). The medium was supplemented with 2% NaCl for MRSA. An overnight culture grown at 37°C in Mueller-Hinton broth (Difco) was diluted to 10⁶ CFU/ml and was inoculated onto a drug-containing agar surface with an inoculum apparatus (Microplanter; Sakuma Seisakusyo, Tokyo, Japan). The final inoculum size was approximately 10⁶ cells per spot. The MIC was defined as the lowest concentration that inhibited visible growth after 18 h of incubation at 37°C for MSSA and 48 h of incubation at 35°C for MRSA.

Stability against renal DHP-I. The stabilities of the carbapenems against renal dehydropeptidase I (DHP-I) were determined by placing purified murine and porcine renal DHP-I. Enzyme was prepared by the procedure described previously (2). The activity of DHP-I was spectrophotometrically determined by

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when the protected least-significant-difference test (8). Data were considered significant viable bacterial counts for the different groups were performed by Fisher’s calculating the arithmetic mean S. aureus appropriate dilutions of the homogenate. The detection limit was 2 log10 CFU/ml with a tissue homogenizer (Ystral, Gottingen, Germany). Viable cells counts muscles were removed and immediately homogenized in ice-cold 0.9% NaCl inoculated with Bacillus subtilis ATCC 12432 as the indicator organism (11), and those of vancomycin were determined by the same method but with 1% sodium citrate-supplemented nutrient agar (Difco) (15). Samples of plasma and urine were appropriately diluted with pooled mouse serum and 10 mM MOPS buffer (pH 7.0), respectively. A total of 26 μl of samples and standards were deposited onto paper disks (8 mm in diameter), and the disks were then placed on the inoculated agar plates in triplicate. The plates were incubated at 37°C overnight, and the zones of inhibition were measured. The potencies of the test samples were calculated from the standard curve.

Pharmacokinetic parameters were calculated by the moment method (25). The maximum concentration of drug in plasma (Cmax) was directly determined from the profiles of the concentration in plasma. The elimination rate constant (k(el)) was calculated from linear regression analysis of the plasma concentration-time curve. The half-life (t1/2) was calculated as ln2/k(el). The area under the plasma concentration-time curve (AUC) was determined by using the trapezoidal rule and was extrapolated to infinity.

RESULTS

Stability to renal DHP-I. The kinetic parameters for murine and porcine renal DHP-I in the hydrolysis of BO-3482 and imipenem are presented in Table 1. The Km, Vmax values of DHP-I for BO-3482 varied with the source of the enzyme. In both cases, BO-3482 showed a higher affinity for DHP-I than imipenem did, since the Km values of murine and porcine DHP-Is for BO-3482 were 1/4 and 1/32 of those for imipenem, respectively. The relative Vmax of porcine DHP-I for BO-3482 when the hydrolysis rate of imipenem was taken as 100 was about one-eighth that of murine DHP-I. The relative Vmax/Km values of murine and porcine DHP-Is were 1.74 and 1.65, respectively, with those for imipenem being taken as 1. Thus, BO-3482 was more readily hydrolyzed by murine and porcine renal DHP-I than imipenem was.

Systemic infection. Because BO-3482 was susceptible to renal DHP-I, its efficacy against MRSA was initially investigated in the presence and absence of cilastatin. The ED50 of BO-3482 alone and with cilastatin were 19.5 and 4.80 mg/kg, respectively, in the murine model of septicaemia caused by the homogeneous strain MRSA BB6221 (Table 2). These results, together with the finding of vulnerability to DHP-I, showed that coadministration with cilastatin was necessary for BO-3482 to exert potent anti-MRSA activity in vivo (10). Therefore, cilastatin was used in the in vivo evaluation of BO-3482 against other S. aureus strains.

Table 2 also indicates the comparative protective efficacies of BO-3482–cilastatin, vancomycin, and imipenem-cilastatin against systemic MRSA and MSSA infections in normal mice. The ED50 of BO-3482–cilastatin against homogeneous high-level MRSA BB6221 and BB6226 were 4.80 and 6.06 mg/kg,

Hydrolysis rate relative to that of imipenem when the hydrolysis rate of imipenem was taken as 100.

Relative values that for imipenem was taken as 1.0.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Parameters of DHP-I derived from the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice</td>
</tr>
<tr>
<td></td>
<td>Km (mM)</td>
</tr>
<tr>
<td>BO-3482</td>
<td>2.00</td>
</tr>
<tr>
<td>Imipenem</td>
<td>7.99</td>
</tr>
</tbody>
</table>

FIG. 1. Chemical structure of BO-3482. Me, methyl.
respectively, while those of vancomycin were 5.56 and 2.15 mg/kg, respectively. BO-3482–cilastatin was as effective as vancomycin in protecting mice against the two homogeneous high-level MRSA strains, despite its lower in vitro activity (MICs, 6.25 versus 1.56 \text{mg/ml}). In these models, imipenem-cilastatin was ineffective (ED\text{50}, >200 mg/kg). BO-3482–cilastatin was as effective as vancomycin and was more effective than imipenem against heterogeneous low-level MRSA BB6156 infection; the ED\text{50} of BO-3482–cilastatin was 0.46 mg/kg. Although the MIC of BO-3482 was eightfold higher than that of imipenem against MSSA Smith, there was no significant difference between BO-3482–cilastatin and imipenem-cilastatin. Similarly, BO-3482–cilastatin was also as active as vancomycin against the infection with MSSA Smith.

The protective effect of BO-3482 against MRSA BB6221 infection was investigated in mice with cyclophosphamide-induced immunosuppression (Table 3). There was no appreciable difference between the ED\text{50} of BO-3482–cilastatin and vancomycin (3.15 versus 1.07 mg/kg).

**Thigh infection.** Figure 2 indicates the therapeutic effect of BO-3482 against thigh infection with MRSA BB6294 in immunosuppressed mice. With time after inoculation, the bacterial counts in the thigh muscle of the control animals showed a significant increase of 1.16 log\text{10} CFU/thigh. The dose of BO-3482 used in this study was based on the possible maximum clinical dose. At all doses, BO-3482–cilastatin was more effective than vancomycin at 40 mg per kg.

BO-3482–cilastatin, vancomycin, and imipenem-cilastatin produced significant \((P < 0.001)\) reductions in bacterial counts (2.34, 1.21, and 0.73 log\text{10} CFU/thigh, respectively). Even at 10 mg per kg per dose, BO-3482–cilastatin caused a significant \((P < 0.001)\) reduction in bacterial count of 1.36 log\text{10} CFU/thigh, while vancomycin produced a nonsignificant reduction of 0.16 log\text{10} CFU/thigh.

**TABLE 2.** Protective efficacy of BO-3482 against systemic staphylococcal infections in normal mice

<table>
<thead>
<tr>
<th>S. aureus strain, challenge dose (CFU/mouse [multiple of LD\text{50}])</th>
<th>Antibiotic</th>
<th>MIC (\text{mg/ml})</th>
<th>ED\text{50} (mg/kg) [95% confidence limit]\text{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB6221, 4.07 \times 10^8 (4.4)</td>
<td>BO-3482</td>
<td>6.25</td>
<td>19.5 (9.51–44.3)</td>
</tr>
<tr>
<td></td>
<td>BO-3482–cilastatin\text{b}</td>
<td>6.25</td>
<td>4.80 (1.66–10.7)</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>1.56</td>
<td>5.56 (3.47–8.92)</td>
</tr>
<tr>
<td></td>
<td>Imipenem-cilastatin</td>
<td>100 \text{&gt;200 (NC)}</td>
<td></td>
</tr>
<tr>
<td>BB6226, 9.23 \times 10^7 (13)</td>
<td>BO-3482–cilastatin</td>
<td>6.25</td>
<td>6.06 (2.58–14.7)</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>1.56</td>
<td>2.15 (0.53–7.62)</td>
</tr>
<tr>
<td></td>
<td>Imipenem-cilastatin</td>
<td>100 \text{&gt;200 (NC)}</td>
<td></td>
</tr>
<tr>
<td>BB6156, 3.98 \times 10^7 (3.5)</td>
<td>BO-3482–cilastatin</td>
<td>3.13</td>
<td>0.46 (0.05–2.17)</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>1.56</td>
<td>1.79 (0.96–3.27)</td>
</tr>
<tr>
<td></td>
<td>Imipenem-cilastatin</td>
<td>12.5</td>
<td>15.9 (7.93–32.6)</td>
</tr>
<tr>
<td>Smith, 6.39 \times 10^6 (29)</td>
<td>BO-3482–cilastatin</td>
<td>0.10</td>
<td>0.11 (0.063–0.194)</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>0.20</td>
<td>0.51 (0.156–0.617)</td>
</tr>
<tr>
<td></td>
<td>Imipenem-cilastatin</td>
<td>0.012</td>
<td>0.052 (0.026–0.096)</td>
</tr>
</tbody>
</table>

\text{a} Mice received a single subcutaneous dose of the antibiotic 1 h after bacterial challenge. The ED\text{50} was expressed as the dose of each antibiotic.

\text{b} Cilastatin was coadministered at 40 mg/kg.

\text{c} NC, not calculated.

**TABLE 3.** Protective efficacy of BO-3482 against systemic staphylococcal infections in immunosuppressed mice

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (\text{mg/ml})</th>
<th>ED\text{50} (mg/kg/dose [95% confidence limit])</th>
</tr>
</thead>
<tbody>
<tr>
<td>BO-3482–cilastatin\text{b}</td>
<td>6.25</td>
<td>3.15 (1.15–7.03)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.56</td>
<td>1.07 (0.34–2.41)</td>
</tr>
<tr>
<td>Imipenem-cilastatin</td>
<td>100</td>
<td>34.5 (12.9–99.5)</td>
</tr>
</tbody>
</table>

\text{a} Mice received subcutaneous doses of the antibiotic 1 and 3 h after bacterial challenge with 1.22 \times 10^7 CFU/mouse (32 LD\text{50}).

\text{b} Cilastatin was coadministered at 40 mg/kg.

**FIG. 2.** Comparative efficacies of BO-3482, vancomycin, and imipenem against MRSA in a murine thigh infection model. The right thighs of mice (\(n = 4\)) with cyclophosphamide-induced immunosuppression were injected subcutaneously with drugs 2 and 6 h after inoculation of homogeneous MRSA BB6294. The treatment regimens were 10, 20, or 40 mg of BO-3482 with 40 mg of cilastatin per kg per dose; 10, 20, or 40 mg of vancomycin (VCM) per kg per dose; or 40 mg of imipenem (IPM) with 40 mg of cilastatin per kg per dose. Four hours after the last treatment, the number of viable cells in the thigh muscles was determined and expressed as the geometric mean \pm standard error. †, \(P < 0.001\) versus controls; *, \(P < 0.001\); **, \(P < 0.01\).
Pharmacokinetic parameters. The pharmacokinetic parameters of BO-3482 in mice are presented in Table 4. The \( C_{\text{max}} \) of BO-3482 given as a single subcutaneous dose of 10 mg/kg was higher than those of imipenem and vancomycin: \( C_{\text{max}} \)s were 25.0, 12.6, and 13.1 \( \mu \text{g/ml} \), respectively, even when BO-3482 was injected alone. Coadministration of BO-3482 with cilastatin extended the \( t_{1/2} \) in plasma, AUC, and urinary recovery of BO-3482 to levels 1.8-, 1.6-, and 1.9-fold higher than those for carbapenem given alone, respectively.

**DISCUSSION**

BO-3482, which has a dithiocarbamate structure at the C-2 position, is a novel 1-\( \beta \)-methyl-carbapenem with potent anti-bacterial activity against MRSA. As reported previously (1, 7), BO-3482 has an improved affinity for PBP2a, as suggested by the results of the present study, compared to imipenem in particular and also to the bactericidal nature of BO-3482, which is superior to that of vancomycin (1).

In conclusion, BO-3482 compared favorably with vancomycin in murine models of systemic and tissue infections caused by MRSA and appears to be a promising anti-MRSA agent.

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**REFERENCES**


