Early and Extended Early Bactericidal Activity of Linezolid in Pulmonary Tuberculosis

Reynaldo Dietze, David Jamil Hadad, Bryan McGee, Lucilia Pereira Dutra Molino, Ethel Leonor Noia Maciel, Charles A. Peloquin, Denise F. Johnson, Sara M. Debanne, Kathleen Eisenach, W. Henry Boom, Moises Palaci, John L. Johnson

Online Data Supplement

Study Design

Patients with suspected pulmonary tuberculosis (TB) were recruited at local TB posts and the Hospital Universitario Cassiano Antonio de Moraes of the Universidade Federal do Espírito Santo (UFES) in Vitória, Brazil. Adults aged 18 to 65 years with newly diagnosed initial episodes of smear-positive TB who weighed more that 75% of their predicted ideal body weight and had relatively normal hematologic, renal and hepatic function (see eligibility criteria below) were eligible. HIV-infected patients, patients with serious medical co-morbidities or significant hemoptysis, and patients with suspected miliary or meningeal TB were ineligible. Patients with self-reported treatment with antituberculosis medications or other antimicrobials with known activity against *M. tuberculosis* during the previous 6 months were ineligible. The institutional review boards of UFES and Case Western Reserve University approved the protocol. All patients gave written informed consent for study participation.

Patients were randomized centrally to receive 7 days of oral (a) INH 300 mg per day (positive control); (b) linezolid 600 mg twice daily; or (c) linezolid 600 mg once daily. Laboratory staff performing smears and cultures were blinded to the patients' treatment arm. Study drugs were purchased in the U.S. and manufactured under Good Manufacturing Practice (GMP).

Patients were hospitalized in a research ward at the Hospital Universitário Cassiano Antônio de Moraes throughout the study for supervised drug administration and specimen collection. At the conclusion of the study period, all patients were treated with standard short course chemotherapy including INH, rifampin, and pyrazinamide for 6 months.

Eligibility Criteria

HIV-non-infected males and females, 18-65 years old, with newly diagnosed, initial episodes of sputum smear-positive, culture-confirmed pulmonary TB were eligible for enrollment in this study. Subjects were enrolled if they meet all of the inclusion criteria and none of the exclusion criteria.

Inclusion Criteria

1. Adults, male or female, age 18 to 65 years
2. Women with child-bearing potential (not surgically sterilized or postmenopausal for less than 1 year) must be using or agree to use an adequate method of birth control during study drug treatment.
3. Newly diagnosed sputum smear-positive pulmonary TB, as confirmed by sputum AFB smear and chest radiographic findings consistent with pulmonary TB
4. Willing and able to provide informed consent
5. Reasonably normal hemoglobin ($\geq$ 8 gm/dL), renal function (serum creatinine < 2 mg/dL), hepatic function (serum AST < 1.5 times the upper limit of normal for the testing laboratory and total bilirubin < 1.3 mg/dL), and random blood glucose < 150 mg/dL.

Exclusion Criteria

1. HIV infection
2. Weight less than 75% of ideal body weight
3. Presence of significant hemoptysis. Patients who cough up frank blood (more than blood streaked sputum) will not be eligible for enrollment.

4. Pregnant or breastfeeding women and those who are not practicing birth control
5. Significant respiratory impairment (respiratory rate greater than 35/minute)
6. Clinical suspicion of disseminated TB or tuberculous meningitis
7. Presence of serious underlying medical illness, such as liver failure, renal failure, diabetes mellitus, chronic alcoholism, decompensated heart failure, hematologic malignancy or patients receiving myelosuppressive chemotherapy.
8. Patients receiving any of the following medications – monoamine oxidase inhibitors (phenelzine, tranylcypromine), adrenergic/serotonergic agonists such as pseudoephedrine and phenylpropanolamine (frequently found in cold and cough remedies), tricyclic antidepressants (amitriptyline, nortriptyline, protriptyline, doxepin, amoxapine, etc), antipsychotics such as chlorpromazine and buspirone, serotonin re-uptake inhibitors (fluoxetine, paroxetine, sertraline, etc.), buproprion, agents known to prolong the QTc interval [erythromycin, clarithromycin, astemizole, type Ia (quinidine, procainamide, disopyramide) and III (amiodarone, sotalol) anti-arrhythmics, carbamazepine, insulin, sulfonylureas, and meperidine.
9. Presence of QTc prolongation (greater than 450 msec) on baseline EKG
10. Allergy or contraindication to use of study drugs
11. Treatment with antituberculosis medications or other antimicrobials with known activity against *M. tuberculosis* during the preceding 6 months
12. Inability to provide informed consent.
13. Total white blood cell count less than $3000/mm^3$
14. Platelet count less than $150,000/mm^3$

15. Patients with suspected drug-resistant TB (e.g., contact to source patient with drug-resistant tuberculosis, patients who have relapsed after previous treatment for TB)
16. Patients unlikely in the opinion of the local investigator to be unable to comply with the requirements of the study protocol

Sputum Collection and Culture

Sputum was collected for 12 hours daily from 8 PM to 8 AM for 2 days prior to the study (baseline) and then for 7 days of study drug administration for all study arms. The morning drug dose was given shortly after completing the previous day's collection. For subjects in the linezolid 600 mg twice-daily arm, the second 600 mg dose was administered at 8PM.

The total volume of the sputum sample was digested with 1% dithiothreitol (DTT) and concentrated by centrifugation. Serial ten-fold dilutions from 10^0 to 10^{-5} were prepared and plated on selective Middlebrook 7H10 agar containing amphotericin, polymyxin B, carbenicillin, and trimethoprim. Plates were incubated at 37°C in 5 to 10% CO_2 for up to 42 days. Plates were examined weekly and colonies counted on plates with dilutions yielding 10 to 50 visible colonies. Data were expressed as $\log_{10}$ cfu per ml of undiluted sputum. Susceptibility testing was performed at pretreatment, day 7 and day 42 (for subjects with positive cultures at the latter time point) isolates from each patient using standard BACTEC methods for INH, rifampicin, ethambutol and pyrazinamide (E1). MIC determinations against linezolid were performed in the BACTEC 460 system using two-fold dilutions from 0.125 to 4 µg/mL. MIC was defined as the lowest concentration for which Δ Growth Index was less than that of the 1:100 control.

Pharmacokinetic Studies

On the fifth day of drug administration and after overnight fasting, plasma samples were collected at 0, 1, 2, 4, 8 and 12 hours post dose for subjects in the linezolid arms and also at 18 and 24 hours for subjects receiving INH. No food was ingested for 2 hours after drug intake. Samples were stored at - 80°C until assay at National Jewish Medical and Research Center, Denver. Serum concentrations of linezolid were determined using a validated high performance liquid chromatography (HPLC) assay. Samples were assayed using a system consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, CA) with model AS1000 fixed-volume autosampler, a model UV2000 ultraviolet detector, a Gateway Series e computer (Poway, CA), and the Chromquest HPLC data management system. The plasma standard curve for linezolid ranged from 0.5 to 30 μg/mL. The absolute recovery of linezolid from plasma was 95%. The within-sample precision (percent coefficient of variation [CV%]) of validation a single standard concentration was 0.69%, and the overall validation precision across all standards was 1.04 to 4.39%. No interferences were observed with the measurement of linezolid with 90 different commonly used medications. Analyses of pharmacokinetic parameters were performed using non-compartmental techniques (WinNonLin PK software Version 4, Pharsight, Mountain View, CA).

Safety

A standard survey of TB symptoms and toxicity was completed daily during the 9-day inpatient study and repeated at Day 42. Complete blood count, urinalysis, and measurement of serum aspartate aminotransferase, total bilirubin, creatinine, and glucose were repeated at Day 4

and Day 7. A time and events schedule showing all study procedures is included below (Table E1).

Endpoints

The primary study endpoint was the proportional difference in sputum bacillary load [colony forming unit (cfu) count] between study arms. Earlier EBA studies have suggested that 10 or fewer patients per arm may be sufficient to detect changes in cfu over time when standard deviations are small (E2-E6). Safety was assessed by comparing the incidence of adverse events and changes in laboratory parameters between arms.

Measures of Bactericidal Activity

Summary measures of bactericidal activity were calculated for each patient.

Early bactericidal activity. Early bactericidal activity (EBA 0-2) was calculated as the rate of fall in sputum cfu (expressed in $\log_{10}$ units) during the first 2 days of study drug using the equation $EBA = (\log_{10} \text{cfu/mL } S_0 - \log_{10} \text{cfu/mL } S_2)/2$, where S_0 and S_2 are the initial cfu (mean of the cfu from the 2 pretreatment sputum samples) and day 2 colony counts, respectively (E7).

Extended early bactericidal activity. A similar equation was used to calculate extended EBA between day 2 and day 7 (EBA 2-7) (E8). The rate of fall in sputum cfu between day 2 and day 7 (b2-7) was estimated by the slope of the linear regression obtained from fitting the 6 sputum cfu values corresponding to days 2 through 7, with the sign of the slope reversed.

REFERENCES

E1. Siddiqi S. Radiometric (BACTEC) tests for slowly growing mycobacteria. In: Isenberg HD, editor. Clinical microbiology procedures handbook. Washington, D.C.: American Society for Microbiology; 1992. p. 5.14.

E2. Botha FJ, Sirgel FA, Parkin DP, van de Wal BW, Donald PR, Mitchison DA. Early bactericidal activity of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater) in patients with pulmonary tuberculosis. *S Afr Med J* 1996;86:155-158.

E3. Donald PR, Sirgel FA, Botha FJ, Seifart HI, Parkin DP, Vandenplas ML, Van de Wal BW, Maritz JS, Mitchison DA. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. *Am J Respir Crit Care Med* 1997;156:895-900.

E4. Sirgel FA, Botha FJ, Parkin DP, Van De Wal BW, Donald PR, Clark PK, Mitchison DA. The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts: A new method of drug assessment. *J Antimicrob Chemother* 1993;32:867-875.

E5. Sirgel FA, Botha FJ, Parkin DP, Van de Wal BW, Schall R, Donald PR, Mitchison DA. The early bactericidal activity of ciprofloxacin in patients with pulmonary tuberculosis. *Am J Respir Crit Care Med* 1997;156:901-905.

E6. Sirgel FA, Donald PR, Odhiambo J, Githui W, Umapathy KC, Paramasivan CN, Tam CM, Kam KM, Lam CW, Sole KM, et al. A multicentre study of the early bactericidal activity of anti- tuberculosis drugs. *J Antimicrob Chemother* 2000;45:859-870.

E7. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1980;121:939-949.

E8. Jindani A, Dore CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *Am J Respir Crit Care Med* 2003;167:1348-1354.

Table E1. Schedule of Study Procedures

	Screening*	Baseline†		Study Drug Treatment‖							Follow-up	
	Days -8 to -1	Baseline/ Day -2	Baseline/ Day- 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 42
Informed consent	x											
Medical history & physical exam	x											
TB symptoms & toxicity survey		x		x	x	x	x	x	x	x	x	x
Vital signs & clinical evaluation	x	x	x	x	x	x	x	x	x	x	x	x
HIV EIA	x*											
Chest radiograph	x*											
12-lead EKG	x											
Sputum AFB smear & culture	x											x§
Storage of MTB culture isolate		x	x									x
Sputum 7H-10 quantitative culture		x	x	x	x	x	x	x	x	x		
MTB drug susceptibility testing		x								x		x‖
CBC, diff & platelet count	x						x			x		
Serum AST, bilirubin, creatinine, glucose	x						x			x		
Plasma drug concentrations								x				
Urinalysis	x*						x			x		
Urine INH metabolite test	x											
Pregnancy test (females only)	x	x										
Standard Chemotherapy											x	

[*] Screening procedures should be completed within 7 days of study entry except chest radiograph, which may be done within the previous 14 days, and HIV EIA, which may be done within the previous 3 months. Urine INH metabolite testing was performed during screening as an additional check that the patient had not been on anti-TB treatment.

[†] Subjects will be hospitalized for 2 days before beginning study drug treatment for collection of 2 pre-treatment sputum specimens.

[] Day 1 is the first day of study drug administration.

[§] For all subjects still spontaneously producing sputum at the Day 42 visit

[‖] For all subjects with a positive sputum culture for *M. tuberculosis* at the Day 42 visit.