Evaluation of T-3811ME (BMS-284756), a New Des-F(6)-Quinolone, for Treatment of Meningitis Caused by Penicillin-Resistant Streptococcus pneumoniae in Rabbits

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T-3811ME (BMS-284756) is a new des-F(6)-quinolone with high levels of activity against gram-positive bacteria, including penicillin-resistant Streptococcus pneumoniae (PRSP) strains. T-3811, the free base of T-3811ME, exhibited potent activity against 28 clinical strains of PRSP isolated clinically (MIC at which 90% of the isolates tested are inhibited, 0.0625 μg/ml). After the intravenous dosing of T-3811ME (20 mg/kg of body weight as T-3811) in rabbits with meningitis caused by PRSP, the area under the concentration-time curve (AUC) of T-3811 in cerebrospinal fluid (CSF) was 5.79 μg · h/ml and was 4.5-fold higher than that of T-3811 in the CSF of rabbits without meningitis. In addition, the AUC/MIC for T-3811ME (20 mg/kg as T-3811) in CSF was 185, which was 4.3-fold higher than that for ceftriaxone (administered intravenously at 100 mg/kg). After the administration of any dose of T-3811ME (5, 10, and 20 mg/kg as T-3811), the viable cell counts in CSF decreased in a dose-dependent manner. In particular, after dosing of 20 mg/kg (as T-3811), the viable cell counts in CSF were significantly less than those in the nontreated group (P < 0.01). By histopathological evaluation, 6 h after the administration of T-3811ME (20 mg/kg as T-3811), the thickening of the cerebral meninx and the infiltration of neutrophils into the cerebral meninx were less severe in the treated group than in the nontreated group. T-3811ME (BMS-284756) may be expected to be evaluated for the management of meningitis caused by highly penicillin-resistant pneumococci.

Streptococcus pneumoniae is a major causative organism of bacterial meningitis and accounts for about 40% of cases of community-acquired meningitis (4). The therapeutic agents currently recommended for the treatment of meningitis caused by S. pneumoniae are mainly β-lactams such as penicillin G, cefotaxime, and ceftriaxone (2, 17). However, penicillin-resistant S. pneumoniae (PRSP) has become an increasing problem worldwide (1), and so new chemotherapeutic agents have been sought for the treatment of pneumococcal meningitis.

Quinolones also show rapid bactericidal activities in vitro and in vivo against susceptible organisms in a highly concentration-dependent manner (5). Because of their lipophilicity, quinolones, as a class, penetrate the cerebrospinal fluid (CSF) better than β-lactam agents (11, 12, 15). New quinolones (e.g., gatifloxacin and moxifloxacin) have increased levels of activity against gram-positive bacteria, including PRSP (6, 9, 14, 20). However, nowadays quinolones are infrequently used for the treatment of meningitis. In addition, quinolones are relatively contraindicated in children, the age group most susceptible to the development of meningitis.

T-3811ME, 1-cyclopropyl-8-(difluoromethoxy)-7-[(1R)-(1-methyl-2,3-dihydro-1H-5-isoxindolyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid methanesulfonate monohydrate, is a new quinolone with high levels of activity against gram-positive bacteria including PRSP (21). In the present study, we evaluated the pharmacokinetics of T-3811ME, including the level of penetration into CSF, in rabbits with meningitis caused by PRSP. We also studied the efficacies of different doses of T-3811ME in the treatment of experimental meningitis and compared the efficacy of T-3811ME to that of ceftriaxone.

MATERIALS AND METHODS

Antibacterial agents. The following agents were used in the study: T-3811ME (BMS-284756), T-3811 (the free base of T-3811ME), trovafloxacin, moxifloxacin, levofloxacin, ciprofloxacin, vancomycin, cefotaxime, ceftriaxone, imipenem, and penicillin G. T-3811, T-3811ME, trovafloxacin, and moxifloxacin were synthesized at the Research Laboratories, Toyama Chemical Co., Ltd. (Tokyo, Japan). Levofloxacin and ciprofloxacin were extracted from commercially available tablelets. The purity of each of these two agents was above 99.8%, as measured by high-performance liquid chromatography. Vancomycin, cefotaxime, ceftriaxone, imipenem, and penicillin G were purchased from Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan); Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan); Nippon Roche Co., Ltd. (Tokyo, Japan); and Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan) (both imipenem and penicillin G, respectively).

Organism. For susceptibility testing with the various antibacterial agents, 28 clinical isolates of PRSP isolated from Japan and Korea were used. PRSP D-979, T-3811, T-3811ME, trovafloxacin, and moxifloxacin were synthesized at the Research Laboratories, Toyama Chemical Co., Ltd. (Tokyo, Japan). Levofloxacin and ciprofloxacin were extracted from commercially available tablelets. The purity of each of these two agents was above 99.8%, as measured by high-performance liquid chromatography. Vancomycin, cefotaxime, ceftriaxone, imipenem, and penicillin G were purchased from Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan); Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan); Nippon Roche Co., Ltd. (Tokyo, Japan); and Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan) (both imipenem and penicillin G, respectively).

Animals. Male Japanese White rabbits (weight, approximately 2.5 kg) were purchased from Chubu-Kagaku Sizai Inc. (Nagoya, Japan), and the rabbits were assigned to the study after an acclimation period of 1 week.

Activities against clinical isolates. T-3811 and the various other antibacterial agents were tested for their in vitro activities against clinical isolates. MICs were determined by the standard agar dilution method recommended by the Japan Society of Chemotherapy (7, 8); Mueller-Hinton medium (Difco, Detroit, Mich.) supplemented with 5% defibrinated sheep blood (Nippon Bio-Test Laboratories, Tokyo, Japan) was used for MIC determinations. Organisms were tested at a final inoculum size of 10^6 CFU/spot by use of a multipoint inoculator (Sakuma Seisakusho, Tokyo, Japan) and were incubated at 37°C for 18 h in air. The MIC was defined as the lowest antibiotic concentration which prevented the visible growth of bacteria.

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were under anesthesia induced with ketamine hydrochloride, a winged catheter T-3811) and ceftriaxone (100 mg/kg) (VOL. 46, 2002 EFFICACY OF T-3811ME IN EXPERIMENTAL MENINGITIS 1761

In vivo therapeutic efficacy against experimental meningitis in rabbits. The therapeutic efficacies of T-3811ME and ceftriaxone were studied.

(i) Experimental meningitis model in rabbits. On the day of infection, rabbits were randomly allocated to groups (n = 5). PRSP D-979 cells, which were prepared from overnight cultures on a heart infusion agar (HIA; Eiken Kagaku Co., Ltd., Tokyo, Japan) plate containing 5% sheep blood, were suspended in brain heart infusion broth (BHIB; Eiken Kagaku Co., Ltd., Tokyo, Japan) to give a final concentration of 10^5 CFU/ml. After 10-fold dilution in BHIB, this bacterial suspension was cultured at 37°C for 4 h with shaking. The inocula were obtained by 500-fold dilution of this culture into BHIB. Experimental meningitis was induced by intracisternal injection of 10^5 CFU of bacteria.

(ii) Administration of antibacterial agents. For evaluation of the antibacterial agents, T-3811ME was dissolved and diluted in 5% mannitol aqua and ceftriaxone was diluted in saline. Antibiotic therapy was started 18 h after infection. T-3811ME (5, 10, and 20 mg/kg of body weight administered as T-3811) and ceftriaxone (100 mg/kg) were administered intravenously via an ear vein.

(iii) Evaluation of therapeutic effects of antibacterial agents. At 0, 2, 4, and 6 h after intravenous administration of the drugs at an injection rate of 10 ml/min, blood was collected from an ear vein contralateral to the ear receiving the antibiotic infusion and CSF was obtained intracisternally. Bacterial titers in CSF were determined by quantitative culture of samples on HIA plates supplemented with 5% defibrinated sheep blood. All results are expressed as means and standard deviations. Comparisons between groups were performed by one-way analysis of variance; in the case of significance, this was followed by Tukey’s test.

Measurement of CSF pressure. CSF pressures were measured 18 h after infection and more than 6 h after administration of T-3811ME (20 mg/kg as T-3811) and ceftriaxone (100 mg/kg) (n = 3 for each group). While the rabbits were under anesthesia induced with ketamine hydrochloride, a winged catheter was injected intracisternally into the occipital region (occipital triangle) of the rabbits. The CSF pressure obtained with the catheter was converted to an electric signal. This was then input into a blood pressure transducer (Nihon-Koden Co., Ltd., Tokyo, Japan) and an amplifier (AG-601G; Nihon-Koden Co., Ltd.). The CSF pressure was then recorded with a pen oscillograph (RECTB-HORIZ-8K; Nihon-Denki-Sanei Co., Ltd., Tokyo, Japan). The CSF pressure was then calculated from the standard pressure for 20 mm Hg obtained with an electric pphysiomonometer (MP-25S; Nihon-Koden Co., Ltd.). The ratio of the CSF pressure change (mm Hg after therapy/mm Hg before therapy) was then calculated.

Determination of drug concentrations in serum and CSF. The concentrations of the study drugs in serum and CSF samples from rabbits with or without meningitis were determined. The following doses were administered: for rabbits with meningitis, 5, 10, and 20 mg/kg as T-3811 for T-3811ME and 100 mg/kg for ceftriaxone; for rabbits without meningitis, 20 mg/kg as T-3811 for T-3811ME and 100 mg/kg for ceftriaxone (n = 4 for each group). An octodecyl silane column was used to measure the concentrations of T-3811ME; and a disk diffusion bioassay, with Klebsiella pneumoniae ATCC 10031 as the test strain and HIA as the medium, was used to measure the concentrations of ceftriaxone. The limits of quantitation in serum and CSF were 0.125 µg/ml for both T-3811ME and ceftriaxone. The area under the concentration-time curve from time zero to infinity (AUCt) and the terminal half-life (t1/2) were calculated from the mean concentrations in serum by noncompartmental analysis with WinNonlin software (Pharsight Corporation, Mountain View, Calif.). The maximum concentration (Cmax), the time to reach Cmax (Tmax), and the time above the MIC (T/MIC) were obtained directly from the actual mean concentrations.

Histopathological examination of central nervous system. At 6 h after the intravenous administration of T-3811ME (20 mg/kg as T-3811) (24 h after infection), the rabbits were anesthetized and euthanized with ketamine hydrochloride. The brain, vertebrae cervicales, and vertebrae thoracicae were removed from the rabbits and fixed in 10% buffered formalin. The vertebrae cervicales and vertebrae thoracicae were then decalcified in Plank-Rychlo solution. These samples were embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined by microscopy.

RESULTS

Antibacterial activities. The MICs at which 50% of the isolates tested are inhibited (MIC90), the MIC90s, and the range of MICs of T-3811 and the reference antibacterial agents for clinical isolates of PRSP are shown in Table 1. T-3811 was the most active against PRSP, with an MIC90 of 0.0025 µg/ml. The activity of T-3811 was 16-fold greater than those of both levofloxacin and ceftriaxone. The MICs of T-3811 and ceftriaxone for PRSP D-979, a strain used to induce experimental meningitis, were 0.031 and 1 µg/ml, respectively.

Efficacy of T-3811ME against meningitis in rabbits caused by PRSP D-979. Table 2 shows the viable cell counts in the CSF

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>MIC/MBC (µg/ml)</th>
<th>Dose (mg/kg)</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>CSF pressure (mm Hg) changea (after therapy/ before therapy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-3811ME 0.031/0.031</td>
<td>5d</td>
<td>10d, 20d</td>
<td>−0.08 ± 0.94</td>
<td>−0.87 ± 0.71</td>
<td>−1.86 ± 0.82</td>
<td>NTb</td>
</tr>
<tr>
<td>Ceftriaxone 1/1</td>
<td>100</td>
<td>2.33 ± 0.72k</td>
<td>−3.60 ± 0.72k</td>
<td>−3.87 ± 1.92</td>
<td>−4.35 ± 1.0f</td>
<td>0.67 ± 0.02b</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>0.56 ± 1.00</td>
<td>−1.73 ± 1.14</td>
<td>−2.82 ± 1.13b</td>
<td>1.59 ± 0.74</td>
<td>1.15 ± 0.27</td>
</tr>
</tbody>
</table>

a n = 5. 
b n = 3. At 6 h after intravenous drug administration. 
c Time after drug administration. 
d Equivalent to T-3811. 
e NT, not tested. 
f P < 0.01 versus the control. 
g P < 0.05 versus ceftriaxone. 
h P < 0.05 versus the control.
of rabbits with experimental meningitis caused by PRSP D-979. The viable cell counts of PRSP D-979 in the nontreated group were about 10^6 CFU/ml from 18 h (the start of drug administration) through 24 h (6 h after drug administration). In the case of ceftriaxone (100 mg/kg), the viable cell counts of PRSP D-979 in CSF decreased by 10^3 to 10^4 CFU/ml at 6 h after administration, becoming significantly less than those of the rabbits in the nontreated groups (P < 0.05). After the administration of T-3811ME at any of the doses (5, 10, and 20 mg/kg as T-3811), the viable cell counts in CSF were decreased in a dose-dependent manner. In particular, after the dosing of T-3811ME (20 mg/kg as T-3811), the viable cell counts in CSF was significantly less than those in the nontreated group (P < 0.01 for 2, 4, and 6 h) and in the ceftriaxone-treated group (P < 0.05 for 2 h).

**CSF pressure.** As shown in Table 2, at 6 h after intravenous administration of T-3811ME (20 mg/kg as T-3811), the CSF pressures of all rabbits were decreased, becoming significantly less than those of the rabbits in the nontreated groups (P < 0.05). However, a decrease in the CSF pressure was not observed after the administration of ceftriaxone (100 mg/kg).

**Drug concentrations in serum and CSF.** Table 3 shows the pharmacokinetic parameters for T-3811ME and ceftriaxone after dosing of rabbits with meningitis with 5, 10, and 20 mg/kg (as T-3811) and 100 mg/kg, respectively. Also shown are the pharmacokinetic parameters for T-3811ME after dosing of rabbits without meningitis with 20 mg/kg as T-3811 and those for ceftriaxone after dosing of rabbits without meningitis with 100 mg/kg. The C_{max} and AUC of T-3811ME (20 mg/kg as T-3811) in the CSF of rabbits with meningitis were 1.48 μg/ml and 5.79 μg · h/ml, respectively, and were 2.2- and 4.5-fold higher than those in the CSF of rabbits without meningitis. The C_{max} of T-3811ME, administered at a dose of 20 mg/kg (as T-3811), in the serum of rabbits with meningitis was 13.3 μg/ml and was lower than that ofceftriaxone (100 mg/kg). The C_{max} of T-3811 in CSF was also lower than that of ceftriaxone. However, the C_{max}/MIC for T-3811 in CSF was 47 and was 8-fold higher than that for ceftriaxone. The AUC of T-3811 in CSF was 5.79 μg · h/ml and was less than that of ceftriaxone (43.0 μg · h/ml). However, the AUC/MIC for T-3811 in CSF was 185 and was 4.3-fold higher than that for ceftriaxone. The T > MIC of T-3811ME (as T-3811), administered at a dose of 20 mg/kg, in CSF was 14.8 h and was comparable to that of ceftriaxone (12.2 h after administration of a dose of 100 mg/kg).

**Histopathological changes in brain.** Figure 1 shows the histopathological findings for the central nervous systems, especially the cerebra, of healthy, nontreated, and T-3811ME-treated rabbits. In the nontreated rabbits, severe thickening of the cerebral and cerebellar meninges (data not shown) was observed (Fig. 1B). Cellular infiltration of neutrophils into the cerebral meninx and dilatation of the meningeal vein were observed. In contrast, at 6 h after the injection of T-3811ME, the thickening of the cerebral meninx and the infiltration of neutrophils into the cerebral meninx were less severe in treated rabbits than in the nontreated group, as was the degree of dilatation of blood vessels (Fig. 1C).

**DISCUSSION**

The pathogens that most commonly cause community-acquired meningitis are *S. pneumoniae*, *Neisseria meningitidis*, and *Listeria monocytogenes*. In particular, bacterial meningitis caused by *S. pneumoniae* is an important cause of neurological morbidity and mortality in both children and adults (4). Broad-spectrum cephalosporins (e.g., ceftriaxone and cefotaxime) are considered the agents of choice for the empirical treatment of bacterial meningitis (2). However, it is reported that the prev-
FIG. 1. Histopathological changes in rabbits with meningitis. (A) Normal cerebral tissue; (B) 18 h after infection (nontreated group); (C) T-3811ME-treated group 6 h after intravenous dosing with 20 mg/kg. cc, cerebral cortex; cm, cerebral meninx.
Alence of penicillin-resistant pneumococci is increasing worldwide (1). The incidence of penicillin resistance in strains of *S. pneumoniae* approaches 40% in some areas of the United States, and the incidence of high-level resistance has increased 60-fold during the past 10 years (19). Also, the emergence of *S. pneumoniae* strains resistant to penicillin and macrolides has significantly complicated the initial management of patients with suspected bacterial meningitis (19). The search for new antibacterial agents in the treatment of bacterial meningitis is justified by a rate of mortality that remains unacceptably high and by the emergence of bacterial resistance. The newer fluoroquinolones may have a potential role in the treatment of central nervous system infections because of their excellent in vitro activities against gram-positive organisms and good penetration into CSF (11).

T-3811, the free base of T-3811ME (2.8-mg/kg) also showed excellent in vitro activity against *S. pneumoniae* including PRSP (21). In the present study, T-3811 had the highest level of in vitro activity against PRSP among the antibacterial agents tested and had activity 16-fold greater than those of ceftriaxone and levofloxacin.

New quinolones with improved antimicrococcal activities have been described in many papers as having excellent activities against experimental pneumococcal meningitis (11, 13, 16, 18). It has been reported that bacterial meningitis increases the permeability of the blood-brain barrier, that all quinolones penetrate the inflamed CSF better than β-lactams, and that the speed of entry into CSF is closely related to their degrees of lipophilicity (10, 12, 15). Quinolones diffuse across transcellular pathways; C_{max} in CSF occur relatively rapidly. T-3811ME also entered CSF readily, with peak values within 15 to 30 min after intravenous infusion. The C_{max} of T-3811ME (20 mg/kg as T-3811) in CSF was 47-fold higher than the MIC for the PRSP strain used. The penetration of T-3811ME into CSF in rabbits with meningitis, calculated as the AUC in CSF versus the AUC in serum, was 13 to 16%. The bacterial counts in the CSF of the group treated with T-3811ME (20 mg/kg as T-3811) were significantly less than those in the CSF of the nontreated group (P < 0.01). By histopathological examination of the brains of T-3811ME-treated rabbits, it was found that the thickening of the cerebral meninx and the infiltration of neutrophils into the cerebral meninx were less severe in the treated group than that in the nontreated group.

The T>MIC of T-3811ME (20 mg/kg as T-3811) in CSF was comparable to that of ceftriaxone (100 mg/kg). The C_{max} and AUC of T-3811ME (20 mg/kg as T-3811) in CSF after intravenous injection were lower than those of ceftriaxone (100 mg/kg). Although it is difficult to compare T-3811ME with ceftriaxone, a β-lactam antibiotic, the C_{max}/MIC and AUC/MIC ratios for T-3811ME were greater than those for ceftriaxone. Experimental studies of pneumonia, peritonitis, and sepsis and clinical trials evaluating fluoroquinolone therapy have shown that AUC/MIC ratios of 100 and C_{max}/MIC ratios of 8 to 10 are almost always associated with satisfactory outcomes (3). The AUC/MIC and C_{max}/MIC ratios for T-3811ME (20 mg/kg as T-3811) in CSF were 185 and 47, respectively. T-3811ME is considered to have a high degree of efficacy as a result of its distribution into CSF and its potent bactericidal activity.


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REFERENCES


