BMS-284756 in Experimental Cephalosporin-Resistant Pneumococcal Meningitis

VIOLETA RODRIGUEZ-CERRATO,* FARYAL GHAFFAR, JESUS SAAVEDRA, IAN C. MICHELOW, ROBERT D. HARDY, JANIE IGLEHART, KURT OLSEN, AND GEORGE H. MCCracken, JR.

Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, Texas

Received 6 February 2001/Accepted 30 July 2001

BMS-284756 is a novel des-fluoro(6) quinolone with a broad antimicrobial activity, including Streptococcus pneumoniae. The purpose of this study was to evaluate the pharmacodynamic profile and effectiveness of BMS-284756 for therapy of experimental meningitis caused by penicillin- and cephalosporin-resistant S. pneumoniae (CRSP). Meningitis was induced in rabbits by intracisternal inoculation of CRSP. BMS-284756 was given intravenously 16 h after intracisternal inoculation in single doses of 2.5 (n = 5 animals), 5 (n = 6), 10 (n = 6), 20 (n = 8), and 30 mg/kg (n = 6) in two doses of 10 mg/kg each separated by 5 h (n = 4), and as a 20-mg/kg dose followed 5 h later by 10 mg/kg (n = 5). The MICs and MBCs of BMS-284756, ceftriaxone, and vancomycin were 0.06 and 0.06, 4 and 4, and 0.25 and 0.25 μg/ml, respectively. After single doses of 10, 20, and 30 mg/kg, the maximum concentrations in cerebrospinal fluid (CSF) (mean ± standard deviation) were 0.32 ± 0.12, 0.81 ± 0.38, and 1.08 ± 0.43 μg/ml, respectively; the elimination half-life in CSF was 4.5 to 6.3 h. The CSF bacterial killing rates (BKR) at 5 h of the single-dose regimens of 10, 20 and 30 mg/kg were −0.84 ± 0.48, −1.09 ± 0.32, and −1.35 ± 0.05 Δlog10 CFU/ml/h. The BKRn50 of the divided regimens (10 mg/kg twice and 20 mg/kg followed by 10 mg/kg) was −0.82 ± 0.52 and −1.24 ± 0.34 Δlog10 CFU/ml/h, respectively. The BKRn50 of the combined therapy with vancomycin and ceftriaxone was −1.09 ± 0.39 Δlog10 CFU/ml/h. The penetration of BMS-284756 into purulent CSF relative to plasma was 14 to 25%. The bactericidal effect of BMS-284756 in CSF was concentration dependent. BMS-284756 at 30 mg/kg as a single or divided dose was as effective as standard therapy with vancomycin and ceftriaxone.

Pneumococcal meningitis is associated with a case fatality rate of 10% in children and up to 30% in adults. Among survivors the morbidity rate is approximately 30%, with neurolologic sequelae in 25% of patients and moderate to severe hearing loss in 32% (1, 4, 8, 16).

The emergence of multidrug-resistant strains of Streptococcus pneumoniae indicates the need to develop and evaluate new antineuropenic agents. BMS-284756 (formerly T-3811ME) is a novel quinolone agent that lacks the fluorine at the C-6 position typical of existing fluoroquinolones. This des-F(6) quinolone displays a broad antimicrobial spectrum and exhibits enhanced in vitro activity against S. pneumoniae indicates the need to develop and eval­

Materials and Methods

Bacterial strain. A highly penicillin- (MIC = 2 μg/ml) and cephalosporin-resistant (ceftriaxone MIC = 4 μg/ml) strain of S. pneumoniae type 6B was used for induction of meningitis in the rabbit model. This strain was originally isolated from an infant with meningitis (5). After intrathecal passage in rabbits, the strain was grown overnight on blood agar plates. The plates were washed with endotoxin-free phosphate-buffered saline (PBS), and aliquots of the resultant suspension were frozen at −70°C. For preparation of the inoculum, aliquots were thawed and diluted in endotoxin-free PBS to a concentration of approximately 1 × 106 to 5 × 106 CFU/ml. The inoculum size was confirmed by quantitative cultures in each experiment.

Susceptibility tests. The MICs and MBCs of antibiotics for this strain were determined using a microdilution method, as recommended by the National Committee for Clinical Laboratory Standards (11).

Meningitis model. The rabbit meningitis model, modified from the original description by Dacey and Sande, was used (3). Young male New Zealand White rabbits weighing 2 to 2.5 kg were anesthetized with intramuscular ketamine (50 mg/kg of body weight) and acepromazine (4 mg/kg) before every procedure and frequently while restrained in the stereotactic frames. Phenoxymethylpenicillin (1.1 mg/kg) was administered intramuscularly every 12 h for analgesia.

Meningitis was induced by intracisternal injection of 250 μl of a pneumococcal suspension containing 1 × 10⁷ to 5 × 10⁸ CFU/ml. Approximately 16 h later, anesthetized animals underwent intracisternal taps to quantify the baseline bacterial concentration in the cerebrospinal fluid (CSF), and then antibiotic therapy was administered via a marginal vein (0 h). Animals were immobilized in stereotactic frames, and a spinal needle remained in the cisterna magna for frequent CSF sampling during the first 3 h after receiving antibiotics. The rate of removal of CSF did not exceed the rate of CSF formation, which is approximately 0.4 ml/h (18). Blood samples were drawn from a central ear artery. The animals were sacrificed with pentobarbital (120 mg/kg) at the end of each experiment or earlier if they were severely lethargic or not recumbent.

Treatment. BMS-284756 (Bristol-Myers Squibb Co., Princeton, N.J.) was administered in single-dose regimens at 2.5 mg/kg (n = 5 animals), 5 mg/kg (n = 6), 10 mg/kg (n = 6), 20 mg/kg (n = 8), and 30 mg/kg (n = 6). Multiple-dose regimens were given as 10 mg/kg twice separated by 5 h (n = 4) and 20 mg/kg followed by 10 mg/kg 5 h later (n = 5). For comparison, four animals received the combination of vancomycin (20 mg/kg every 5 h, three doses) and ceftriaxone (125 mg/kg, single dose). As controls, five animals were inoculated with S. pneumoniae and did not receive antibiotic therapy.

Sample collection and processing. After a single dose of antibiotic was given, CSF (150 μl) and blood (700 μl) samples were obtained at 0.5, 1, 2, 3, 5, 10, and 24 h. In the case of multiple-dose regimens, CSF and blood were also collected at 6 h (1 h post-second dose). Blood and CSF samples were centrifuged at 5,000 × g for 10 and 5 min, respectively, and the supernatants were stored at

* Corresponding author. Mailing address: Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9063. Phone: (214) 648-3720. Fax: (214) 648-2961. E-mail: rodriguez_cerrato@yahoo.com.
−70°C for determination of antibiotic concentrations. An additional 100 to 150 μl of CSF was collected at 3, 5, 10, and 24 h after initiation of therapy for quantification of bacterial titers. Bacterial concentrations were determined by plating undiluted and serial dilutions of CSF (100 μl) on sheep blood agar and incubating in 5% CO$_2$ at 35°C for 24 h. The lowest bacterial concentration detectable by this method was 10 CFU/ml. For purposes of analysis, specimens with <10 CFU/ml were assigned a value of 1 (0 log$_{10}$) CFU/ml. Bacterial killing rates (BKR) were calculated as the difference between bacterial concentrations at the start of therapy and 3, 5, 10, and 24 h later divided by time.

**Antibiotic concentration determination.** BMS-284756 concentrations in CSF and plasma were measured by disk diffusion bioassay using *Bacillus subtilis* ATCC 6633 as described previously (17). BMS-284756 standards for CSF and plasma were measured by disk diffusion bioassay using the linear trapezoidal rule and the logarithmic trapezoidal rule, respectively. Values lower than the limit of detection were not used for the pharmacokinetic calculations. BMS-284756 standards for CSF and plasma were prepared with rabbit CSF and plasma, respectively. The intra- and interassay coefficients of variation were 2.3 and 7.4% for plasma and 2.7 and 6.8% for CSF, respectively.

**Pharmacokinetic and pharmacodynamic indices.** The maximum concentrations ($C_{max}$) of BMS-284756 in plasma and CSF were the highest measured values. Pharmacokinetic analyses were performed using the computer program TopFit V2 (Karl Thomae, Boehringer, Ingelheim, Germany). A noncompartmental model was used for calculations of CSF pharmacokinetic indices, and a two-compartment model was used for plasma pharmacokinetics. The areas under the concentration-time curves (AUC) for plasma and CSF were estimated from 0 h (initial antibiotic therapy) to the last quantifiable concentration (24 h) using the linear trapezoidal rule and the logarithmic trapezoidal rule, respectively.

**TABLE 1. Concentrations of BMS-284756 in serum and CSF after single-dose regimens during experimental meningitis caused by cephalosporin-resistant *S. pneumoniae***

<table>
<thead>
<tr>
<th>Dose (n)</th>
<th>Sample</th>
<th>Mean BMS-284756 concn ± SD (μg/ml) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 (5)</td>
<td>Serum</td>
<td>0.85 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>5 (6)</td>
<td>Serum</td>
<td>1.61 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>10 (6)</td>
<td>Serum</td>
<td>3.03 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>0.32 ± 0.12</td>
</tr>
<tr>
<td>20 (8)</td>
<td>Serum</td>
<td>5.27 ± 2.71</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>0.77 ± 0.41</td>
</tr>
<tr>
<td>30 (6)</td>
<td>Serum</td>
<td>7.66 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>1.05 ± 0.40</td>
</tr>
</tbody>
</table>

Mean BMS-284756 concentrations in CSF and plasma were calculated to be between 0.1 and 4.0 and between 0.2 and 10.0 μg/ml respectively. Some samples showed zones of inhibition that could be extrapolated to calculate concentrations lower than the limits of detection. The lowest extrapolated values for plasma and CSF were 0.04 and 0.02 μg/ml, respectively. Values lower than the limit of detection were not used for the pharmacokinetic calculations. BMS-284756 standards for CSF and plasma were prepared with rabbit CSF and plasma, respectively. The intra- and interassay coefficients of variation were 2.3 and 7.4% for plasma and 2.7 and 6.8% for CSF, respectively.

**FIG. 1. Concentration-time curves of BMS-284756 in plasma (left) and CSF (right) after single intravenous doses for experimental cephalosporin-resistant pneumococcal meningitis.** Mean concentrations (± SD) of BMS-284756 are illustrated at 5 mg/kg (●), 10 mg/kg (○), 20 mg/kg (▲), and 30 mg/kg (□).
The log-trapezoidal method was used for CSF values because it is believed to have less distortion of the AUC computation at both the ascending and descending parts of the concentration-time curve (7). The percentage of time above the MBC (T > MBC) was calculated as described by Turnbull (20). The ratio of CSF C\text{max} to MBC (C\text{max}/MBC) and the AUC/MBC ratio were calculated. The MBC value was used in this study because bacterial activity is critical for clearance of organisms from CSF (14). The penetration of BMS-284756 across the blood-brain barrier (expressed as a percentage) was calculated as the ratio of CSF to plasma AUC\text{CSF}/AUC\text{plasma}.

The relationship between pharmacodynamic indices (C\text{max}/MBC and AUC/MBC) and BKR was established according to a sigmoid E\text{max} model with the computer program WinNonlin version 1.5. (Scientific Consulting, Inc.). The log-trapezoidal method was used for CSF values because it is believed to have less distortion of the AUC computation at both the ascending and descending parts of the concentration-time curve (7).

In vitro susceptibility. The MICs and MBCs of the study antibiotics for this strain of S. pneumoniae were as follows: BMS-284756, 0.06 and 0.06 μg/ml, respectively; ceftriaxone, 4 and 4 μg/ml, respectively; and vancomycin, 0.25 and 0.25 μg/ml, respectively.

Single-dose pharmacokinetics. Mean serum and CSF concentrations of BMS-284756 after single-dose regimens are presented in Table 1. The concentration-time curves of BMS-284756 in plasma and CSF after single-dose regimens are shown in Fig. 1. Maximum CSF concentrations of BMS-284756 were reached between 30 min and 1 h after receiving therapy. The highest serum concentrations were measured at 30 min after drug administration. Initial mean bacterial concentrations were similar for all groups. Pharmacokinetic indices over a 24-h period after single-dose regimens of BMS-284756 are shown in Table 2. Among infected animals, the penetration rate of BMS-284756 into the CSF, expressed as AUC\text{CSF}/AUC\text{plasma}, was 14 to 25% with doses of 10 to 30 mg/kg. A good linear correlation was found between the total dose and CSF C\text{max} values (r = 0.84), AUC (r = 0.84), and T > MBC (r = 0.94; P < 0.001).

Results

In vitro susceptibility. The MICs and MBCs of the study antibiotics for this strain of S. pneumoniae were as follows: BMS-284756, 0.06 and 0.06 μg/ml, respectively; ceftriaxone, 4 and 4 μg/ml, respectively; and vancomycin, 0.25 and 0.25 μg/ml, respectively.

Single-dose pharmacokinetics. Mean serum and CSF concentrations of BMS-284756 after single-dose regimens are presented in Table 1. The concentration-time curves of BMS-284756 in plasma and CSF after single-dose regimens are shown in Fig. 1. Maximum CSF concentrations of BMS-284756 were reached between 30 min and 1 h after receiving therapy. The highest serum concentrations were measured at 30 min after drug administration. Initial mean bacterial concentrations were similar for all groups. Pharmacokinetic indices over a 24-h period after single-dose regimens of BMS-284756 are shown in Table 2. Among infected animals, the penetration rate of BMS-284756 into the CSF, expressed as AUC\text{CSF}/AUC\text{plasma}, was 14 to 25% with doses of 10 to 30 mg/kg. A good linear correlation was found between the total dose and CSF C\text{max} values (r = 0.84), AUC (r = 0.84), and T > MBC (r = 0.94; P < 0.001).
maximal BKR of −1.35 CFU/ml/h was observed with the 30-mg/kg dose, which produced an AUC/MBC of 128, \( C_{\text{max}}/\text{MBC} \) of 18, and \( T > \text{MBC} \) of 87.7%. For 5 h after administration, the concentrations of BMS-284756 in CSF after the 20-mg/kg and 30-mg/kg doses were 6.2- to 8.2-fold higher than the MBC. The concentrations of BMS-284756 in CSF after the 20-mg/kg and 30-mg/kg dose, which produced an AUC/MBC of 128, \( C_{\text{max}}/\text{MBC} \) of 18, and \( T > \text{MBC} \) of 87.7%. For 5 h after administration, the concentrations of BMS-284756 in CSF after the 20-mg/kg and 30-mg/kg doses were 6.2- to 8.2-fold higher than the MBC.

### Table 4. Bacterial killing in CSF over 24 h with different dosing regimens of BMS-284756 therapy for experimental \( S. \text{pneumoniae} \) meningitis and comparison with standard therapy

<table>
<thead>
<tr>
<th>Regimen and dose (mg/kg)</th>
<th>CSF indices (mean ± SD)</th>
<th>( \Delta \log_{10} \text{CFU/ml} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3 h</td>
<td>0-5 h</td>
</tr>
<tr>
<td>Single dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>−0.3 ± 0.1</td>
<td>−0.4 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>−0.4 ± 0.2</td>
<td>−0.4 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>−0.8 ± 0.3</td>
<td>−0.8 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>−1.3 ± 0.7</td>
<td>−1.1 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>−0.8 ± 0.1</td>
<td>−1.3 ± 0.05</td>
</tr>
<tr>
<td>Two doses(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>−0.6 ± 0.3</td>
<td>−0.8 ± 0.5</td>
</tr>
<tr>
<td>20→10</td>
<td>−0.9 ± 0.2</td>
<td>−1.2 ± 0.3</td>
</tr>
<tr>
<td>VAN/CRO(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/125</td>
<td>−0.8 ± 0.3</td>
<td>−1.1 ± 0.4</td>
</tr>
</tbody>
</table>

\(^a\) Two 10-mg/kg doses given every 5 h, or a first dose of 20 mg/kg followed by a 10-mg/kg dose after 5 h.
\(^b\) Vancomycin (VAN) at 20 mg/kg every 5 h for three doses plus ceftriaxone (CRO) at 125 mg/kg as a single dose.

The pharmacodynamic indices in CSF and the bacteriologic effectiveness are summarized in Tables 3 and 4. The CSF concentrations of BMS-284756 were below the MBC at 24 h for all animals treated with 10 mg/kg given twice and for three of five animals treated with 20 mg/kg followed by 10 mg/kg. However, bacterial clearance (<10 CFU/ml) in the CSF was obtained at 24 h for all animals treated with divided-dose regimens. At 10 h, three of four animals treated with 10-mg/kg × 2 therapy and all animals treated with the 20-mg/kg followed by 10-mg/kg regimen achieved CSF sterilization. The bacterial killing rate of BMS-284756 with divided-dose regimens was concentration dependent at all time points.

**Comparison of BMS-284756 with standard therapy.** The bacterial killing of the 20-mg/kg plus 10-mg/kg regimen was slightly higher than that of vancomycin and ceftriaxone, but the difference was not statistically significant (Table 4). The CSF concentrations of BMS-284756 were below the MBC at 24 h for all animals treated with 10 mg/kg given twice and for three of five animals treated with 20 mg/kg followed by 10 mg/kg.
bacterial concentrations after the 30-mg/kg single dose and 20-mg/kg plus 10-mg/kg regimen of BMS-284756 are displayed and compared to that of vancomycin and ceftriaxone in control animals (M) compared to all antibiotic groups was significant (P < 0.05).

DISCUSSION

Because of their excellent antibacterial activity and favorable CSF penetration, the new fluoroquinolones (e.g., trovafloxacin, gatifloxacin, and moxifloxacin) have been shown to be effective for treatment of resistant pneumococcal meningitis in rabbits (9, 12, 15). We demonstrated that BMS-284756 is highly active against cephalosporin-resistant \textit{S. pneumoniae} in this meningitis model. BMS-284756 displayed good penetration into inflamed meninges. Protein binding of BMS-284756 has been described to be as high as 80% (D. P. Nicolau, H. M. Mattoes, M. A. Banevicius, D. Xuan, and C. H. Nightingale, Program Abstr. 40th ICAAC, 2000, abstr. 290). As with other fluoroquinolones, the bacterial killing of BMS-284756 was concentration dependent (2). The effectiveness of BMS-284756 in CSF was correlated with the three pharmacodynamic indices, AUC/MBC, \( C_{\text{max}}/\text{MBC} \), and \( T > \text{MBC} \), but the AUC/MBC ratio correlated best with bacterial killing in CSF.

In the neutropenic thigh infection model, maximal bactericidal activity of BMS-284756 was achieved when serum AUC/MIC ratios were 150 to 200 and therefore the free drug AUC/MIC was 30 to 40 (Nicolau et al., abstr.). In our study, total drug AUC/MBC ratios of 114 to 128 (free AUC/MBC of 23 to 26) produced maximal bactericidal activity in CSF.

In this study, bacterial clearance at 24 h was observed despite the fact that CSF concentrations of BMS-284756 were below the MBC for this strain at 24 h in animals treated with the divided-dose regimens. This suggests a potential sub-MBC effect in CSF, contrary to previous observations for other fluoroquinolones (9, 10, 13).

Recently, data have been presented on safety, tolerability, and pharmacokinetics in humans after prolonged oral administration (D. Grasela, D. Gajjar, A. Bello, Z. Ge, and L. Christopher, Program Abstr. 40th ICAAC, 2000, abstr. 2260). Mean plasma concentrations ranged from 1.7 to 25 \( \mu \text{g/ml} \) after oral doses of 100 to 1,200 mg. Based on the long elimination \( t_{1/2} \) in plasma (13 to 17 h), once-daily dosage of BMS-284756 may be suitable in humans. However, the CSF pharmacokinetics of BMS-284756 has not been studied in humans. Based on our findings of similar \( t_{1/2} \) values in serum and CSF of rabbits, a single daily dose equivalent to our 30 mg/kg or a regimen equivalent to 20 mg/kg followed by 10 mg/kg dose in rabbits given every 12 h could be effective for therapy of pneumococcal meningitis. Clinical trials are necessary to confirm this hypothesis.

In conclusion, we demonstrated that BMS-284756 as a single agent was as effective as the standard regimen with vancomycin and ceftriaxone for therapy of experimental highly cephalosporin-resistant pneumococcal meningitis.

ACKNOWLEDGMENTS

Violeta Rodriguez-Cerrato is the recipient of a Fellowship Grant from the European Society of Paediatric Infectious Diseases (ESPID) sponsored by Wyeth-Lederle Vaccines and Pediatrics. This work was supported in part by a grant from Bristol-Myers Squibb Co., Princeton, N.J.

REFERENCES


