

## In Vitro and In Vivo Efficacies of T-3811ME (BMS-284756) against *Mycoplasma pneumoniae*

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**T-3811, the free base of T-3811ME (BMS-284756), a new des-F(6)-quinolone, showed a potent in vitro activity (MIC at which 90% of the isolates tested are inhibited [MIC<sub>90</sub>], 0.0313 µg/ml) against *Mycoplasma pneumoniae*. The MIC<sub>90</sub> of T-3811 was 4-fold higher than that of clarithromycin but was 4- to 8-fold lower than those of trovafloxacin, gatifloxacin, gemifloxacin, and moxifloxacin and was 16- to 32-fold lower than those of levofloxacin, ciprofloxacin, and minocycline. In an experimental *M. pneumoniae* pneumonia model in hamsters, after the administration of T-3811ME (20 mg/kg of body weight as T-3811, once daily, orally) for 5 days, the reduction of viable cells of *M. pneumoniae* in bronchoalveolar lavage fluid was greater than those of trovafloxacin, levofloxacin, and clarithromycin (20 and 40 mg/kg, orally) ( $P < 0.05$ ).**

*Mycoplasma pneumoniae* is a major causative organism of pneumonia and accounts for as many as 20 to 30% of pneumonia cases (20). For the clinical treatment of *M. pneumoniae* infections, macrolides and tetracyclines are commonly used (9, 18, 25). However, mutants resistant to macrolides have been reported over the last 10 years (17, 21, 23). Thus, a viable alternative to macrolides in the treatment of pneumonia caused by *M. pneumoniae* is needed. New quinolones developed recently, such as moxifloxacin and gatifloxacin (5, 10), possess a wide antimicrobial spectrum and potent activity against various pathogens, including *M. pneumoniae*, and are expected to be useful in the treatment of this organism (4, 5, 6). T-3811ME (BMS-284756), a new des-F(6)-quinolone, which exhibits excellent activity against respiratory pathogens such as *Streptococcus pneumoniae*, *Chlamydia pneumoniae*, and *M. pneumoniae*, is under development for both oral and parenteral administration (24). In the present study, we studied in vitro and in vivo antimycoplasma activity of T-3811ME. The in vivo efficacy of T-3811ME was evaluated in hamsters with experimental pneumonia caused by *M. pneumoniae* and compared with those of trovafloxacin, levofloxacin, and clarithromycin.

**Determination of MIC.** The following agents were employed: T-3811ME (T-3811 methanesulfonate monohydrate, also known as BMS-284756), T-3811 (the free base of T-3811ME), ciprofloxacin, levofloxacin, trovafloxacin, gatifloxacin, gemifloxacin, moxifloxacin, clarithromycin, and minocycline; T-3811, T-3811ME, trovafloxacin, gatifloxacin, gemifloxacin, and moxifloxacin were synthesized at Research Laboratories, Toyama Chemical Co., Ltd., Tokyo, Japan. Ciprofloxacin, levofloxacin, and clarithromycin were extracted from commercially available tablets. The purity of each of these three agents was above 99.8% as measured by high-performance liquid chromatography (HPLC). Minocycline was

purchased from Lederle-Japan Ltd., Tokyo, Japan. T-3811, the free base of T-3811ME, was used for all in vitro studies.

*M. pneumoniae* FH, a standard strain, was used for experimental pneumonia studies with hamsters. Fifty clinical isolates of *M. pneumoniae* were used for MIC determination of nine antimicrobial agents. MICs were determined using a microdilution method (phenol red method) in 96-well microplates (12). A broth microdilution procedure was used according to the method of Whithear et al. (26). The medium used for MIC determination and dilution of agents was PPLO (Difco Inc., Detroit, Mich.) broth supplemented with 30% Mycoplasma Supplement S (Difco), 0.5% glucose (Wako Pure Chemicals Industries, Ltd., Osaka, Japan), and 0.002% phenol red (Tokyo Kasei Co., Ltd., Tokyo, Japan). Preculture was carried out with the same medium for 6 to 9 days of incubation at 37°C. Then, 10 µl of suspension (10<sup>6</sup> CFU/ml) was inoculated into 96-well microplates containing 90 µl of the antibiotic solution. The MICs were determined after 5 days of incubation at 37°C by color change from red to yellow. Phenol red indicated a yellow color (acidification) after cell growth resulting in consumption of glucose.

**Therapeutic efficacies of T-3811ME, trovafloxacin, levofloxacin, and clarithromycin.** (i) **Experimental pneumonia model in hamsters.** For the experimental pneumonia model, hamsters were used as in previous reports (2, 3, 11). Four-week-old male Syrian hamsters were purchased from Japan SLC Inc., Shizuoka, Japan, and were assigned to the study after an acclimation period of 3 days. On the day of infection, the hamsters were randomly allocated to each group (control,  $n = 11$ ; treated groups,  $n = 5$  to 6). *M. pneumoniae* FH was cultured in PPLO broth at 37°C for 7 days and stored at -40°C (approximately 10<sup>8</sup> CFU/ml). The frozen cells were thawed at 37°C before use. Pulmonary infections were induced in hamsters anesthetized with sodium pentobarbital (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) by intratracheally injecting 0.1 ml of the suspension of *M. pneumoniae*.

(ii) **Administration of antimicrobial agents.** T-3811ME, levofloxacin, trovafloxacin, and clarithromycin were suspended in 0.5% methylcellulose SM-400 (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan). Oral administration of antimicrobial

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TABLE 1. Activities of antibacterial agents against 50 clinical isolates of *M. pneumoniae* and *M. pneumoniae* FH

| Antibacterial agent | MIC ( $\mu\text{g/ml}$ ) for <i>M. pneumoniae</i> <sup>a</sup> |        |        | MIC ( $\mu\text{g/ml}$ ) for <i>M. pneumoniae</i> FH <sup>b</sup> |
|---------------------|--|--------|--------|---|
|                     | Range  | 50%    | 90%    |   |
| T-3811              | 0.0156–0.0625  | 0.0313 | 0.0313 | 0.0313  |
| Ciprofloxacin       | 0.5–2  | 1      | 1      | 1   |
| Levofloxacin        | 0.25–1   | 0.5    | 0.5    | 0.5   |
| Trovafloxacin       | 0.0625–0.25  | 0.125  | 0.25   | 0.125   |
| Gatifloxacin        | 0.0625–0.25  | 0.125  | 0.125  | 0.125   |
| Gemifloxacin        | 0.0625–0.25  | 0.25   | 0.25   | 0.125   |
| Moxifloxacin        | 0.0625–0.125   | 0.125  | 0.125  | 0.0625  |
| Clarithromycin      | 0.002–0.0156   | 0.0078 | 0.0078 | 0.002   |
| Minocycline         | 0.0625–1   | 0.5    | 1      | 0.0625  |

<sup>a</sup> 50%, MIC<sub>50</sub>; 90%, MIC<sub>90</sub>.

<sup>b</sup> The strain used for experimental pneumonia.

agents at 20 mg/kg of body weight (T-3811ME-treated groups) and 20 and 40 mg/kg (all other groups) was initiated on the 7th day after infection by *M. pneumoniae* and continued once daily for 2 and 5 days.

**(iii) Evaluation of therapeutic effects of antimicrobial agents.**

Animals were sacrificed at 24 h after final administration following anesthetization with ether. Bronchoalveolar lavage fluid (BALF) was obtained by injection into and subsequent suction

of 2 ml of Hanks solution (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) from the trachea. This BALF and its 10-fold serial dilutions were used for viable cell count. The viable cell counts were measured on the plate of PPLO agar containing supplements. The limit of assay of CFUs in BALF by this method was 2.30 log CFU/ml.

**(iv) Statistical analysis.** The significance of each group in terms of the viable cell count in BALF was analyzed by Tukey's

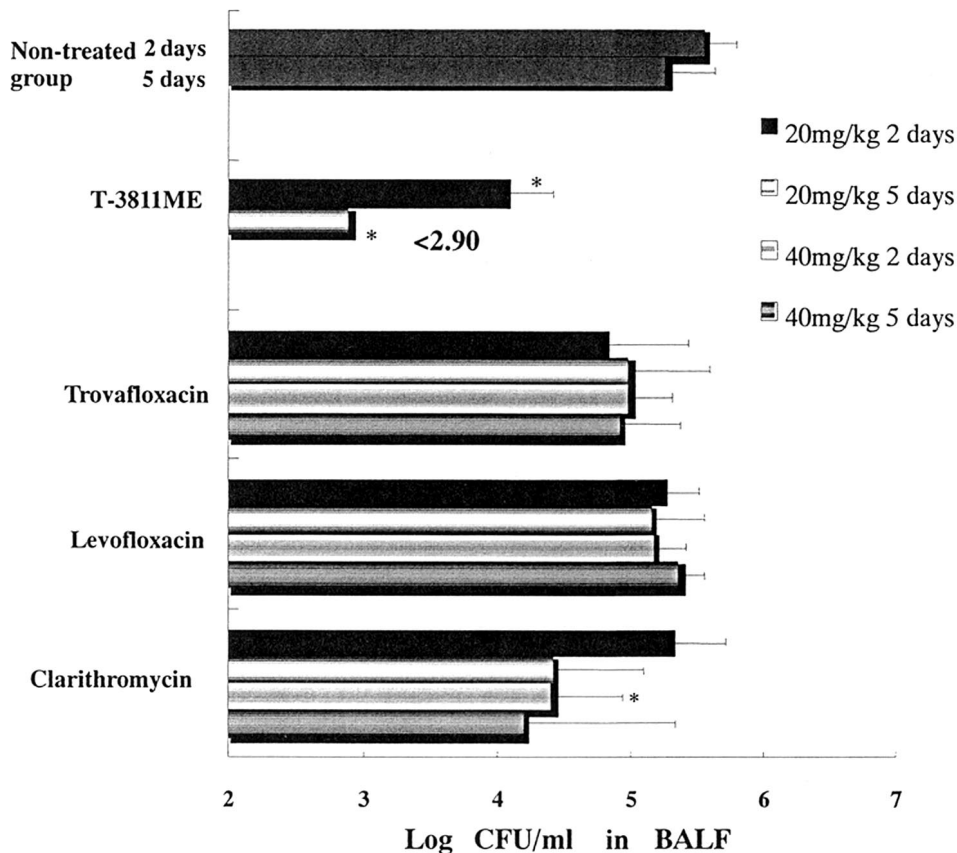


FIG. 1. In vivo efficacy of T-3811ME and other antibacterial agents against experimental pneumonia caused by *M. pneumoniae* FH in hamsters. For results at 2 days an asterisk indicates a *P* of <0.05 for T-3811ME versus control, trovafloxacin, levofloxacin, and clarithromycin (except clarithromycin at 40 mg/kg), or for clarithromycin (40 mg/kg) versus control. For results at 5 days, an asterisk indicates a *P* of <0.05 for T-3811ME versus control, trovafloxacin, levofloxacin, and clarithromycin. Results are presented as Means + standard deviations (error bars) (untreated group, *n* = 11; treated groups, *n* = 5 to 6), for 2 or 5 days of therapy.

TABLE 2. Pharmacokinetic parameters after oral administration of antibacterial agents (20 mg/kg) to hamsters with experimental pneumonia caused by *M. pneumoniae* FH

| Fluid | Antibacterial agent   | $C_{\max}$ ( $\mu\text{g/ml}$ or $\text{g}$ ) | $T_{\max}^a$ (h) | $t_{1/2}^b$ (h) | $\text{AUC}_{0-\infty}$ ( $\mu\text{g} \cdot \text{h/ml}$ or $\text{g}$ ) | $C_{\max}/\text{MIC}^c$ | $\text{AUC}_{0-\infty}/\text{MIC}^c$ |
|-------|-----------------------|---|------------------|-----------------|---|-------------------------|--------------------------------------|
| Serum | T-3811ME <sup>d</sup> | 0.85  | 0.5              | 3.0             | 4.3   | 27.2                    | 137                                  |
|       | Trovaflaxacin         | 1.1   | 0.5              | 1.7             | 3.1   | 8.80                    | 24.8                                 |
|       | Levofloxacin          | 2.2   | 0.5              | 2.0             | 8.4   | 4.40                    | 16.8                                 |
|       | Clarithromycin        | 0.62  | 1                | 1.3             | 1.0   | 310                     | 500                                  |
| Lung  | T-3811ME              | 2.1   | 1                | 2.4             | 7.7   | 67.1                    | 246                                  |
|       | Trovaflaxacin         | 1.6   | 0.5              | 1.1             | 3.5   | 12.8                    | 28.0                                 |
|       | Levofloxacin          | 2.5   | 0.25             | 2.2             | 10.3  | 5.00                    | 20.6                                 |
|       | Clarithromycin        | 9.6   | 1                | 2.3             | 27.5  | 4,800                   | 13,750                               |
| BALF  | T-3811ME              | 0.09  | 0.5              | 3.9             | 0.27  |                         |                                      |
|       | Trovaflaxacin         | 0.08  | 1                | 1.1             | 0.22  |                         |                                      |
|       | Levofloxacin          | 0.17  | 0.5              | 2.1             | 0.46  |                         |                                      |
|       | Clarithromycin        | <0.20   |                  |                 |   |                         |                                      |

<sup>a</sup>  $T_{\max}$ , time to  $C_{\max}$ .<sup>b</sup>  $t_{1/2}$ , terminal half-life.<sup>c</sup> MIC for *M. pneumoniae* FH.<sup>d</sup> Equivalent to T-3811.

multiple comparisons procedures in an imbalanced two-way ANOVA (SAS version 6.12 software).

The concentrations in serum, BALF, and lung tissue after administration at a dose of 20 mg/kg orally were studied for T-3811ME, trovafloxacin, levofloxacin, and clarithromycin (each group,  $n = 3$  to 4). At 0.25, 0.5, 1, 2, 4, and 6 h after drug administration, blood, BALF, and lung tissue samples were collected. Animals were sacrificed by exsanguination from the cephalic vein under ether anesthesia at each sampling time. The blood serum (collected from the cephalic vein) was measured for drug concentrations. After blood was kept in Sepaclear-A-5 (Eiken Kizai Co., Ltd., Tokyo, Japan) at room temperature for more than 30 min, serum was obtained by centrifugation. Lung tissue was removed and homogenized in fourfold volumes of phosphate-buffered solution (PBS) (pH 7.0, 1/15 mol/liter). BALF was obtained by injection into and subsequent suction of PBS (1 ml) from the trachea. We used this method because the volume of BALF was very small. However the actual concentration of BALF would be much higher if corrected for dilution. The homogenized tissue and BALF were centrifuged. The supernatants and serum were frozen at  $-40^\circ\text{C}$  until required for HPLC assay. An equal volume of methanol was added to each of the samples, which were then mixed and centrifuged. Supernatants were used for measurement of T-3811, trovafloxacin, and levofloxacin concentrations by HPLC (LC series device [Shimadzu Co., Ltd., Tokyo, Japan] or L series device [Hitachi Co., Ltd., Tokyo, Japan]). The concentration of clarithromycin was measured by a bioassay method (paper disk method) using *Micrococcus luteus* ATCC 9341 as a test strain and heart infusion agar (Eiken Kagaku Co., Ltd., Tokyo, Japan) as a medium. Area under the plasma concentration-time curve from 0 h to  $\infty$  ( $\text{AUC}_{0-\infty}$ ) and the terminal half-life were calculated from the mean concentrations in plasma by noncompartmental analysis with WinNonlin software (Scientific Consulting, Inc.). The maximum plasma concentration ( $C_{\max}$ ) and the time to reach  $C_{\max}$  were obtained directly from the actual mean concentrations in plasma.

The MIC at which 90% of the isolates tested are inhibited

(MIC<sub>90</sub>) of T-3811 against 50 clinical isolates was 0.0313  $\mu\text{g/ml}$  and was 4-fold higher than that of clarithromycin but was 4- to 8-fold lower than those of trovafloxacin, gatifloxacin, gemifloxacin, and moxifloxacin and was 16- to 32-fold lower than those of levofloxacin, ciprofloxacin, and minocycline. The MIC of T-3811 for *M. pneumoniae* FH was 0.0313  $\mu\text{g/ml}$  and was 2- to 32-fold lower than those of other quinolones tested (Table 1).

Figure 1 shows the viable cell counts in BALF from hamsters with experimental pneumonia caused by *M. pneumoniae* FH. The viable cells of *M. pneumoniae* in the nontreated group were  $10^5$  to  $10^6$  CFU/ml from 7 days (start of administration) through 12 days (1 day after administration over 5 days) after infection. After the administration of trovafloxacin (20 and 40 mg/kg), levofloxacin (20 and 40 mg/kg), and clarithromycin (20 mg/kg) once daily for 2 days, the viable cells of *M. pneumoniae* in BALF were comparable to those of the nontreated group. On the other hand, T-3811ME (20 mg/kg as T-3811) and clarithromycin (40 mg/kg) reduced the viable cell count in BALF, and the viable cells of *M. pneumoniae* in these groups were significantly fewer than in the nontreated group ( $P < 0.05$ ). The viable cells of *M. pneumoniae* in BALF after administration of T-3811ME (20 mg/kg as T-3811) once daily for 5 days were significantly fewer than those in the clarithromycin-, trovafloxacin-, and levofloxacin-treated groups (20 and 40 mg/kg) and the nontreated groups ( $P < 0.05$ ).

Table 2 shows the pharmacokinetic parameters. The  $C_{\max}$  of T-3811 in serum was 0.85  $\mu\text{g/ml}$  and was lower than that of levofloxacin and comparable to those of trovafloxacin and clarithromycin. The  $C_{\max}$  of T-3811 in lung tissue was 2.1  $\mu\text{g/g}$  and was lower than that of clarithromycin and comparable to those of levofloxacin and trovafloxacin. The  $C_{\max}$  of T-3811 in BALF was 0.09  $\mu\text{g/ml}$  and was lower than that of levofloxacin and comparable to that of trovafloxacin. The  $C_{\max}$  of clarithromycin in BALF was lower than the limit of assay. The  $\text{AUC}_{0-\infty}$  of T-3811 in lung tissue was 7.7  $\mu\text{g}\cdot\text{h/g}$  and was greater than that of trovafloxacin and lower than those of levofloxacin and clarithromycin. The  $C_{\max}/\text{MIC}$  and  $\text{AUC}_{0-\infty}/\text{MIC}$  of T-3811 in lung tissue were 67.1 and 246, respectively and were greater

than those of trovafloxacin and levofloxacin. Clarithromycin possessed a potent in vitro activity against *M. pneumoniae* and showed a significant therapeutic effect (40 mg/kg, for 2 days) in the present study. However, the therapeutic effect of clarithromycin was inferior to that of T-3811ME in other cases. Because *M. pneumoniae* is an extracellular parasite (19), it was considered that the low distribution in BALF and weak bactericidal activity of clarithromycin compared with T-3811 (data not shown) resulted in low efficacy.

Macrolides and tetracyclines are the most-active antibiotics against mycoplasmas in vitro (1, 16, 22, 25). However, improvements in therapy are needed to accommodate specific characteristics of the microorganism or unusual manifestations of the illness. Until now, it was reported that quinolones are less effective in mycoplasmal and chlamydial infection (16). The newer fluoroquinolones, such as moxifloxacin and gatifloxacin, which have recently undergone substantial development, exhibit in vitro activity against a broadened spectrum, including *M. pneumoniae* (5, 6, 7, 8, 10, 15). T-3811, the free base of T-3811ME (BMS-284756), also showed excellent in vitro activity against *M. pneumoniae*. The in vitro activity of T-3811 was four- to eight-fold greater than those of other new quinolones tested. Also, the in vivo efficacy of T-3811ME against experimental pneumonia caused by *M. pneumoniae* was superior to those of trovafloxacin, levofloxacin, and clarithromycin. Although the existing data on in vivo efficacy are only preliminary (11, 13, 14), the newer fluoroquinolones are promising agents for the treatment of *M. pneumoniae* infection and, more generally, for that of community-acquired pneumonia. Newer quinolones have attracted interest as potential therapy for community-acquired pneumonia because they are active against a wide range of pathogens, including *M. pneumoniae*.

In conclusion, T-3811ME possessed potent in vitro antimycoplasma activity and had a significantly superior therapeutic effect compared to clarithromycin, trovafloxacin, and levofloxacin on experimental pneumonia caused by *M. pneumoniae* FH. These data indicate that T-3811ME (BMS-284756) could be a viable alternative to macrolides, such as clarithromycin, in the treatment of pneumonia caused by *M. pneumoniae*.

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