Early Bactericidal Activity and Pharmacokinetics of the Diarylquinoline TMC207 in Treatment of Pulmonary Tuberculosis


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Tibotec Medicinal Compound 207 (TMC207) is a novel diarylquinoline with a unique mode of action that targets mycobacterial ATP synthase. TMC207 exhibits high in vitro activity against mycobacterial strains either susceptible or resistant to all first-line and many second-line drugs, including fluoroquinolones, and has shown exceptional in vivo activity against several mycobacterial species in different animal models. In this early bactericidal activity study, 75 treatment-naïve patients with smear-positive pulmonary tuberculosis were randomized to once-daily oral TMC207 (25 mg, 100 mg, or 400 mg), 600 mg rifampin (RIF), or 300 mg isoniazid (INH) for 7 days. Sixteen-hour overnight sputum collected at baseline and on each treatment day was plated in serial dilutions on selective agar plates. The bactericidal activity was expressed as the log10 decrease in CFU/ml sputum/day. Pharmacokinetic sampling was performed on day 7 of TMC207 administration up to 24 h postdose. The decreases in log10 CFU counts (± standard deviation) from baseline to day 7 were 0.04 ± 0.46 for 25 mg TMC207 (n = 14), 0.26 ± 0.64 for 100 mg TMC207 (n = 14), 0.77 ± 0.58 for 400 mg TMC207 (n = 14), 1.88 ± 0.74 for INH (n = 11), and 1.70 ± 0.71 for RIF (n = 14). Significant bactericidal activity of 400 mg TMC207 was observed from day 4 onward and was similar in magnitude to those of INH and RIF over the same period. The pharmacokinetics of TMC207 were linear across the dose range. In summary, TMC207 demonstrated bactericidal activity with a delayed onset and was well tolerated, and no study drug-related serious adverse events occurred.

Tuberculosis (TB) has reemerged as one of the most deadly infectious diseases worldwide, killing approximately 1.7 million people in 2004 (25). In Africa, more than 30% of new adult TB cases are coinfected with human immunodeficiency virus (HIV) (5). Widespread efforts to control the resurgence of TB, such as the implementation of outcome-driven treatment programs (16) and the introduction of “directly observed therapy short-course” (26), have had limited success, in part due to constrained public health resources and the length of treatment needed to sterilize infectious TB lesions. The global situation is deteriorating further with the spread of multidrug-resistant (MDR) TB (7, 8) and, more recently, extensively drug-resistant TB (9). There is an urgent need for new anti-TB agents that can shorten treatment duration and be effective in patients with drug-susceptible or drug-resistant TB.

This paper reports the results of a phase IIa, open-label, randomized clinical trial designed to evaluate the pharmacokinetics, safety, tolerability, and extended early bactericidal activities of three different daily oral doses of TMC207 administered as monotherapy over a treatment period of 7 days in treatment-naïve patients with sputum smear-positive pulmonary TB. TMC207 is the first novel anti-TB compound to be studied in patients in nearly 4 decades.

MATERIALS AND METHODS

Study design, study setting, and patients. This was a parallel-group, open-label, randomized phase IIa trial with three different doses of TMC207 (25 mg, 100 mg, and 400 mg once a day [q.d.]), RIF (600 mg q.d.), and INH (300 mg q.d.), that was conducted simultaneously at two centers in Cape Town and Durban, South Africa. Patients were randomized centrally. TMC207 doses were selected to provide exposure above MIC with the higher doses potentially permitting less-than-daily dosing in future clinical trials. TMC207 up to 400 mg daily had been generally safe and well tolerated in phase 1 trials. The protocol was...
approved by the Medicines Control Council of South Africa, the Biomedical Ethics Committee of the University of KwaZulu-Natal (reference T014/05), and Pharma-Ethics Independent Research Ethics Committee for the Cape Town site (reference 05021225). The trial was conducted in accordance with South African Guidelines for Good Clinical Practice and the Helsinki Declaration of 1975, as revised in 1983. The trial is registered at http://www.clinicaltrials.gov. All patients provided written informed consent.

Treatment-naive sputum-smear-positive patients (≥1+ [in this study, 1+ = 1 to 9 acid-fast bacilli per 10 microscopic fields examined on Ziehl-Neelsen-stained smears at 1,000 × oil magnification]) between 18 and 65 years of age were included if they were found to be free of underlying medical conditions that would make participation inadvisable, such as drug or alcohol abuse, disseminated TB, or diabetes mellitus necessitating insulin treatment. HIV-positive individuals on antiretroviral therapy were excluded. Subjects with an estimated overnight sputum production of less than 15 ml and those with bacilli resistant to RIF as determined by the FASTPlaque TB assay (Biotec, Ipswich, United Kingdom) were not included (1). Recent exposure to INH was excluded by urine testing (BBL Taxo INH test strips; Becton Dickinson, Franklin Lakes, NJ).

Study procedures. The patients were hospitalized for the whole period of study medication intake. Sputum was collected for 16 h overnight at baseline, for 7 nights following study drug intake, and for one additional night following the first medication intake. Sputum was collected for 16 h overnight at baseline, for 7 nights following study drug intake, and for one additional night following the first medication intake. Sputum was collected for 16 h overnight at baseline, for 7 nights following study drug intake, and for one additional night following the first medication intake. Sputum was collected for 16 h overnight at baseline, for 7 nights following study drug intake, and for one additional night following the first medication intake.

Microbiology. Sputum smears, CFU counts, and susceptibility testing for first-line drugs (MGIT SIRE kit; Becton Dickinson, Franklin Lakes, NJ) were performed at both centers (Durban, J.A.; Cape Town, A.V.) using standardized protocols. For CFU counting, the sputum was homogenized by magnetic stirring. Dithiothreitol (1:20 dilution; Sputasol; Oxoid, Cambridge, United Kingdom) was added to a maximum of 10 ml of homogenized sputum in equal volume, vortexed for 20 seconds, and left to digest at room temperature for 20 min; 1 ml of this mixture was used to prepare a range of 10-fold dilutions from 10^0 to 10^-7. One hundred microliters from each dilution was plated in quadruplicate on TH11 agar plates (Becton Dickinson, Franklin Lakes, NJ) that contained 200 units/ml of polymyxin B, 10 µg/ml of amphotericin B, 100 µg/ml of tetracyclin, and 10 µg/ml of trimethoprim (Selectatab; Mast, Merseyside, United Kingdom). CFU were counted after 3 to 4 weeks of incubation at 37°C at the dilution that yielded 20 to 200 visible colonies (6). Cultures from the baseline and the last overnight sputum collection were used for susceptibility testing. TMC207 sensitivity was analyzed with a Resazurin Microtiter Assay (Institute of Tropical Medicine, Antwerp, Belgium) (20). Mycobacteria were identified with a Mycobacterium tuberculosis Complex Direct Detection assay (Becton Dickinson, Franklin Lakes, NJ; performed by Covance, Geneva, Switzerland) (24).

Pharmacokinetic analysis. Medication intakes were directly observed once daily within 30 min after breakfast. Pharmacokinetic profiles for TMC207 and its active N-monodesmethyl metabolite (M2) were determined up to 24 h postdose on day 7. Plasma was collected immediately before drug intake and 1, 2, 3, 4, 5, 6, 8, 12, and 24 h postdose. In addition, predose samples were collected on days 5 and 6, TMC207 and M2 concentrations in heparinized plasma were determined centrally using a validated liquid chromatography-tandem mass spectrometry (MS/MS) method as briefly described below. One hundred-microliter aliquots of plasma were spiked with a mixture of two stable-isotope-labeled internal standards (one for TMC207 and one for M2), followed by protein precipitation with 400 µl of a 70% ethanol solution. A 4-µl aliquot of the supernatant was analyzed by liquid chromatography-MS/MS. Chromatography was on a Polaris C18-A column operating at 40°C with a mobile phase of 0.01 M ammonium formiate (pH 4)/acetonitrile at a flow rate of 1.5 ml/min. Detection was by MS/MS (API4000) with ion spray operated in the positive ion mode. Analysts and internal standards were monitored at mass transitions m/z 555.2 to 58, 561.2 to 64, 541.1 to 480, and 545.2 to 480 for TMC207 and its internal standard and M2 and its internal standard, respectively. The validated range was 1 to 2,000 ng/ml for both analytes. Interrun accuracy from quality control samples analyzed along with the study samples ranged from 103.3% to 107.7% for TMC207 and from 101.5% to 105.3% for M2. Interrun precision (percent coefficient of variation) ranged from 2.2% to 12.6% for TMC207 and 2.3% to 7.2% for M2. Pharmacokinetic parameters were estimated with noncompartmental analysis. The area under the plasma concentration-time curve from 0 to 24 h was determined using the linear trapezoidal rule.

Statistical methods. The efficacy of treatments was assessed by the change in log_{10} CFU/ml sputum from baseline. The average decrease per treatment day was calculated as log_{10} (CFU/ml sputum − log_{10} CFU/ml sputum day x). For a CFU count of 0, the log_{10} CFU count was set to 0. The population for analysis was defined as all patients who received at least one dose of study drug (intention-to-treat population). All statistical tests were interpreted at the two-tailed 5% significance level. Statistical analysis was conducted using SAS (version 9.1.3; SAS Institute Inc., Cary, NC). As this was an exploratory study, no formal sample size calculation was performed.

RESULTS

Study population. From 1 June 2005 to 6 September 2005, 75 out of 120 screened individuals were randomized to five treatment groups. Sixty-seven subjects (89%) completed the 7-day treatment period (Fig. 1). The patients were 60% male with a median age of 34 years (range, 18 to 61 years). Fifty-seven percent were black. The majority of patients were active smokers (56%). One-third (31%) were HIV positive, with a median CD4 T-cell count of 510 cells/ml. The median body mass index was 19.3 kg/m² (range, 15 to 33 kg/m²). Demographic and disease characteristics were similarly distributed among treatment groups.

Bactericidal activity. The mean changes in CFU counts from baseline are presented in Table 1 and Fig. 2. Pretreatment CFU counts were comparable between treatment groups and in agreement with known values in patients with smear-positive TB. All patients’ isolates were susceptible to the investigational drug at baseline and at day 7. As expected, RIF and INH showed clear bactericidal activity, with the largest decrease in CFU counts observed during the first 3 days of treatment. The bactericidal activity for TMC207 became apparent comparatively late and resulted in a smaller absolute decrease in log_{10} CFU counts. Over days 4 to 7, 400 mg TMC207 induced daily CFU falls similar in magnitude to those with INH and RIF over the same period. TMC207 at 400 mg reached a statisti-
cally significant fall in CFU at the end of the 7-day period compared to baseline sputum CFU counts. Mean CFU falls with 400 mg TMC207 were significantly different from baseline from day 4 onward (P < 0.05; two-sided Wilcoxon signed-rank test). As expected, the addition of standard antituberculous treatment on day 8 resulted in an additional CFU fall, which was more pronounced in TMC207-treated subjects than in RIF- and INH-treated subjects. The fall in CFU counts from baseline to day 7 for individual study subjects by treatment group is illustrated in Fig. 3.

**Pharmacokinetics.** The exposure to TMC207 and the N-monodesmethyl metabolite increased proportionally to the dose across the range evaluated. Maximal plasma concentrations were reached after about 4 h postdose across the dosages. Based on plasma predose concentrations on days 5, 6, and 7, steady-state conditions were not yet reached after 7 days of treatment. The extent of N-demethylation of TMC207 was dose independent within the dose range of 25 mg to 400 mg q.d. (data not shown). Detailed pharmacokinetic data are given in Table 2.

**Safety.** The TMC207 treatment regimens were generally safe and well tolerated. The incidences of adverse events (AE) were similar across all treatment groups. The most commonly reported AE was hemoptysis, reported by one patient (6.3%) on 100 mg TMC207, three patients (21.4%) on 400 mg TMC207, and two patients (13.3%) on 300 mg INH. All AE were considered not related or doubtfully related to the study medication, except for rash (n = 1; 7%) in the 100 mg TMC207 group and diarrhea (n = 1; 7%) and somnolence (n = 1; 7%) in the 400 mg TMC207 group, which were rated possibly related to the study drug. Two patients treated with 400 mg TMC207 died 14 and 34 days after the end of TMC207 treatment, respectively. The deaths were due to hemoptysis secondary to TB, complications of TB, and AIDS and were not considered related to the study drug. Electrocardiogram assessments determined average increases of more than 10 ms in the QT interval corrected by the Fredericia method postdose on day 7 in the RIF, INH, 400 mg TMC207, and 100 mg TMC207 groups (mean increase ± standard deviation for RIF, 13 ± 21.9 ms; INH, 20.2 ± 13.2 ms; 25 mg TMC207, −1.9 ± 14.8 ms; 100 mg TMC207, 7.1 ± 9.5 ms; 400 mg TMC207, 18.8 ± 27.7 ms). No

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**TABLE 1. Mean changes in sputum CFU counts from baseline**

<table>
<thead>
<tr>
<th>Day</th>
<th>25 mg q.d.</th>
<th>100 mg q.d.</th>
<th>400 mg q.d.</th>
<th>RIF 600 mg q.d.</th>
<th>INH 300 mg q.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
</tr>
<tr>
<td>Baseline</td>
<td>15</td>
<td>6.66 (0.68)</td>
<td>16</td>
<td>6.32 (1.14)</td>
<td>14</td>
</tr>
<tr>
<td>After starting monotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>−0.06 (0.52)</td>
<td>16</td>
<td>−0.11 (0.52)</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.02 (0.27)</td>
<td>14</td>
<td>−0.20 (0.68)</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>−0.04 (0.36)</td>
<td>15</td>
<td>−0.37 (0.66)</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>−0.22 (0.57)</td>
<td>14</td>
<td>−0.24 (0.62)</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>−0.09 (0.52)</td>
<td>15</td>
<td>−0.13 (0.48)</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>−0.06 (0.59)</td>
<td>14</td>
<td>−0.09 (0.76)</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>−0.04 (0.46)</td>
<td>14</td>
<td>−0.26 (0.64)</td>
<td>12</td>
</tr>
</tbody>
</table>

* n, number of individuals with values. All values are in log_{10} CFU/ml sputum.

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**FIG. 2.** Bactericidal activities for days 0 to 7 by treatment regimen. The activities of 300 mg isoniazid and 600 mg rifampin are of immediate onset and continuous over 7 days. TMC207 shows delayed onset of activity from day 4. The values are means. Log Fall, change in log_{10} CFU/ml sputum from baseline to day 7. The error bars are 95% confidence intervals.

**FIG. 3.** Decline in CFU from baseline to day 7 for individual subjects by treatment group. Log Fall, change in log_{10} CFU/ml sputum from baseline to day 7.
TABLE 2. Pharmacokinetics of TMC207 after oral administration at different dosesa

<table>
<thead>
<tr>
<th>Parameterb</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMC207 25 mg</td>
</tr>
<tr>
<td>n (day 7)</td>
<td>12</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>104.3 ± 41.57</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>98.29 ± 36.76</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>319.2 ± 97.65</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>3.93 (2.00–6.17)</td>
</tr>
<tr>
<td>AUC0–24 (µg h/ml)</td>
<td>3,973 ± 1,238</td>
</tr>
<tr>
<td>Cav (µg/ml)</td>
<td>165.5 ± 51.68</td>
</tr>
<tr>
<td>FI (%)</td>
<td>135.7 ± 33.13</td>
</tr>
</tbody>
</table>

a All doses q.d. for 7 days. Data are expressed as mean ± SD, for tmax: median (range).
b n, number of subjects with values; Cav: plasma concentration at hour zero, i.e., predose; Cmax, minimum concentration of drug in serum; Cmax, maximum concentration of drug in serum; tmax, time to maximum concentration of drug in serum; AUC0–24, area under the concentration-time curve, 0 to 24 h; Cav, average steady-state plasma concentration; FI, fluctuation index.

patienty prolonged QT and QTc values were observed in any treatment group.

DISCUSSION

This study confirmed bactericidal activity of TMC207 in treatment-naive patients with sputum smear-positive pulmonary TB. While none of the TMC207 doses demonstrated a clear response in the initial days of treatment, a decline in CFU was observed from day 4 onward for the 400 mg TMC207 group. As expected, INH showed early bactericidal response from the first day onward, and a similar response was also found with RIF. No serious AE attributable to the study drug occurred during the trial. The exposure to TMC207 was linearly related to the dose. Steady-state plasma concentrations were not reached within the 7-day period of drug administration.

Early bactericidal activity (EBA) studies, traditionally carried out over 2 days, measure the ability of a single anti-TB agent to kill rapidly metabolizing mycobacteria present in tuberculous pulmonary cavities (6, 13, 14). EBA studies also offer the opportunity to evaluate the short-term toxicity and dose ranging of TB drugs. Mycobacteria in cavities exist as heterogeneous populations that are either actively replicating or in a hypometabolic state (10). Semireplicating, or hypometabolic, bacteria are more resistant to killing by conventional bactericidal anti-TB drugs. Although it is clear that the results of 2-day EBA studies bear little relationship to the ability of an agent to sterilize TB lesions, extending the period of drug treatment to 5 days or longer potentially offers the opportunity to measure the killing of hypometabolic organisms and thus might provide a preliminary assessment of the sterilizing activity of an agent (11, 15). This may also be indicative of the ability of a drug to prevent relapse of TB (13). Drugs that have effective sterilizing properties are essential to shorten the treatment duration of TB. Interestingly, similar to TMC207, pyrazinamide (PZA) also has almost no bactericidal action during the first 2 to 4 days of treatment (3, 13, 14) but nonetheless kills consistently thereafter and is one of the most important components of TB standard-of-care regimens (27). Significant syn-

energy is found between PZA and TMC207 in mice, which, together with the similar EBA profile in humans, might suggest possible similarities in the mechanisms of mycobacterial killing (12). The ability to deplete mycobacterial energy stores may give TMC207 the potential to become an important sterilizing agent of TB lesions and thus further shorten TB treatment. It was shown that the ATP synthase enzyme complex is down-regulated during dormancy as a result of overall shutdown of metabolic pathways (23). This results in lower ATP stores, which make dormant bacteria exquisitely vulnerable to further ATP depletion. The log kill obtained with a single dose of standard therapy following the experimental phase was greater in TMC207-treated subjects than in RIF- and INH-treated subjects. This suggests that TMC207 may be acting on a population distinct from the one targeted by the existing first-line drugs and may indicate that increased activity could be expected if TMC207 were added to the standard treatment regimen.

The reasons for the slow onset of action of TMC207 in patients remain speculative. It may be suggested that energy depletion and disruption of intracellular pH homeostasis as a result of the action of TMC207 (2) may require a few days to affect mycobacterial viability. This might be because at any given time point in a cell there is a cellular ATP pool present that may allow bacteria to survive transiently. This probably interferes, albeit for a short period, with any block of ATP synthesis mediated by TMC207’s inhibition of the ATP synthase enzyme. The activity of TMC207 found in humans in the present study fits in well with in vitro and animal data. In vitro, TMC207 has time-dependent activity driven by the time over MIC (2). TMC207 at 10× MIC or 100× MIC in 7H9 broth was highly bactericidal against M. tuberculosis, but this was only observed after 6 days of incubation and not earlier. The most impressive activity of TMC207 in animal models was observed after treatment for at least 1 month in studies conducted in mice infected with drug-sensitive TB and nonestablished (2) and established infection (2, 12) or MDR-TB (19). In guinea pigs, where TB bacilli are preferentially found extracellularly and not intracellularly as in mice, similar activity was found (18). These animal studies demonstrated killing in 1 week of treatment with TMC207 in the same range as found in the present study. The activity of TMC207 beyond 7 days in humans remains unknown and needs further investigation.

Having confirmed the bactericidal activity of TMC207 in the context of an EBA study in patients with tuberculosis, the next step is evaluation of its activity over a longer period. The rapid development of resistance of M. tuberculosis to agents given in monotherapy limits the duration of single-agent EBA studies. In a previous study that included the sterilizing drugs PZA and RIF, 90 to 95% of patients demonstrated culture negativity at 2 months following treatment, superior to results in regimens that did not include these agents (21). In a recent study, substitution of the fluoroquinolone moxifloxacin for ethambutol in a regimen containing RIF and PZA did not result in a significant difference in the 2-month sputum culture-negativity, although the moxifloxacin regimen did lead to a significantly higher rate of culture negativity at 1 month (4). The potency of RIF and PZA to sterilize sputum during the first 2 months of TB treatment complicates the evaluation of new anti-TB agents in combination therapy with these drugs. An alternative
approach would be the addition of TMC207 to second-line agents in the treatment of MDR TB, where the use of first-line agents is limited and the remaining drugs would be expected to be less efficient. It is possible that the addition of TMC207 to an MDR TB regimen, even those containing a fluoroquinolone, would enhance the ability to determine the intrinsic sterilizing activity of TMC207. A trial to assess the activity, safety, and tolerability of TMC207 in patients with MDR pulmonary TB is currently in progress.

In conclusion, the present study has shown that oral once-daily administration of TMC207 has bactericidal activity at a dose of 400 mg when administered as monotherapy for 7 days in patients with pulmonary TB. Compared to INH and RIF, dose of 400 mg of TMC207 started later but was of similar magnitude on days 4 to 7. Serious AE related to the bactericidal activity of 400 mg of TMC207 started later but in patients with pulmonary TB. Compared to INH and RIF, daily administration of TMC207 has bactericidal activity at a pulmonary TB is currently in progress.

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