Substitution of ethambutol with linezolid during the intensive phase of treatment of pulmonary tuberculosis: a prospective, multicentre, randomised, open-label, phase 2 trial

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Lancet Infect Dis 2019; 19: 46–55

Published Online November 23, 2018 http://dx.doi.org/10.1016/S1473-3099(18)30480-8

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Summary

Background Linezolid improves the treatment outcomes of multidrug-resistant tuberculosis substantially. We investigated whether use of linezolid instead of ethambutol increases the proportion of sputum culture conversion at 8 weeks of treatment in patients with pulmonary tuberculosis.

Methods We did a phase 2, multicentre, randomised, open-label trial for patients with pulmonary tuberculosis at the three affiliated hospitals to Seoul National University and National Medical Center (Seoul–Seongnam, South Korea). Patients, aged 20–80 years, with a positive sputum for pulmonary tuberculosis, but without resistance to rifampicin, and current treatment administered for 7 days or fewer, were randomly assigned at a 1:1:1 ratio into three groups. The control group received ethambutol (2 months) with isoniazid, rifampicin, and pyrazinamide. The second group used linezolid (600 mg/day) for 2 weeks and the third group for 4 weeks instead of ethambutol for 2 months. We used a minimisation method to randomise, and stratified according to institution, cavitation on chest radiographs, and diabetes. The primary endpoint was the proportion of patients with negative culture conversion of sputum in liquid media after 8 weeks of treatment. The results of this trial were analysed primarily in the modified intention-to-treat population. The trial is registered with ClinicalTrials.gov, number NCT01994460.

Findings Between Feb 19, 2014, and Jan 13, 2017, a total of 429 patients were enrolled and 428 were randomly assigned into either the control group (142 patients), the linezolid 2 weeks group (143 patients), or the linezolid 4 weeks group (143 patients). Among them, 401 were eligible for primary efficacy analyses. In the modified intention-to-treat analyses, negative cultures in liquid media at 8 weeks of treatment were observed in 103 (76·9%) of 134 control patients, 111 (82·2%) of 135 in the linezolid 2 weeks group, and 100 (75·8%) of 132 in the linezolid 4 weeks groups. The difference from the control group was 5.4% (95% CI −4·3 to 15·0, p=0·28) for the linezolid 2 weeks group and −1·1% (−11·3 to 9·1, p=0·83) for the linezolid 4 weeks group. Numbers of patients who experienced at least one adverse event were similar across the groups (86 [62·8%] of 137 in control, 79 [57·2%] of 138 in the linezolid 2 weeks group, and 75 [62·0%] of 121 in the linezolid 4 weeks group). Resistance to linezolid was not identified in any patient.

Interpretation Higher rates of culture conversion at 8 weeks of treatment with short-term use of linezolid were not observed. However, safety analyses and the resistance profile suggested the potential role of linezolid in shortening of treatment for drug-susceptible tuberculosis.

Funding Ministry of Health and Welfare, South Korea.

Introduction

The standard short-course treatment for drug-susceptible pulmonary tuberculosis consists of a 2-month intensive phase of treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol, and a subsequent 4-month continuation phase of treatment with isoniazid and rifampicin. Although this regimen is highly effective, the long duration of treatment increases the likelihood of adverse events (AEs) while decreasing patients' adherence to anti-tuberculosis drugs. The proportion of non-adherence among patients with tuberculosis treated with the 6-month regimen has been reported to be 16·8% in a clinical trial setting[1] and 48% in a real-world setting.[2] Patients with pulmonary tuberculosis who take their medication irregularly could spread tuberculosis to other people,[3] and their disease could progress to drug-resistant tuberculosis.[2]

To shorten the treatment duration of drug-susceptible pulmonary tuberculosis, later generation fluoroquinolones, which have shown high mycobactericidal activity,[4] have been tried. Several 4-month

Research in context

Evidence before this study

We searched PubMed on May 3, 2018, for published clinical trials addressing the possibility of pulmonary tuberculosis treatment shortening, using the search terms of "tuberculosis", "treatment shortening", and "clinical trial". The search yielded several randomised controlled trials of 4-month regimens adopting moxifloxacin or gatifloxacin instead of isoniazid or ethambutol. None of those 4-month treatment regimens showed non-inferiority to the current 6-month regimen for treating drug-susceptible pulmonary tuberculosis. A phase 2b clinical trial showed superior bactericidal activity of the combination of moxifloxacin, pretomanid, and pyrazinamide in drug-susceptible tuberculosis during 8 weeks of treatment.

Added value of this study

Linezolid, an oxazolidinone, has been shown to improve the treatment outcomes of multidrug-resistant tuberculosis substantially. We carried out a prospective, multicentre, randomised, open-label, phase 2 trial to ascertain if use of linezolid instead of ethambutol could increase the proportion of sputum culture conversion at 8 weeks of treatment in patients with drug-susceptible pulmonary tuberculosis. We did not observe higher proportion of culture conversion at 8 weeks of treatment with 2 weeks or 4 weeks use of linezolid in the modified intention-to-treat analyses. However, per-protocol analyses showed favourable results for the linezolid, especially 2 weeks, groups. In addition, resistance to linezolid was not developed in any patient.

Implications of all the available evidence

Higher proportion of culture conversion at 8 weeks of treatment was not observed in our trial; however, we believe that a role for linezolid in the treatment shortening of drug-susceptible tuberculosis remains a possibility. Various combinations including repurposed (eg, linezolid, fluoroquinolones) as well as newly developed (eg, bedaquiline, delamanid, pretomanid) drugs could be tested to shorten treatment duration of drug-susceptible tuberculosis in the future.

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regimens adopting moxifloxacin or gatifloxacin instead of isoniazid or ethambutol (in addition to rifampicin and pyrazinamide) have been tested through randomised controlled trials. Unfortunately, none of those 4-month treatment regimens showed non-inferiority to the current 6-month regimen.[5–8] Several trials adopting shorter regimens with high dose rifamycins or new drugs including pretomanid are ongoing.[9]

Linezolid, an oxazolidinone, has antibacterial activity by inhibiting protein synthesis through binding the 23S ribosomal RNA portion of the bacterial 50S ribosomal subunit.[10] Despite modest early bactericidal activity against *Mycobacterium tuberculosis*,[11] linezolid can improve the treatment outcomes of multidrug-resistant tuberculosis (MDR-TB) substantially.[12] Adding linezolid to the treatment regimen has been shown to result in a proportion of culture conversion of 89% by 6 months in patients with extensively drug-resistant tuberculosis (XDR-tuberculosis) refractory to previous treatment.[13]

On the basis of its impressive effects among patients with MDR-TB, we hypothesised that linezolid could increase the proportion of 2-month negative culture conversion of sputum in patients with drug-susceptible pulmonary tuberculosis. We aimed to ascertain if use of linezolid instead of ethambutol could increase the proportion of sputum culture conversion at 8 weeks of treatment in patients with drug-susceptible pulmonary tuberculosis.

Methods

Study design and participants

We did a phase 2, multicentre, randomised, open-label trial with three arms. Patients were assigned randomly at a 1:1:1 ratio to three groups: control; linezolid 2 weeks group and linezolid 4 weeks group (figure 1).

The study protocol was reviewed and approved by the institutional review board of each institution and the Ministry of Food and Drug Safety, South Korea. The protocol was published previously.[14] A data and safety monitoring board consisting of two pulmonologists and one biostatistician reviewed trial data and provided recommendations every 6 months throughout the study.[14]

Patients were recruited at four hospitals in South Korea. Three of these hospitals are affiliated with the Seoul National University (Seoul National University Hospital, Seoul, South Korea; Seoul Metropolitan Government–Seoul National University Boramae Medical Center, Seoul, South Korea; and Seoul National University Bundang Hospital, Seongnam, South Korea) and the other is the National Medical Center, Seoul, South Korea. All hospitals are in an urban area.

Patients with pulmonary tuberculosis satisfying the inclusion criteria, who provided written informed consent, were enrolled by investigators in outpatient and inpatient settings in the four participating hospitals.

The inclusion criteria were men and women aged 20–80 years, documented positivity by sputum Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for pulmonary tuberculosis at screening, and administration of current tuberculosis therapy (if any) for less than or equal to 7 days at the time of enrolment.

Patients with resistance to rifampicin as detected by an Xpert MTB/RIF assay, and women of childbearing potential who were pregnant, breastfeeding, or unwilling to avoid pregnancy were excluded from this study. In addition, any of the following factors led to exclusion:

absolute neutrophil count of less than $2{\cdot}0\times10^3$ cells/mL, white blood cell count of less than $3{\cdot}0\times10^3/\mu L$, haemoglobin concentrations of less than 7·0 g/dL, serum creatinine concentrations of greater than 2·0 mg/dL, aspartate aminotransferase concentrations of greater than 100 IU/L, alanine aminotransferase of greater than 100 IU/L, total bilirubin concentrations of greater than 2·0 mg/dL, history of optic neuritis or peripheral neuropathy, or other substantial laboratory abnormalities as judged by each investigator. We also excluded patients who needed ongoing therapy with selective serotonin reuptake inhibitors, tricyclic antidepressants, serotonin 5-hydroxytryptamine 1 receptor agonists, meperidine, buspirone, monoamine oxidase inhibitors, sympathomimetic agents (eg, pseudoephedrine), vasopressive agents (eg, epinephrine and norepinephrine), or dopaminergic agents (eg, dopamine and dobutamine).

Randomisation

Patients were randomly assigned at a 1:1:1 ratio into three groups. A minimisation method was used for patient allocation to minimise the imbalance between the numbers of patients in each treatment group over several stratification factors: the institution, presence or absence of cavitation on chest radiographs at baseline, and presence or absence of diabetes. Randomisation was done and disclosed to investigators by an independent statistician using the centralised, internet-based system of the Medical Research Collaborating Center of Seoul National University Hospital (Seoul, South Korea).

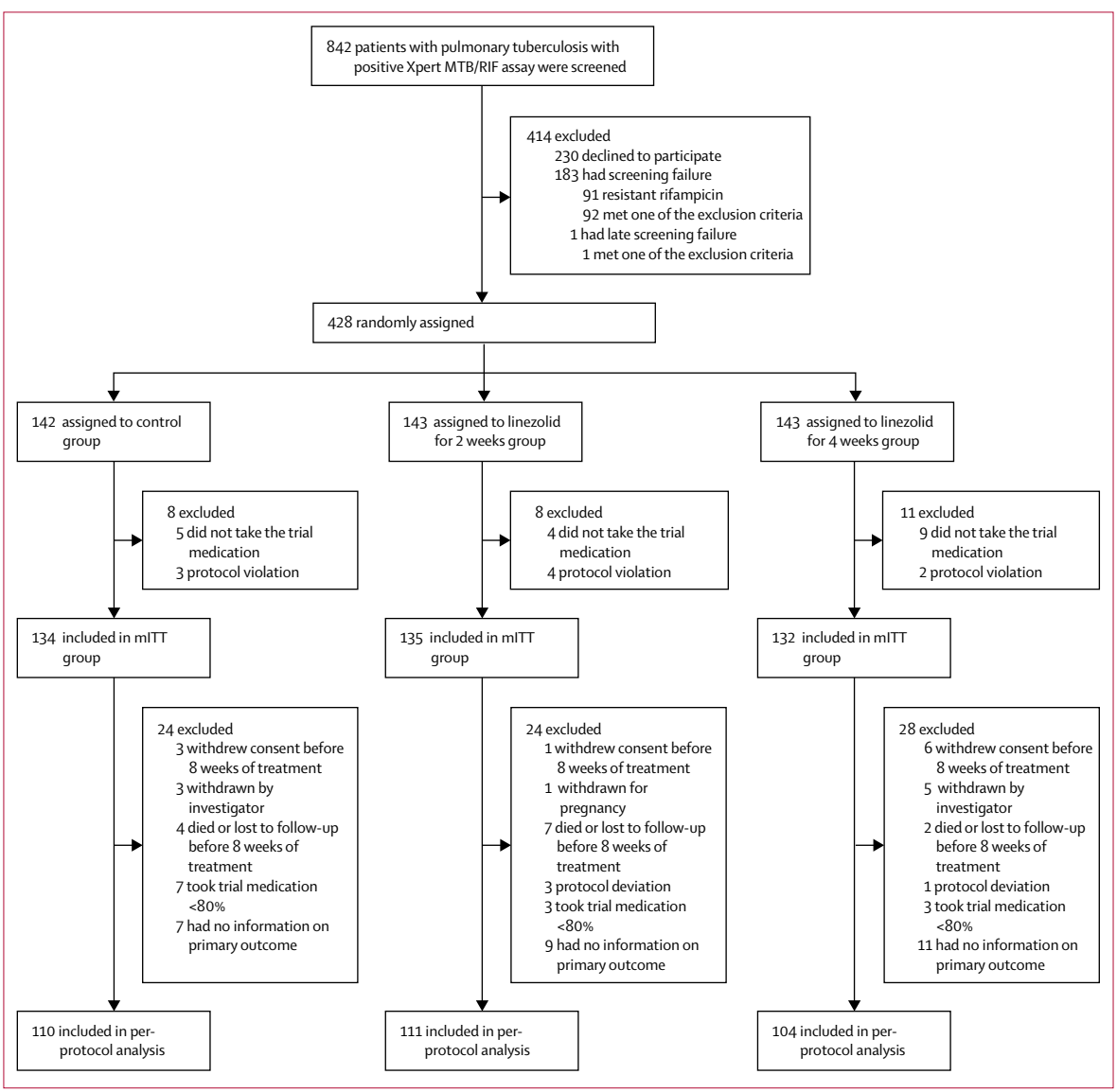

Figure 1: **Trial profile**
mITT=modified intention to treat.

Treatment adherence was evaluated during each visit by research nurses who kept track of packages and returned drugs and recorded the unused pill count. If scheduled visits were delayed or cancelled, the study team attempted to contact patients as soon as possible by telephone.

Procedures

Patients in the control group treated with a standard regimen for drug-susceptible pulmonary tuberculosis used isoniazid (6 months), rifampicin (6 months), pyrazinamide (2 months), and ethambutol (2 months) as recommended by Korean National Guidelines.[15]

Patients in the linezolid 2 weeks group were treated with isoniazid (6 months), rifampicin (6 months), pyrazinamide (2 months), and linezolid (600 mg/day, 2 weeks), instead of ethambutol for 2 months.

Patients in the linezolid 4 weeks group were treated with isoniazid (6 months), rifampicin (6 months), pyrazinamide (2 months), and linezolid (600 mg/day, 4 weeks), instead of ethambutol for 2 months.

Clinical and laboratory evaluations were carried out at baseline, and week 1, 2, and 4, and then every 4 weeks until 24 weeks after treatment initiation. One sputum sample was obtained for acid-fast smear and culture in liquid and solid media at each visit. Drug-susceptibility tests were requested with the first culture of *M tuberculosis*. Complete blood count as well as levels of total bilirubin, aspartate transaminase, alanine transaminase, and creatinine were measured at each visit. Optic tests using Ishihara plates were done at baseline, and weeks 1, 2, 4, and 8 weeks after randomisation. Development of peripheral neuropathy was screened through history, analysing patients' symptoms. On every visit, patients were asked about paraesthesia, numbness, tingling, pain, and weakness in feet and hands by investigators and research nurses (appendix).

M tuberculosis isolates from the patients who had not had conversion to culture negativity at 8 weeks of treatment were subjected to determination of the minimal inhibitory concentration (MIC) and nucleotide sequence analyses to investigate the development of resistance to linezolid.

We determined the MIC values of the isolates using the resazurin microtitre assay plate method (appendix).[16] In addition, three genes, *rrl*, *rplC*, and *rplD*, previously known to be associated with linezolid resistance in *M tuberculosis* and other bacteria,[13] were analysed by Sanger sequencing of DNA fragments amplified by PCR. The primer pairs used for PCR amplifications were: gatagtggttgcgagcatca/catgtccctgactcgca, aagcctaaatactcctcga/accacaaggtggatgtg, atgtgcagtcgcaaaga/ccgagttccttaaccat, ggtgatcctctgctgccaa/tcgcagtcaagctcccttg, and ttagggacagtgcctgg/gcaaatttgttctggtg for *rrl*; gacatcatcgatcccacgcc/ggcgtcttgacgtcgatttt for *rplC*; and cataaggtcgatgccgagaa/ccagcaacccataggatttc for *rplD*.

Outcomes

The primary efficacy endpoint was the proportion of patients with negative culture conversion of sputum in liquid media (MGIT tube; Becton-Dickinson and Co, Sparks, MD, USA) after 8 weeks of treatment. Secondary efficacy endpoints were: the proportion of patients with sputum culture conversion on solid media (Ogawa medium, Shinyang Diagnostics, Seoul, South Korea) after 8 weeks of treatment, the interval from enrolment to sputum culture conversion in liquid and solid media, the proportion of cured patients, and the proportion of patients with treatment success. Treatment success was the achievement of a cure or treatment completed according to the Korean TB Guidelines,[15] which are similar to those of WHO,[17] as follows: cure, a negative sputum culture after (or during the last months of) treatment combined with one or more previous negative sputum cultures; treatment completed, a negative sputum culture after or at the end of treatment, but without a previous negative sputum culture; or a negative sputum culture during treatment, but no negative sputum culture after or during the last month of treatment.

We defined culture conversion as two consecutive negative sputum cultures in liquid or solid media. The date of culture conversion was defined as the date of the initial negative culture. Negative sputum cultures followed by contaminated cultures without subsequent positive culture were also regarded as culture conversion. Culture conversion was also defined as a patient who could not expectorate sputum after one negative sputum culture.

Safety endpoints were grade greater than or equal to 3 AEs and serious AEs according to the Common Terminology Criteria for Adverse Events (version 4.03)[18] that were considered possibly, probably, or definitely related to the study drug.

Statistical analysis

See Online for appendix

Our hypothesis was that the use of linezolid instead of ethambutol increases the proportion of sputum culture conversion in liquid media after 8 weeks of anti-tuberculosis treatment. A primary comparison was made between the control group and the linezolid 4 weeks group. The proportion of sputum culture conversion in liquid media at 8 weeks in patients who were treated at Seoul National University Hospital is about 75%.[19] On the basis of an α=0·05 level of significance, a power of 85%, and a 15% difference in the culture conversion rate after 8 weeks between the control group and the linezolid 4 weeks group (75% *vs* 90%), we estimated a sample size per arm of 114 (342 in total) with a two-sided Z test without continuity correction. The difference in the proportion of culture conversion after 8 weeks of 15% was assumed on the basis of previous phase 2 trials.[20,21] After consideration of a proportion of 10% default and 10% culture failure in patients with a positive Xpert MTB/RIF assay result, the final number per group was calculated to be 143 (429 in total).

The results of this trial were analysed primarily in the modified intention-to-treat (mITT) population. This

population comprised participants who satisfied the inclusion and exclusion criteria, those who were randomly assigned to a group, and those who took the trial drug at least once. If the information on the results of mycobacterial culture was unavailable at some point, it was considered that they remain without reaching the negative conversion of sputum culture for the missing imputation. Per-protocol analyses were done secondarily. The per-protocol groups comprised patients from the mITT population satisfying the following conditions: patients took the trial drug (ethambutol or linezolid) at greater than or equal to 80% of the planned dose until the point of the primary endpoint estimation; patients who completed the clinical trial according to the protocol. Sensitivity analyses were done based on patients with no resistance to isoniazid, rifampicin, ethambutol, or pyrazinamide. In addition, subgroup analyses according to sputum smear positivity at baseline visit were done. Safety analyses were done based on the safety population, which consisted of patients who took the trial drug at least once. Patients who took ethambutol and linezolid during the treatment course were excluded from the safety group. Patients randomly assigned to the linezolid 4 weeks group, but who took ethambutol and linezolid for less than or equal to 18 days, were included in the linezolid 2 weeks group in safety analyses. No interim analyses of the data were planned. As post-hoc analyses, proportions of culture conversion at 2 weeks and monthly were summarised and compared.

The proportion of culture conversion was estimated as the proportion and differences between the control group and investigational groups. It was established using the χ^2 test or Fisher's exact test. Comparisons were made by two-tailed tests with a significance of 5%. The confidence interval for the proportional difference was estimated using the Wald confidence interval without continuity correction.

The median time to culture conversion was calculated in each group with the Kaplan-Meier method and compared by use of a log-rank test. Cure, treatment completed, and treatment success proportions were compared between the three groups using the χ^2 test or Fisher's exact test. For safety endpoints, frequencies and fractions of total AEs, grade 3 or greater AEs, and serious AEs were described. In addition, the relatedness of AEs to the trial drug and their seriousness was summarised. Frequencies of each AE were compared with the χ^2 test or

	Control group (n=134)	Linezolid 2 weeks group (n=135)	Linezolid 4 weeks group (n=132)	All patients (n=401)
Demographics				
Men	95 (71%)	93 (69%)	89 (67%)	277 (69%)
Women	39 (29%)	42 (31%)	43 (33%)	124 (31%)
Age (years), median (IQR)	56 (43–67)	56 (40–70)	55 (38–66)	55 (41–68)
Body-mass index (kg/m²), median (IQR)	20·9 (18·6–22·4)	20·4 (19·0–22·4)	21·0 (18·7–23·5)	20·8 (18·8–22·7)
Comorbidities				
History of previous tuberculosis	26 (19%)	23 (17%)	19 (14)	68 (17)
Diabetes	27 (20%)	28 (21%)	24 (18%)	79 (20%)
Positive HIV antibody	0/132 (0%)	0/135 (0%)	2/130 (2%)	2/397 (<1%)
Bacteriological examinations				
Acid-fast staining of sputum				
Negative	75 (56%)	90 (67%)	92 (70%)	258 (64%)
Trace	4 (3%)	3 (2%)	1 (1%)	9 (2%)
≥1	14 (11%)	13 (10%)	15 (11%)	42 (11%)
≥2	14 (11%)	13 (10%)	8 (6%)	36 (9%)
≥3	11 (8%)	10 (7%)	8 (6%)	26 (7%)
≥4	16 (12%)	6 (4%)	8 (6%)	30 (8%)
Positive MTB culture of sputum on liquid media	101 (78%)	101 (78%)	83 (67%)	285 (74%)
Positive MTB culture of sputum on solid media	104 (78%)	104 (77%)	90 (69%)	298 (75%)
Drug resistance				
Isoniazid	6 (4%)	3 (2%)	4 (3%)	13 (3%)
Rifampicin	1 (1%)	0 (0%)	0 (0%)	1 (<1%)
Pyrazinamide	2 (1%)	0 (0%)	0 (0%)	2 (<1%)
Radiographical examinations				
Bilateral involvement	60 (45%)	62 (46%)	49 (37%)	171 (43%)
Presence of cavity	56 (42%)	49 (36%)	54 (41%)	159 (40%)

MTB=*Myobacterium tuberculosis*.

Table 1: **Baseline characteristics of the patients included in modified intention-to-treat analyses**

Fisher's exact test. All analyses were done with SAS (version 9.2) software package.

The trial is registered with ClinicalTrials.gov, number NCT01994460.

Role of the funding source

The sponsor of the study, the Ministry of Health and Welfare, South Korea, had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 19, 2014 and Jan 13, 2017, a total of 429 patients were enrolled: 170 at Seoul National University Boramae Medical Center, 107 at Seoul National University Hospital, 93 at the National Medical Center, and 59 at Seoul National University Bundang Hospital. Except for one patient with late screening failure, 428 patients were randomly assigned a group. A total of 401 were included in mITT analyses. The most common reason for exclusion of patients from mITT analyses was non-adherence to medication (18 patients). A total of 325 patients were included in per-protocol analyses. The

	Control group	Linezolid 2 weeks group	Linezolid 4 weeks group
Modified intention-to-treat analysis			
Patients with culture conversion	103/134 (77%)	111/135 (82%)	100/132 (76%)
Difference (95% CI)	ref	5·4 (−4·3 to 15·0)	−1·1 (−11·3 to 9·1)
p values	ref	0·28*	0·83*
Per-protocol analysis			
Patients with culture conversion	90/110 (82%)	106/111 (96%)	94/104 (90%)
Difference (95% CI)	ref	13·7 (5·5 to 21·9)	8·6 (−0·6 to 17·7)
p values	ref	0·001*	0·07*

*χ^2 test. Data are n/N (%) or difference (95% CI), unless otherwise stated.

Table 2: **Primary outcome (sputum culture negativity on liquid media at completion of 8 weeks of treatment) classification according to treatment group for modified intention-to-treat and per-protocol analyses**

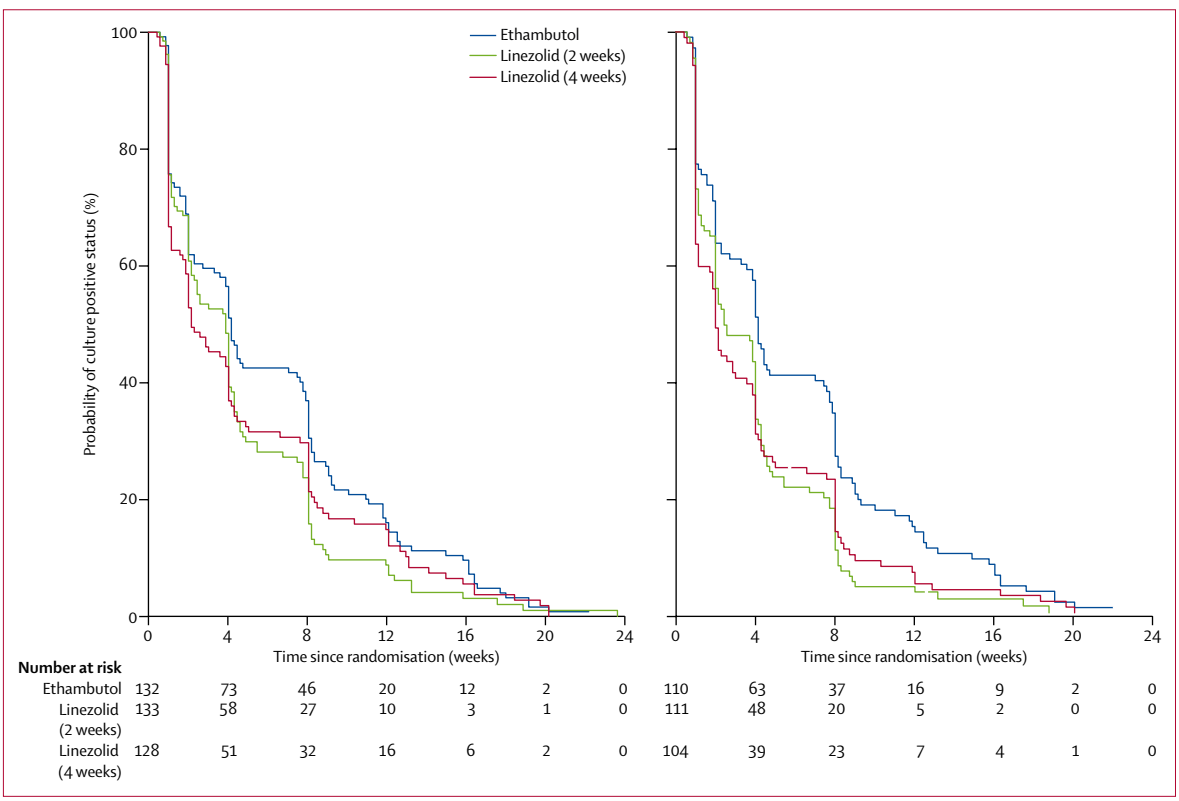

Figure 2: **Kaplan-Meier estimates of the time to conversion from enrolment to culture-negative status in liquid media**
(A) Modified intention-to-treat analysis. (B) per-protocol analysis.

most common reason for exclusion from per-protocol analyses was absence of information on the primary outcome (27 patients; figure 1). The baseline characteristics of patients were similar across all three groups except for culture positivity at enrolment. The proportion of patients with positive *M tuberculosis* culture in liquid media at enrolment was 78% in both the control group and the linezolid 2 weeks group and 67% in the linezolid 4 weeks group (table 1).

In mITT analyses, negative cultures in liquid media at 8 weeks of treatment were achieved in 103 (77%) of 134 patients in the control group, 111 (82%) of 135 in the linezolid 2 weeks group, and 100 (76%) of 132 in the linezolid 4 weeks groups. The difference in the proportion of culture conversion was 5% (95% CI –4·3 to 15·0, p=0·28) between the linezolid 2 weeks group and control group, and –1% (–11·3 to 9·1, p=0·83) between the linezolid 4 weeks group and control group (table 2).

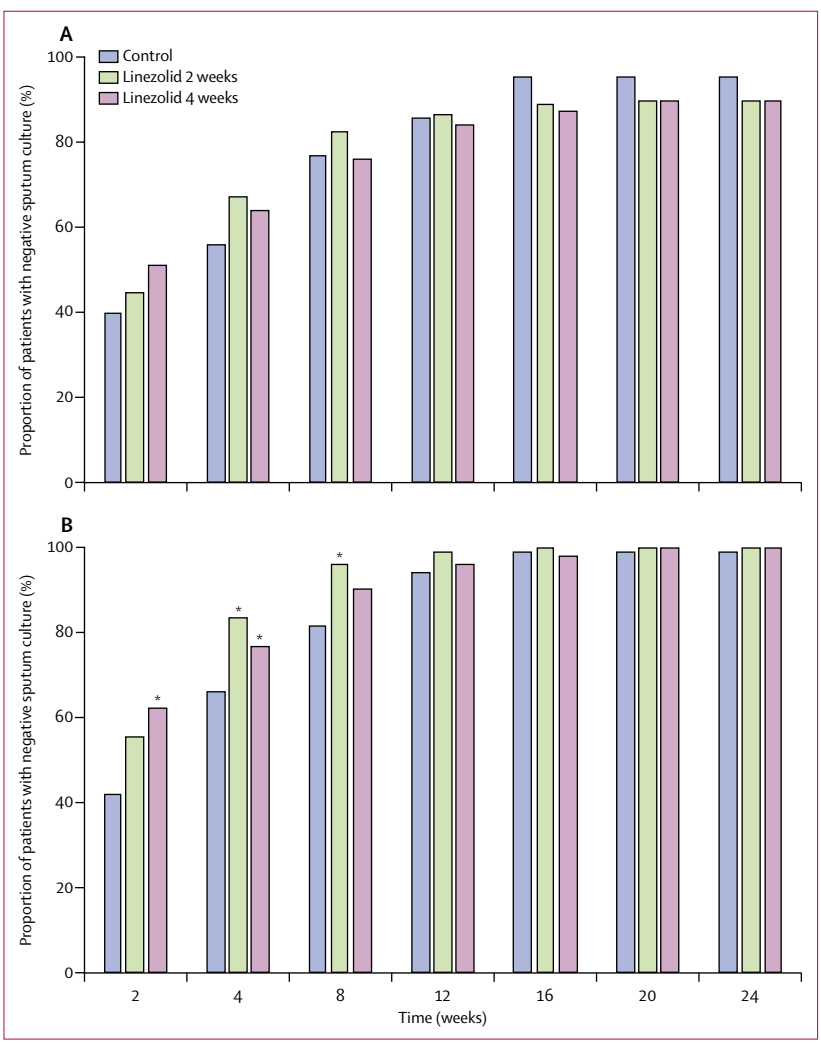

Figure 3: **Comparison of 2 weeks and monthly culture negativity in liquid media**
(A) Modified intention-to-treat analysis. (B) Per-protocol analysis. *p<0·05.

In the per-protocol analyses, negative cultures in liquid media at 8 weeks of treatment were observed in 90 (82%) of 110 patients in the control group, 106 (96%) of 111 in the linezolid 2 weeks group, and 94 (90%) of 104 in the linezolid 4 weeks groups. The difference in the proportion of culture conversion was 14% (95% CI 5·5–21·9, p=0·001) between the linezolid 2 weeks group and the control group, and 8·6% (–0·6 to 17·7, p=0·07) between the linezolid for 4 weeks group and the control group (table 2).

Sensitivity analyses excluding patients with resistance to any of isoniazid, rifampicin, ethambutol, and pyrazinamide (three in the control, three in the linezolid 2 weeks, and four in the linezolid 4 weeks groups) as well as subgroup analyses according to the sputum smear positivity at the baseline visit, showed similar results (appendix).

The proportion of culture conversion on solid media at 8 weeks of treatment was not different between the control group and linezolid 2 weeks or linezolid 4 weeks group (appendix). The median time to culture conversion from randomisation in liquid media was 29 days (IQR 8, 63) in the control group, 27 days (8–54, p=0·03) in the linezolid 2 weeks group, and 15 days (7–56, p=0·12) in the linezolid 4 weeks group in mITT analyses (figure 2A). In the per-protocol analyses, the median time to culture conversion in liquid media was 29 days (IQR 11–58), 17 days (7–33, p=0·0009), and 14 days (7–40·5, p=0·008), respectively (figure 2B).

Proportion of cure was lower in the linezolid 4 weeks group (56·8%, p=0·046) than in the control group (68·7%), but not in the linezolid 2 weeks group (65·9%, p=0·63) in the mITT population. However, the proportions of treatment success were not different among treatment groups (appendix). In per-protocol analysis, proportions of cure as well as of treatment success were similar (appendix).

We carried out post-hoc analyses comparing 2 weeks and monthly culture negativity in liquid media between groups. In the linezolid 2 weeks group, sputum cultures were more likely to be negative than in the control group at earlier time points (2 weeks, 4 weeks, and 8 weeks), especially in per-protocol analyses. Among the linezolid 4 weeks group in per-protocol analyses, a higher proportion of culture conversion was identified at 2 weeks and 4 weeks of treatment, but was not maintained from 8 weeks (figure 3; appendix).

A total of 396 patients were included in the safety analyses: 137 in the control group, 138 in the linezolid 2 weeks group, and 121 in the linezolid 4 weeks group. There was no significant difference between groups for the number of patients with at least one grade greater than or equal to 3 AEs (p=0·89), in nine (6·6%) patients in the control group, ten (7·2%) patients in the linezolid 2 weeks group, and seven (5·8%) patients in the linezolid 4 weeks group. Optic neuropathy was not identified in any patient and numbers of patients who had paraesthesia

and polyneuropathy were similar across groups. In addition, the numbers of patients who experienced haematological abnormalities were small and similar among the three groups (table 3).

Five patients in the linezolid 2 weeks group and ten patients in the linezolid 4 weeks group did not convert to culture negativity at 2 months of treatment. Among them, 13 patients (five in the linezolid 2 weeks group and eight in linezolid 4 weeks group) were investigated for the development of resistance to linezolid using culture isolates at greater than or equal to 8 weeks. None of the isolates were confirmed to have developed definite resistance to linezolid according to MIC measurement. 11 isolates showed a MIC of 0·5 mg/mL, which is in the MIC range of susceptible strains reported earlier.[13] A marginal MIC value, 1·0 mg/mL, was observed in two isolates. The baseline isolates from the respective patients, however, showed the same MIC value, suggesting there had been no change in the resistance level. In accordance with the results based on MIC values, no mutational change was identified by sequence analyses of the three genes, *rrl*, *rplC*, and *rplD*, known previously to be associated with linezolid resistance.

	Control group (n=137)	Linezolid 2 weeks group (n=138)	Linezolid 4 weeks group (n=121)
At least one adverse event	86 (63%)	79 (57%)	75 (62%)
At least one grade 3 or greater adverse event	9 (7%)	10 (7%)	7 (6%)
At least one grade 3 or greater adverse event, considered probably related or definitely related	2 (2%)	4 (3%)	4 (3%)
Serious adverse events	14 (10%)	21 (15%)	11 (9%)
Serious adverse events, considered probably related or definitely related	4 (3%)	5 (4%)	4 (3%)
Death			
Any	1 (0·7%)	1 (0·7%)	1 (0·8%)
Tuberculosis related	0 (0%)	0 (0%)	0 (0%)
Patients with selected symptoms (any grade)			
Optic neuropathy*	0	0	0
Paraesthesia	5 (4%)	2 (2%)	7 (6%)
Polyneuropathy	2 (2%)	0 (0%)	0 (0%)
Anaemia	0 (0·0%)	0 (0·0%)	1 (1%)
Leukopenia	1 (1%)	0 (0·0%)	1 (1%)
Thrombocytopenia	1 (1%)	1 (1%)	0 (0·0%)
Hepatotoxicity†	5 (4%)	1 (1%)	4 (3%)
Nausea	13 (10%)	15 (11%)	19 (16%)
Vomiting	4 (3%)	9 (7%)	5 (4%)
Diarrhoea	2 (2%)	5 (4%)	7 (6%)
Arthralgia	10 (7%)	5 (4%)	11 (9%)
Pruritis	21 (15%)	16 (12%)	23 (19%)
Urticaria	2 (2%)	2 (2%)	3 (3%)

Data are n (%). *Defined when patients read incorrectly 3 or more of 24 Ishihara's plates in subsequent tests compared with the baseline test. Subsequent tests were missing in 9 patients. †Defined as an increase in the level of aspartate transaminase or alanine transaminase >5 times the upper normal limit at any time during treatment.

Table 3: **Safety analyses**

Discussion

We showed that substitution of 8 weeks of ethambutol with linezolid for 2 weeks or 4 weeks did not affect negative conversion of sputum culture at 8 weeks of anti-tuberculosis treatment in terms of the primary endpoint in the mITT population. However, in the per-protocol analyses, the linezolid 2 weeks group (95·5%) and the 4 weeks group (90·4%) showed higher proportions of culture conversion than in the control group (81·8%). The proportions of AEs across groups were similar.

The higher and faster proportions of sputum culture conversion observed among patients in the investigational arms with the per-protocol analyses, but not in mITT analyses could be explained by the different attrition of patients between groups. This was an open-label trial and the effectiveness and safety of conventional treatment regimens is well known. Hence, patients assigned to use a linezolid-containing regimen (especially the linezolid 4 weeks group) could withdraw consent or default readily if they had AEs. Similarly, investigators could withdraw patients randomly assigned to a linezolid group more readily if their treatment response was not satisfactory. In fact, instances of patients withdrawing consent or being withdrawn by the investigator were most common in the linezolid 4 weeks group (figure 1). Furthermore, the conservative imputation method for missing values for the primary outcome could decrease proportion of culture conversion at 8 weeks of treatment, especially for the linezolid 4 weeks group.

The highest proportion of culture conversion at 8 weeks of treatment (95·5%) was observed in the linezolid 2 weeks group by per-protocol analyses. However, this proportion still falls short of the 99% and 97% proportions of culture conversion of new treatment regimens with shortened duration of 4 months or 5 months, as suggested by a meta-analysis of 24 studies.[22] Given that linezolid-including regimens yielded higher proportions of culture conversion at 8 weeks, as well as at earlier time points, linezolid could be considered for new shorter regimens for drug-susceptible tuberculosis. Various combinations, including linezolid and other existing or newly developed anti-tuberculosis drugs, could be tested in the future.

The differences in the proportions of culture conversion at 8 weeks were more obvious in per-protocol analysis than in mITT analyses. In particular, the proportion of higher culture conversion at 8 weeks of treatment was more prominent in the linezolid 2 weeks group than in the 4 weeks group. Among the 4 weeks group, higher proportions of culture conversion were identified at earlier time points but were maintained until 8 weeks of treatment even in per-protocol analyses. The reason for the absence of efficacy or inferior results of linezolid use beyond 2 weeks merits explanation. It is well known that rifampicin is a strong inducer of the cytochrome P450

system and results in a decrease in the serum concentration of many drugs.[23] The accelerated clearance of drugs by co-administration of rifampicin increases rapidly up to 2 weeks and is maintained thereafter.[24] Administration of linezolid with rifampicin beyond 2 weeks might be redundant in terms of efficacy. However, the absence of pharmacokinetic analysis among participants in this trial prevented further investigation.[25] Better understanding is needed of synergism and antagonism between linezolid and rifampicin according to the stages of tuberculosis treatment.

Although linezolid is highly effective for MDR-tuberculosis treatment, its long term use is frequently accompanied by AEs such as peripheral neuropathy and bone marrow suppression. In a previous trial, as many as 87% of XDR-tuberculosis patients reported clinically significant AEs.[13] In addition, a meta-analysis reported AEs in 58·9% of MDR or XDR-tuberculosis patients who used linezolid.[12] In our trial, the proportions of AEs were similar between the three groups. Contrary to results from studies on patients with MDR or XDR-TB, our trial showed that use of linezolid (600 mg/day) for 2 weeks or 4 weeks with isoniazid, rifampicin, and pyrazinamide in the intensive phase of anti-tuberculosis treatment was safe in terms of AEs. A much shorter duration of linezolid use compared with that among patients with MDR-TB, as well as induction of linezolid metabolism by concomitant use of rifampicin,[26] could explain this safety profile.

The emergence of resistance to linezolid has been reported among patients with MDR or XDR-TB.[13] However, linezolid resistance was not identified in 15 patients who did not culture convert until 8 weeks of treatment. Given that linezolid resistance was confirmed after 12–26 weeks of linezolid use among patients with XDR-tuberculosis in previous trials,[13] our results suggest that 2 weeks or 4 weeks of linezolid use with active accompanying drugs have strong sterilising activities in patients with tuberculosis, potentially protecting against the emergence of resistance.

To appreciate the results of this trial correctly, we should take into account issues regarding the dose and duration of linezolid use. Because the pharmacokinetic and pharmacodynamic data for linezolid among patients with tuberculosis are scarce,[25] various dosing schedules are adopted in ongoing clinical trials for MDR-TB (eg, 600 mg twice daily for NiX-TB [NCT02333799]; 600 mg once daily for 2 months, 300 mg daily thereafter for MDR-END [NCT02619994]; 600 mg once daily for 4 months, 300 mg daily or intermittent dosing thereafter for EndTB [NCT02754765]).[9] Our decision on the dose of linezolid, 600 mg/day, was primarily based on our experience among patients with MDR-TB, either in real practice[27] or in a clinical trial setting.[13] Although the hollow fibre system model[28] proposed that half a dose of linezolid (300 mg/day) could be effective in patients with MDR-tuberculosis, we chose the dose of 600 mg/day taking into account the reduced concentration of linezolid from co-administration with rifampicin.[26]

Longer use of linezolid, (eg, for 8 weeks) might provide different results. The duration of linezolid use (ie, 4 weeks as a primary comparison) for this trial was determined by considering the drug–drug interaction between rifampicin and linezolid as well as minimising the possibility of AEs. Given that little is known about the sterilising activity of linezolid in humans, we prioritised the safety of the participants in designing this trial because we were set to compare the new regimen with the previously established effective regimen for drug-susceptible tuberculosis. We decided on a duration of administration of linezolid of 4 weeks for this trial, which is the permitted duration of its use.

In addition, the proportion of culture conversion at 8 weeks of treatment as a primary outcome for this study should be reappraised. It has been proposed as a risk factor for relapse in patients with tuberculosis[29] as well as a predictor for the proportion of relapse of a specific regimen.[22] However, none of those 4-month treatment regimens including fluoroquinolones, motivated by phase 2 data adopting proportions of culture conversion at 8 weeks as a primary outcome, showed non-inferiority to the current 6-month regimen.[5–8] Data from more reliable animal model studies and information regarding lesion penetration of anti-tuberculosis drugs should be incorporated in the design of clinical trials in the future.[30]

In conclusion, we did not observe higher proportions of culture conversion at 8 weeks of treatment with 2 weeks or 4 weeks of use of linezolid instead of ethambutol during the intensive phase of anti-tuberculosis treatment in mITT analyses. However, per protocol, safety analyses, and the resistance profile, suggested the potential role of short-term use of linezolid in shortening of treatment for drug-susceptible tuberculosis.

Contributors

J-JY conceived the study and participated in the design of the study. SH, JHL, and DKK contributed to the design of the study. HA and HSK constructed a database for this study and screened data. YRH, HK, JK, and HSC participated in data collection. J-KL, JYL, DKK, HIY, IJ, EYH, YSP, YSJ, SSP, JSP, JK, S-ML, J-SJ, C-HL, JL, SMC, J-HP, SHL, Y-JC, YJL, SJK, NK, YRH, HK, JK, JNL, HSC, ML, TS, JH, SH, and J-JY participated in data collection, analysis, and interpretation. All the authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health and Welfare, South Korea (HI13C0844). Linezolid (Zyvox) was donated by Pfizer Inc (NY, USA).

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