Pharmacodynamic Characterization of Ceftobiprole in Experimental Pneumonia Caused by Phenotypically Diverse *Staphylococcus aureus* Strains

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Ceftobiprole (BPR) is an investigational cephalosporin with activity against *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA) strains. The pharmacodynamic (PD) profile of BPR against *S. aureus* strains with a variety of susceptibility phenotypes in an immunocompromised murine pneumonia model was characterized.

The BPR MICs of the test isolates ranged from 0.25 to 2 μg/ml. Pharmacokinetic (PK) studies were conducted with infected neutropenic BALB/c mice; and the BPR concentrations were measured in plasma, epithelial lining fluid (ELF), and lung tissue. PD studies with these mice were undertaken with eight *S. aureus* isolates (two methicillin-susceptible *S. aureus* strains, three hospital-acquired MRSA strains, and three community-acquired MRSA strains). Subcutaneous BPR doses of 2 to 125 mg/kg of body weight/day were administered, and the change in the number of log10 CFU/ml in lungs was evaluated after 24 h of therapy. The PD profile was characterized by using the free drug exposures (fT > MIC) determined from the following parameters: the percentage of time that the concentration was greater than the MIC (T > MIC), the maximum concentration in serum/MIC, and the area under the concentration-time curve/MIC. The BPR PK parameters were linear over the dose range studied in plasma, and the ELF concentrations ranged from 60 to 94% of the free plasma concentration. fT > MIC was the parameter that best correlated with efficacy against a diverse array of *S. aureus* isolates in this murine pneumonia model. The 80% effective dose (ED80), ED50, and stasis exposures appeared to be similar among the isolates studied. BPR exerted maximal antibacterial effects when fT > MIC ranged from 6 to 22%, regardless of the phenotypic profile of resistance to β-lactam, fluoroquinolone, erythromycin, clindamycin, or tetracycline antibiotics.

Pneumonia has been recognized as a difficult-to-treat infection and is associated with high rates of morbidity and mortality, especially in critically ill and immunocompromised hosts (3). At present, *Staphylococcus aureus* has been identified as the foremost gram-positive pathogen that causes hospital-acquired (HA) pneumonia and has increasingly been reported as a cause of community-acquired (CA) pneumonia in recent years (12, 16, 18, 23). Methicillin-resistant *S. aureus* (MRSA) has become a cause for concern in both the hospital and the community settings, as these MRSA infections have been associated with increased rates of mortality, lengths of stay, and costs of care (8, 12, 16, 19, 20).

In the wake of the increasing occurrence of MRSA and, particularly, the increasing rates of occurrence of pneumonia caused by this organism, treatments are limited. The treatments recommended for health care-associated MRSA pneumonia include vancomycin and linezolid as the preferred agents (23). While vancomycin is considered the “gold standard” treatment, it has been associated with poor clinical outcomes in cases of pneumonia caused by MRSA strains with MICs for susceptibility of 2 μg/ml, presumably due to poor penetration into the lung (24).

Ceftobiprole (BPR) is the first cephalosporin with anti-MRSA activity that has completed phase III clinical trials (5, 6). In vitro, BPR is also active against vancomycin-intermediate *S. aureus*, vancomycin-resistant *S. aureus*, and enterococci, as well as some gram-negative pathogens, including *Pseudomonas aeruginosa* and non-extended-spectrum β-lactamase-producing members of the family *Enterobacteriaceae* (13, 17). The in vivo efficacy of BPR against MRSA has been confirmed with several animal models (9). Results from phase III studies of complicated skin and skin structure infections confirmed the efficacy of BPR against MRSA (26). Further phase III studies are under way to evaluate the clinical efficacy of BPR for the treatment of other serious infections, such as nosocomial pneumonia (1, 6).

BPR appeared to exert in vivo activity comparable to that of the commercially available expanded-spectrum cephalosporins when it was studied in a model of mouse pneumonia caused by *Streptococcus pneumoniae* (4) and gram-negative pathogens (28). In a murine thigh infection model, BPR has also demonstrated time-dependent antimicrobial activity against MRSA and *Streptococcus pneumoniae* strains (2, 4).

The pharmacodynamic (PD) characteristics of BPR in staphyloccocal pneumonia have not yet been studied; thus, the aim of this current study was to characterize the PD profile of BPR against *S. aureus* isolates, including methicillin-susceptible *S. aureus* (MSSA), HA-MRSA, and CA-MRSA isolates with a variety of resistance phenotypes in a murine pneumonia model.

MATERIALS AND METHODS

Antimicrobials. BPR (BAL9141) and BPR medocaril (BAL5788, prodrug of BPR) were supplied by Johnson & Johnson Pharmaceutical Research & Devel-
opment (Raritan, NJ) for in vitro and in vivo experiments, respectively. Compound BAL9141 is water insoluble; thus, the water-soluble prodrug BAL7588 (BPR medocaril) was used for the in vivo studies. Vancomycin, erythromycin, doxycycline, clindamycin, and trimethoprim-sulfamethoxazole were obtained from Sigma-Aldrich (St. Louis, MO). Levofloxacin and linezolid were provided by Johnson & Johnson Pharmaceutical Research & Development and Pharmacia & Upjohn (Pharmacia Corp., Kalamazoo, MI), respectively.

**Bacteria.** Eight *S. aureus* isolates (two MSSA, three CA-MRSA, and three HA-MRSA isolates) were used for the PD evaluation of BPR. The two MSSA isolates were ATCC 29213 and ATCC 25923. The HA-MRSA strains (strains 56, 149, and 152) and the CA-MRSA strains (strains 144, 146 and 147) were clinical isolates that have been phenotypically and genotypically characterized (15, 20, 22, 30). All isolates were maintained in double-strength skim milk medium (BD Biosciences, Sparks, MD) at ±8°C. Before they were used in experiments, the isolates were subcultured twice on Trypticase soy agar with 5% sheep blood agar and Columbia nutrient agar (for the prevention of coagulase-negative *S. aureus*) isolation. The MICs of BPR and the other compounds tested against these *S. aureus* isolates were determined in triplicate by broth microdilution methods, according to the guidelines of the Clinical and Laboratory Standards Institute (10).

**Animals.** Pathogen-free inbred female BALB/cAnNCr mice (ages, 7 to 9 weeks; weight range, 15 to 22 g) were obtained from the National Cancer Institute, Frederick, MD. The study protocol was approved by the Hartford Hospital Institutional Animal Care and Use Committee. The animals were accelerated for 7 to 14 days before the experiments were initiated and were adequately supplied with water and chow throughout the study. Two separate injections of cyclophosphamide (Cytoxan; Bristol-Myers Squibb, Princeton, NJ) were used to create neutropenia in the mice. The first dose of cyclophosphamide was administered intraperitoneally at 250 mg/kg of body weight 4 days before injection to provide a wide range of BPR exposures, dosages of 1 to 25 mg/kg were used in each dose-exposure group. At approximately 6 h after inoculation (0 h), lungs were collected from a group of untreated controls to provide a baseline measurement of the lung bacterial density. BPR or sham treatment (sterile water for injection) was provided by the study sponsor, Johnson & Johnson Pharmaceutical Research & Development. Graphs of the log10 change in the number of CFU at 24 h versus the PD parameters were constructed by using the level of free drug exposure (C/F), were plotted using the sigmoid maximum-effect model. The 80% effective dose (ED80), ED95, and stasis exposure values were calculated from the individual curve for each *S. aureus* isolate as well as from a composite curve for all eight isolates.

**TABLE 1. In vitro susceptibilities of *Staphylococcus aureus* strains to BPR and other compounds**

<table>
<thead>
<tr>
<th>Drug*</th>
<th>MSSA ATCC 29213</th>
<th>MSSA ATCC 25923</th>
<th>CA-MRSA 144</th>
<th>CA-MRSA 146</th>
<th>CA-MRSA 147</th>
<th>HA-MRSA 56</th>
<th>HA-MRSA 149</th>
<th>HA-MRSA 152</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPR1</td>
<td>0.250</td>
<td>0.250</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>LZD</td>
<td>2.0</td>
<td>4.0</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>VAN</td>
<td>1.0</td>
<td>2.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ERY</td>
<td>0.125</td>
<td>0.125</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>32</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>CLI</td>
<td>0.125</td>
<td>0.125</td>
<td>&gt;16</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>LVX</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>8</td>
<td>8</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>TMP-SXT</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>DOX</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* LZD, linezolid; VAN, vancomycin; ERY, erythromycin; CLI, clindamycin; LVX, levofloxacin; TMP-SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline.

* MIC range, 0.25 to 1 μg/ml according to CLSI guidelines (10).
RESULTS

Table 1 displays the phenotypic resistance profiles to antimicrobial compounds of the eight *S. aureus* isolates. The BPR MICs for the isolates ranged from 0.25 to 2 µg/ml. The MICs of BPR were lower than those of vancomycin for the majority of the isolates.

In plasma, a linear pharmacokinetic profile was noted for BPR over the range of doses studied. The half-life of BPR in mice was estimated to be 0.25 to 0.45 h. The values for other pharmacokinetic parameters are summarized in Table 2. The total plasma concentration for each single dose is displayed in Fig. 1. In plasma, \( fT_{\text{MIC}} \) ranged from 3 to 58%, while \( fC_{\text{max}}/\text{MIC} \) and \( fAUC/\text{MIC} \) ranged from 1 to 63 and 2 to 262, respectively, with the dosage regimens used.

Overall, the concentrations of BPR in ELF and lung tissue increased with escalating dosages, and the concentrations in ELF exceeded those observed in whole lung tissue for all dosages studied (Fig. 2). The AUC\(_{0-4}\) values of BPR in ELF were estimated by use of the trapezoidal rule and ranged from 60 to 94% of the free drug concentration in plasma. The level of lung tissue penetration, as estimated by the ratio of the AUC\(_{0-4}\) for free BPR in the lung to the AUC\(_{0-4}\) for free BPR in plasma, was approximately 25% (range, 17 to 40%).

The starting (0-h) bacterial density in the lungs of the controls was consistently 10⁵ to 10⁶ CFU/ml (5.80 ± 0.22, mean ± standard deviation) between each experiment for all of the *S. aureus* isolates. At 24 h after inoculation, the bacterial density had increased 1.3 to 1.9 log units in the untreated control mice. The maximal change in bacterial density in the lungs at 24 h after BPR treatment was approximately a 2.5-log decrease compared to the initial numbers of CFU.

The PD profiles of BPR appeared to be similar against the eight *S. aureus* isolates. The relationship between the antimicrobial activity of BPR and each PD parameter was assessed for each individual *S. aureus* isolate separately, as well as for a composite of all eight isolates (Fig. 3). The correlations (\( R^2 \)) of the composite curves for the eight isolates tested between the change in the log₁₀ numbers of CFU and the three PD parameters \( fT_{\text{MIC}} \), \( fC_{\text{max}}/\text{MIC} \), and \( fAUC/\text{MIC} \) were 0.831, 0.771, and 0.807, respectively. Overall, in comparison to \( fC_{\text{max}}/\text{MIC} \) and \( fAUC/\text{MIC} \), \( fT_{\text{MIC}} \) was the parameter that best correlated with efficacy by the determination of \( R^2 \) and the distribution of the data along the fitted curve. As demonstrated in Table 3, the individually generated ED₈₀, ED₅₀, and stasis exposure values appeared to be similar among the eight *S. aureus* isolates studied. The maximum changes in the numbers of CFU were determined by measurement of the reductions in the numbers of CFU in the treatment groups in relation to the numbers of CFU in the control animals at 24 h. For all test isolates, the maximum lung bacterial titer reduction occurred

![FIG. 1. Total plasma drug concentration of BPR after a single s.c. dose.](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Dosing regimen (mg/kg)</th>
<th>( C_{\text{max}} ) (mg/liter)</th>
<th>( T_{\text{max}} ) (h)</th>
<th>AUC(_{0-4}) (mg · h/liter)</th>
<th>( V_F ) (liter/kg)</th>
<th>Half-life (h)</th>
<th>CL (ml/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.74</td>
<td>0.48</td>
<td>1.84</td>
<td>0.19</td>
<td>0.25</td>
<td>0.54</td>
</tr>
<tr>
<td>2.5</td>
<td>2.91</td>
<td>0.41</td>
<td>2.57</td>
<td>0.63</td>
<td>0.45</td>
<td>0.97</td>
</tr>
<tr>
<td>10</td>
<td>19.75</td>
<td>0.15</td>
<td>15.79</td>
<td>0.38</td>
<td>0.42</td>
<td>0.63</td>
</tr>
<tr>
<td>25</td>
<td>38.59</td>
<td>0.47</td>
<td>35.25</td>
<td>0.41</td>
<td>0.40</td>
<td>0.71</td>
</tr>
</tbody>
</table>

\( a \) \( T_{\text{max}} \) time required to achieve \( C_{\text{max}} \); AUC\(_{0-4}\) AUC from time zero to infinity; \( V_F \), volume of distribution; CL, clearance.
MIC values of 6 to 22%, with an average fT > MIC for an ED_{so} of 15% ± 5%.

Likewise, BPR displayed similar effects against this consortium of S. aureus isolates when the data were taken together. When they were analyzed as one data set, the fT > MICs required to achieve ED_{so}, ED_{so}, and stasis, as determined from the composite curve for the eight isolates (Fig. 3), were 17%, 12%, and 11%, respectively. The fC_{max} / MICs required to achieve ED_{so} and ED_{so} for the eight isolates ranged from 2 to 21 (15 ± 7) and 2 to 87 (19 ± 28), respectively. The fAUC / MICs required to achieve ED_{so} and ED_{so} for the eight isolates ranged from 3 to 34 (21 ± 12) and 3 to 420 (72 ± 154), respectively.

**DISCUSSION**

While the S. aureus isolates used in the current study displayed diverse phenotypic profiles, the BPR MICs ranged from 0.25 to 2 μg/ml and were consistent with the values previously reported for the compound (17).

In pneumonia, ELF is believed to be the primary site of infection for extracellular organisms like S. aureus (27). The...
pharmacokinetic data from this study showed that BPR sufficiently penetrated the ELF and achieved concentrations in excess of the MICs for the isolates. As whole lung tissue contains both extracellular and intracellular fluid that may dilute the antibiotic concentration, lower concentrations in lung tissue compared to those in ELF are not unexpected. The concentrations of BPR in the lung tissue in our study with infected, neutropenic mice were lower than those obtained with uninfected, nonneutropenic mice reported by Azoulay-Dupuis et al. (4). The use of different strains of mice, especially a strain with a functional immune system, may have heightened the dissimilarity in the values of the pharmacokinetic parameters obtained between the studies. The concentrations of BPR in ELF and lung tissue obtained from four sampling time points in our study provided an estimation of BPR’s rate and extent of penetration into the target sites of infection.

In the current study, we found that $fT > MIC$ was the PD parameter which best defined the efficacy of BPR against a diverse array of *S. aureus* isolates in a murine pneumonia model. BPR exerted maximal antibacterial effects when $fT > MIC$ was approximately 20%, regardless of the phenotype of resistance to other antimicrobial compounds, after 24 h of drug exposure. Our results bear similarity to those previously determined with a *S. aureus* murine thigh infection model (2) and *S. pneumoniae* lung infection model (4). Against two MRSA isolates in a neutrophilic thigh infection model, BPR displayed time-dependent killing, as an exposure with a $T > MIC$ equal to 23 to 33% resulted in a static effect; however, the authors did not specify whether these results were calculated with total or free drug exposures (2). In the study with the *S. pneumoniae* pneumonia model, $T > MICs$ between 9 and 18% were required for efficacy (4).

While other cephalosporin antibiotics require $fT > MICs$ of approximately 30 to 40% for stasis or 60 to 70% for bacterialidal effects (11), our study demonstrated that BPR exerted consistent killing activity against diversely resistant *S. aureus* isolates at a lower level of free drug exposure in neutropenic hosts. PD studies conducted with neutropenic models, such as that used in the current study, are challenging trials for BPR since the animals lack a functioning immune system. Several studies have supported the differences in antibiotic treatment outcomes between neutropenic and nonneutropenic hosts (7, 25). The antimicrobial effects of BPR were predictable for both MSSA and MRSA isolates and were not affected by resistance to other classes of antibiotics. The low level of drug exposure in plasma required for BPR may be related in part to the low percentage of protein binding, which improved penetration into target tissues. The good penetration of BPR into ELF and lung tissue potentially accounts for its reliable efficacy in this pneumonia model; thus, this agent should offer an attractive option for the treatment of serious MRSA infections, including pneumonia, in critically ill or immunocompromised patients. *S. aureus* has been identified as a common causative organism in HA pneumonia and, more recently, as a pathogen in CA pneumonia (16, 19, 23). Moreover, Kollef et al. identified *S. aureus* as the leading pathogen in pneumonia and as the only pathogen independently associated with mortality (19). For the treatment of either CA pneumonia or HA pneumonia, the compound must not only display microbiological activity but must also achieve sufficient antimicrobial exposures at the site of infection (27). Our study has shown the penetration of BPR into target tissues and its resultant efficacy against *S. aureus*. Moreover, this agent displayed consistent activity not only against MSSA isolates but also against MRSA isolates, including both HA-MRSA and CA-MRSA genotype isolates. BPR appears to have several important characteristics such as potent in vitro activity, low levels of protein binding, and good penetration into the lungs; thus, it should prove to be a valuable tool in the armamentarium for the management of bronchopulmonary infections due to *S. aureus* strains, including MRSA strains, possessing diverse phenotypic profiles.

**ACKNOWLEDGMENTS**

This study was funded by a grant from Johnson & Johnson Pharmaceutical Research & Development. We thank Darren Abbanat at Johnson & Johnson Pharmaceutical Research & Development for assistance with the determination of BPR concentrations in biological samples and providing protein binding data.

**REFERENCES**


**TABLE 3. $fT > MICs$ for corresponding EDs of BPR against all *S. aureus* isolates in an immunocompromised murine pneumonia model.**

<table>
<thead>
<tr>
<th><em>S. aureus</em> strain</th>
<th>% $fT &gt; MIC$ for:</th>
<th>Maximum log$_{10}$ CFU reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$</td>
<td>ED$_{50}$</td>
</tr>
<tr>
<td>MSSA 25923</td>
<td>10.2</td>
<td>8.1</td>
</tr>
<tr>
<td>MSSA 29213</td>
<td>19.5</td>
<td>14.8</td>
</tr>
<tr>
<td>HA-MRSA 56</td>
<td>15.5</td>
<td>13.7</td>
</tr>
<tr>
<td>HA-MRSA 149</td>
<td>13.8</td>
<td>8.6</td>
</tr>
<tr>
<td>HA-MRSA 152</td>
<td>15.9</td>
<td>14.0</td>
</tr>
<tr>
<td>CA-MRSA 144</td>
<td>14.3</td>
<td>13.8</td>
</tr>
<tr>
<td>CA-MRSA 146</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>CA-MRSA 147</td>
<td>21.6</td>
<td>17.1</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14.6 (4.9)</td>
<td>11.9 (4.0)</td>
</tr>
</tbody>
</table>
antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 7th ed. CLSI publication M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.