JAC Antimicrob Resist
https://doi.org/10.1093/jacamr/dlaf072

JAC-Antimicrobial Resistance

High-dose isoniazid for TB with low-to-moderate isoniazid resistance after 1 week of treatment

U.D. Ranganathan[1], S.E. Smith-Jeffcoat[2], S. Balaji[1], K. Devika[1], D. Nair[1], B. Devaleenal Daniel[1], R. Srinivasan[1], M. Baskaran[1], D. RaviKumar[1], D. Sangamithirai[1], G. Radhika[1], G. Mangalambal[1], J. Lavanya[3], K. Maheswaran[4], S. Kumar[4], R. Sridhar[4], S. Tripathy[1], S. Swaminathan[1], K.D. Eisenach[5] and J.P. Cegielski[2,6]*

[1]Departments of Microbiology, Immunology and Clinical Research, Indian Council for Medical Research–National Institute of Research for Tuberculosis, Chennai, India; [2]Division of Global HIV and TB, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA; [3]State Tuberculosis Cell, Greater Chennai Corporation, Chennai, Tamil Nadu, India; [4]Government Hospital for Thoracic Medicine, Tambaram, Tamil Nadu, India; [5]TB or Not TB Consulting, LLC, Little Rock, AR, USA; [6]Rollins School of Public Health, Emory University, Atlanta, GA, USA

*Corresponding author. E-mail: peter.cegielski@emory.edu

Received 10 November 2024; accepted 17 February 2025

Objectives: To evaluate the effect of high-dose isoniazid in patients with isoniazid-resistant TB by its bactericidal activity after 1 or more weeks of treatment.

Subjects and methods: Using the rapid direct method of phenotypic drug susceptibility testing, we screened persons with positive sputum microscopy results and genotypic drug resistance for isoniazid resistance. Those with no growth at a critical concentration of 2.0 mg/L were invited to participate in a trial of high-dose isoniazid monotherapy lasting 6 days. After 3 days of no treatment, patients received isoniazid 15 mg/kg and were followed with serial quantitative sputum cultures from Days 0 to 6.

Results: We enrolled 15 patients after a median of 2 weeks standard first-line treatment. Their median bacillary count on Day 0 was 4.9 $\log_{10}$ cfu/mL on solid agar, and the time to detection (TTD) was 200 h in liquid medium. Neither metric showed meaningful change in bacillary burden over 6 days, declining by a non-significant 0.08 $\log_{10}$ cfu/mL/d on solid media and slowing TTD by 23 h. These effects did not differ by degree of isoniazid resistance or specific *Inhibin Subunit Alpha* (*inhA)* gene mutations.

Conclusions: The utility of high-dose isoniazid against low-level isoniazid resistance beyond the first 2 weeks of chemotherapy should be reconsidered.

Introduction

Treatment of drug-susceptible TB is effective and safe. Treatment of drug-resistant TB is difficult, and outcomes are not as good.[1] Resistance to isoniazid is the most common type, affecting an estimated 1.4 million people per year (~13.1% of incident cases).[2]

Isoniazid is exceptional in its early bactericidal activity (EBA), killing ≥99% of susceptible bacilli in the first 2 days.[3] After that, its bactericidal activity slows one $\log_{10}$ to a pace like other first-line drugs, ~0.11 $\log_{10}$ cfu/day.[4] Because multidrug treatment is mandatory for TB, EBA studies were developed in the late 1970s to measure the effect of a single agent by delaying treatment for 2–14 days while administering the experimental drug and monitoring its effect with serial quantitative sputum cultures. In previous work, we observed that 43% (554/1278) of initial isolates from MDR TB patients remained susceptible to isoniazid at high concentrations of 5.0 mg/L on Middlebrook agar despite resistance at standard concentrations of 0.2 and 1.0 mg/L.[5–7] At higher doses of 10–15 mg/kg, peak serum concentrations exceed 10–15 mg/L, remaining above 5 mg/L for several hours.[8] Favourable pharmacokinetics and *in vitro* activity, however, do not prove clinical efficacy. To investigate the potential for higher doses of isoniazid to treat patients whose isolates remain susceptible at this higher concentration in a manner that resembles clinical practice, we developed a novel variant on EBA study methodology in which patients started standard first-line treatment immediately and, at the same time, rapid phenotypic drug susceptibility testing (DST) using the direct method (directly from sputum, not from a cultured isolate) was carried out. Patients were recruited into the study once these DST results demonstrated isoniazid resistance.

Subjects and methods

Public sector TB clinic patients in Chennai, India, were invited to participate if they had ≥2+ acid-fast bacilli (AFB) microscopy results from unconcentrated sputum plus rapid molecular results (GenoType MTBDR*plus*; Hain Lifescience GmbH, Nehren, Germany) indicating isoniazid resistance. After informed consent, sputum specimens ≥7 mL had phenotypic DST directly from sputum in MGIT 960 (Becton Dickinson, Franklin Lakes, NJ, USA) at the National Institute of Research for Tuberculosis (NIRT). They were treated according to national guidelines while awaiting DST results (Table 1).

Patients were eligible if their *Mycobacterium tuberculosis* isolates were susceptible to isoniazid at 2.0 mg/L, resistant at 0.1 or 0.4 mg/L, and sputum microscopy remained positive. Due to changes in national policies near the end of the enrolment period, we added a criterion that patients could be enrolled based on GenoType MTBDR*plus* results showing mutations associated with isoniazid resistance in the *Inhibin Subunit Alpha (inhA)* gene or its promoter region. Exclusion criteria included: age <18 years, HIV-positive with CD4 count <50 cells/μL, pregnant/lactating women, oxygen tension <90%, resting respiratory rate >25 breaths per minute, Karnofsky score <60, hepatic enzyme level >3× the upper limit of normal, estimated glomerular filtration rate <60 mL/min/1.73 m^2, or unable to provide an adequate sputum specimen.

Table 1. Baseline characteristics of 15 patients having serial quantitative sputum cultures over 6 days of high-dose isoniazid monotherapy

Characteristic	*n*	Median (min–max)
Age, y	15	50 (33–63)
Male sex	14	
BMI, kg/m^2	15	16.8 (12.3–24.8)
Karnovsky score:		
80	6	
70	6	
60	3	
Chest X-ray (unilateral/bilateral):		
Infiltrates	3/12	
Cavities	2/1	
Fibrosis	5/6	
Treatment before enrolment		
INH, RIF, Z, E	6	19 days (13–180)
INH, RIF, Z, E, STR	5	20 days (19–42)
RIF, Z, E, STR	1	28 days
INH, RIF, Z, E, KAM, MXF	2	5 and 18 days
RIF, E, Z, MXF	1	5 days
Days of standard first-line treatment before enrolment	15	19 days (5–180)
AFB sputum microscopy		
1+	2	
2+	8	
3+	5	
Isoniazid-resistant at indicated mg/L		
0.1	10	
0.4	2	
2.0	1	
Contaminated/insufficient growth	2	
*inh*A mutations		
MUT1	12	
MUT3a	2	
No mutation detected	1	
Baseline $\log_{10}$ cfu/mL		4.9 (3.3–5.6)
Baseline TTD, h	13	168 (65–359)
'Baseline' specimen not timely	2	

E, ethambutol; INH, isoniazid; KAM, kanamycin; MXF, moxifloxacin (quinolone); RIF, rifampicin; STR, streptomycin; Z, pyrazinamide.

Once DST results were reported, anti-TB drugs were stopped for a median of 4 days (IQR 3–6). After this 'washout' period, a baseline 16 h overnight sputum specimen was collected the night preceding the first dose of isoniazid monotherapy. Patients received 600 mg/day (<45 kg) or 900 mg/day (≥45 kg) directly observed for 6 days. Serial 16 h overnight sputum specimens were collected and transferred on ice to the NIRT. After the last specimen was collected, the patient resumed standard therapy. Follow-up DST at 1 and 2 months tested for acquired isoniazid resistance.

Safety was assessed by history, physical examination and laboratory tests before starting monotherapy, then again on Day 5, including complete blood count, biochemistry panel, electrolytes, serum creatinine and hepatic enzyme levels. In addition, screening for enrolment included HIV testing with CD4 counts and pregnancy test.

Direct method MGIT 960 phenotypic DST

Sputum was processed with standard decontamination procedures (1.5% final NaOH concentration).[9] Resuspended sediment (0.5 mL) was inoculated into MGIT tubes (BD, Sparks, Maryland), a drug-free growth control and three concentrations of isoniazid (0.1, 0.4 and 2.0 mg/L) and incubated in the MGIT 960 as per DST protocol. Once the growth control turned positive, results of the isoniazid tubes were interpreted as susceptible if the growth unit was <100 and resistant if it was ≥100.

Overnight sputum specimen collection and processing

On Days 0, 1, 2, 4 and 6 patients collected sputum overnight for 16 h. It was retrieved at 08:00, transported to the NIRT on ice and processed. Sputa were homogenized with 1:10 proportion by volume of 0.1% dithiothreitol (DTT; final concentration 0.01% DTT) by vortex for 30 s and shaken mechanically at 60 rpm for 15–20 min.

Quantitative culture on Middlebrook 7H11S agar

Middlebrook 7H11S selective medium contained amphotericin B (5 μg/mL), carbenicillin (25 μg/mL), polymyxin B (100 units/mL) and trimethoprim (10 μg/mL). Five serial 10-fold dilutions of the homogenized sputum specimen were prepared in PBS. Homogenized, undiluted sputum and all five dilutions from each sample (100 μL) were inoculated on each side of duplicate 7H11S biplates. Plates were sealed with carbon dioxide–permeable tape and incubated with CO_2 (5%–10%) at 37°C. Plates were examined weekly for 6 weeks to count visible

colonies. Once growth stopped or 6 weeks passed, the dilution with 50–150 colonies was chosen as most reliable. The average number of colonies was calculated for four biplate sides. The cfu/mL of sputum was calculated as: average number of colonies × 10 (100 µL inoculum/side) × dilution factor.

MGIT 960 liquid culture and smear microscopy

Remaining homogenized overnight sputum was decontaminated with a final NaOH concentration of 1.5%. Sputum sediment was resuspended to 2 mL in PBS. A 0.05 mL volume of resuspended sediment was examined microscopically for AFB. MGIT tubes were inoculated with 0.5 mL of resuspended sediment according to manufacturer's instructions. Positive MGIT cultures were identified as *M. tuberculosis* using MPT64 antigen detection test (Bioline, Abbott) and Ziehl-Neelsen smear. Contamination was ruled out with microscopy and cultivation on brain heart infusion agar plates. Instrument-generated time to detection (TTD) in days and hours was recorded for positive cultures.

Data and statistical methods

We tabulated baseline demographic and clinical data. Underweight was defined as a BMI <18.5 kg/m^2. We assessed distributions and trends of culture results with frequency tables for categorical variables, and means (SD) or median (IQR) for continuous variables. We report one-way descriptive frequencies for

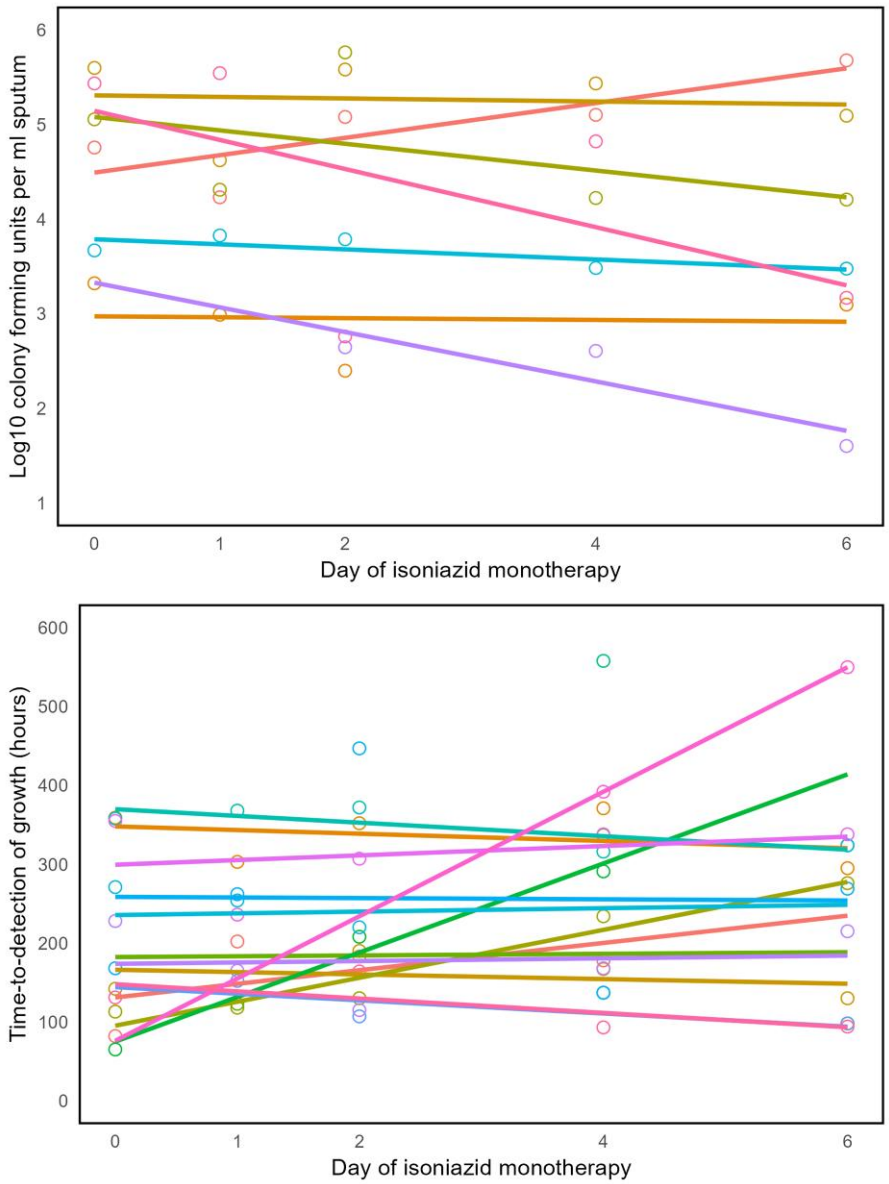

Figure 1. Serial quantitative sputum bacteriology of patients having low-level isoniazid-resistant pulmonary TB before and during 6 days of high-dose isoniazid monotherapy. (a) Log_{10} cfu/mL; (b) time to detection (hours) in MGIT 960. Each color is one patient.

characteristics of the participants and their successive isolates. We $\log_{10}$-transformed cfu/mL to normalize the distribution. Scatterplots and trendlines for each patient are summarized with box-and-whisker plots of $\log_{10}$ cfu/mL and of TTD (in hours) for five serial overnight sputum specimens. Scatter plots of $\log_{10}$ cfu/mL and of TTD across serial specimens are displayed in separate graphs with least squares regression lines estimating the overall mean change per day. We estimated the change in $\log_{10}$ cfu/mL and TTD per day (95% CI) with linear mixed-effects regression models with serial specimens treated as repeated outcome measures. Patients were modelled as random effects, whereas day of treatment was the primary independent variable.

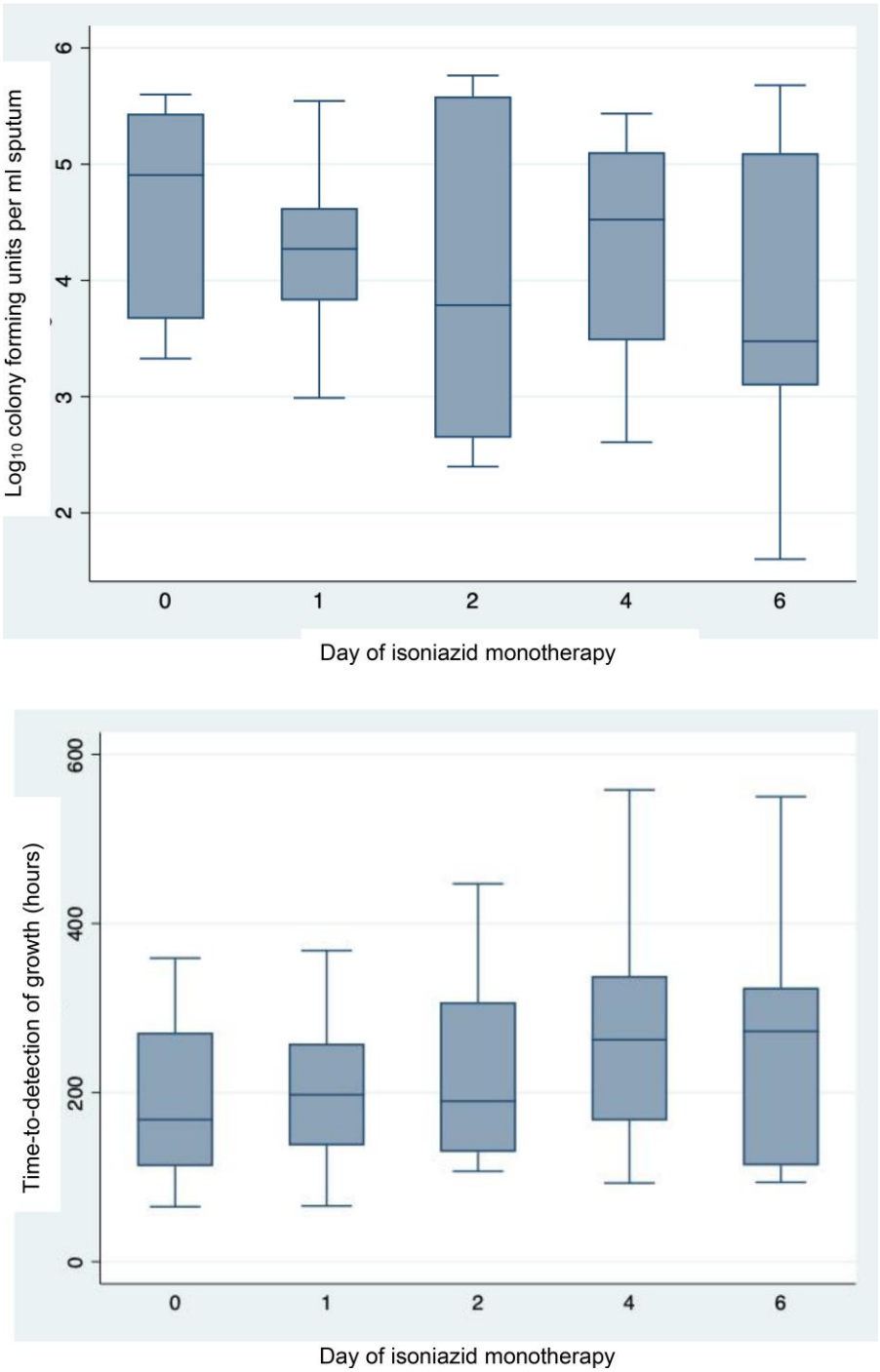

Figure 2. Box-and-whisker plots of logCFU/ml on Middlebrook 7H11S agar (upper panel) and of time to detection in MGIT960 (lower panel) during 6 days of high-dose INH monotherapy. The whiskers indicate 95% confidence limits.

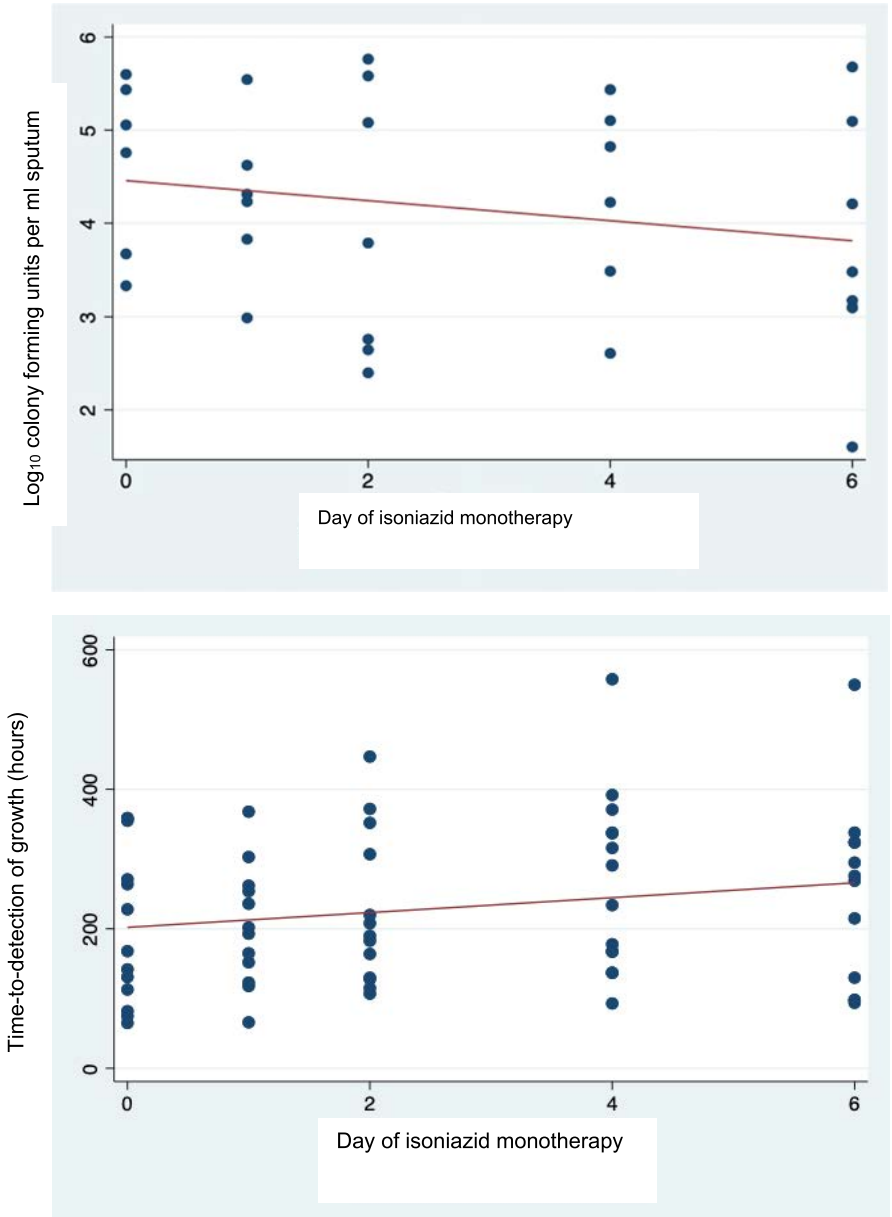

Figure 3. Scatterplot and fitted linear regression trendline of $\log_{10}$ cfu/mL on Middlebrook 7H11S agar (upper panel) and time-to-detection in MGIT 960 (lower panel) during 6 days of high-dose isoniazid monotherapy.

Three covariates were included as fixed effects: isoniazid resistance at 0.4 mg/L (susceptible at 2.0 mg/L), absence of *inh*A mutations by line probe assay (LPA), and isoniazid dose <14 mg/kg. The impact of baseline bacterial concentration was assessed graphically. Bactericidal activity was calculated as the mean slope (95% CI) over 6 days of $\log_{10}$ cfu/mL and of TTD. *P* values <0.05 were considered statistically significant.

Human subjects and registration

The study was approved by the institutional review board at US CDC and by NIRT's Institutional Ethics Committee (NIRT-IEC No. 2016004). All participants provided written informed consent. The study is registered in clinicaltrials.gov (NCT02236078).

Results

We enrolled 15 patients aged 33–63 years (median = 50); 14/15 were men, most were underweight (median BMI 16.8 kg/m^2) (Table 1). None had HIV infection. All 15 participants had infiltrates radiographically, 12 were bilateral; 3 had cavities. Participants were treated with standard first-line anti-TB treatment as per national guidelines for a median of 14 days (range 5–26 days).

Phenotypically, 10 isolates had low-level resistance to isoniazid at 0.1 mg/L, remaining susceptible at 0.4 and 2.0 mg/L (Table S1, available as Supplementary data at *JAC-AMR* Online). Two isolates had resistance at 0.4 mg/L, remaining susceptible at 2.0 mg/L. Overall, 12 isolates had *inh*A-MUT1, 2 isolates had *inhA*-MUT3a, and 1 isolate with low-level resistance had no mutations detected. Three of these 12 were enrolled based on the MTBDR*plus* assay of *inh*A mutations.

Baseline median bacterial burden was 4.9 $\log_{10}$ cfu/mL (range: 3.3–5.6) and 168 h TTD (range: 65–359).

The median dose of isoniazid was 15 mg/kg/day (range 12–17). In three patients $\log_{10}$ cfu/mL decreased by at least 1 log, three patients had smaller decreases (0.2–0.5 log), and in one patient it increased by 1 log (not those with 0.4 mg/L resistance) (Figure 1a). The overall mean decreased from 4.6 (3.3–5.6 log) to 3.8 (1.6–5.7 log) between Days 0 and 6. TTD slowed by 23–286 h in six patients, whereas in eight patients it was 13–86 h faster (Figure 1b). Between Days 0 and 6, the amplitude of day-to-day variability in counts exceeded the overall change in counts in patients with serial cfu data and with serial TTD data.

Contrary to expectation, neither box-and-whisker plots nor least squares regression lines over scatter plots indicated meaningful or statistically significant changes in bacterial burden (Figures 2 and 3), with CIs across days overlapping almost entirely. The overall slope of $\log_{10}$ cfu/mL/day was −0.08 (95% CI −0.19 to 0.04; $P=0.2$) and of TTD was +8.5 h/day (95% CI −0.9 to 18.0; $P=0.08$). There were no meaningful differences in cfu or TTD between patients according to prior treatment, baseline bacterial burden, or either phenotypic or molecular susceptibility test results.

None of the patients experienced adverse reactions to high-dose isoniazid, and follow-up DST results did not show increased resistance to isoniazid.

Discussion

Contrary to our study hypothesis, daily isoniazid at 15 mg/kg did not demonstrate meaningful bactericidal effect in patients with low- or moderate-level isoniazid resistance when it was administered after 1 or more weeks of standard four-drug first-line treatment. Two patients whose isolates had moderate-level resistance did not differ in this respect nor did one patient without *inh*A mutations. The response did not differ according to baseline bacterial burden or isoniazid dose per kg.

This study differs from standard EBA studies that enrol treatment-naive patients based on sputum microscopy or molecular detection of *M. tuberculosis* DNA, delaying treatment until after 2 to 14 days of experimental therapy with the study drug(s).[4,8–14] In clinical practice, in contrast, first-time patients start first-line treatment immediately without waiting for DST results. Treatment is adjusted after DST results are reported. Our novel approach of waiting for phenotypic DST results replicates this sequence of events. We wanted to test high-dose isoniazid the same way it would be used in clinical practice because of the sharp decrease in isoniazid's bactericidal activity after the first few days.[3,15–17]

Our study introduced another novel method: testing at 2.0 mg/L in broth (5.0 mg/L on agar), 5-fold higher than the higher of two standard critical concentrations (0.4 mg/L in broth, 1.0 mg/L on agar). Our previous work showed >40% of isolates demonstrating resistance at standard concentrations remained susceptible at 2.0 mg/L (broth)/5.0 mg/L (agar).[5–7] With higher doses, serum levels easily exceed 2.0 mg/L for many hours.

Dooley *et al.*[12] recently reported that high-dose isoniazid has significant bactericidal activity in the first 7 days of treatment, although their results may have been confounded by a coincidental difference in baseline bacterial concentrations in the two high-dose limbs (~10^7 cfu/mL) compared with the two standard-dose limbs (~10^5 cfu/mL). Our study differs from and extends that of Dooley *et al*. in several respects. First, Dooley *et al*. tested isoniazid's activity in treatment-naive patients over the first 7 days of therapy. Second, they enrolled patients based on LPA results. However, *inhA*/promoter and *katG* (*catalase-peroxidase*) mutations do not entirely correlate with phenotypic resistance.[18–20] Our understanding of isoniazid's clinical efficacy comes from decades of clinical trials that relied on phenotypic DST. The categories of low-, moderate- or high-level resistance that correlate with *in vivo* efficacy are defined by growth-based tests. Further, most patients worldwide are not treated initially based on isoniazid resistance–associated mutations. Third, Dooley *et al*. included invaluable pharmacokinetic results, documenting maximum drug concentrations, AUC and clearance half-life, which our study did not. Their results support our choice of dosing at 15 mg/kg.

These considerations highlight important limitations of our study. The first is sample size, although most published EBA studies enrol 15 patients per limb, the prevailing norm in EBA studies. Second, fungal contamination of agar plates was problematic as in all EBA studies. Third, variability in quantitative culture results between patients likely reflected variability in actual bacillary load not in laboratory technique because of extensive training and validation of laboratory methods before starting enrolment. Fourth, we did not include a comparison group because (i) Without treatment, bacterial numbers increase. (ii) The dose-dependent bactericidal effect of isoniazid against isoniazid-susceptible TB is well established; higher doses have an even greater effect.[3] (iii) The bactericidal effect of first-line anti-TB drugs other than isoniazid (e.g. against isoniazid-resistant TB) is also well established.[10,11,17] In each case, treatment leads to a progressive, log-linear decrease in bacterial burden.

Our patients had AFB 2+ and 3+ sputum microscopy results before starting treatment, corresponding roughly to ~10^6 to 10^7 cfu/mL,[21–23] although it averaged ~10^5 cfu/mL the night before starting high-dose isoniazid monotherapy. Even conventional doses of 300 mg isoniazid per day may be effective against *M. tuberculosis* with only low-level resistance.[7] This suggests their first-line treatment before starting the experimental protocol may have eliminated the most susceptible bacilli, leaving behind less-susceptible bacilli. Such bacilli would normally be eliminated by the relatively slower bactericidal activity of rifampicin plus pyrazinamide. Therefore, the value of high-dose isoniazid past the first week or two of treatment may be questioned as it adds little bactericidal activity, increases the risk of toxicity, and antagonizes rifampicin in a concentration-dependent manner.[24–26]

Conclusion

In previous work we observed that over 40% of MDR *M. tuberculosis* isolates remained susceptible to 5.0 mg/L isoniazid on agar

plates.[27] We introduced a novel variant of EBA study methodology to test the hypothesis that high-dose isoniazid would continue killing *M. tuberculosis* with low to moderate levels of resistance after phenotypic DST results were reported—in a manner reflecting clinical practice—because serum concentrations over 15 mg/L lasting several hours are easily attainable with 15 mg/kg dosing. We believed it would be safe because hepatic toxicity tends to be idiosyncratic, not dose dependent, and peripheral neuropathy can be prevented with adjuvant pyridoxine. Isoniazid was safe in our study, but we found no evidence to support the thinking that 15 mg/kg isoniazid continues killing *M. tuberculosis* having low or moderate resistance after 1–4 weeks of standard four-drug treatment. Consequently, the benefit versus risk of continuing isoniazid after that period should be re-examined. Although pharmacodynamics over the first 2 weeks of treatment in drug-susceptible TB has been well studied, the pharmacodynamics of higher-dose isoniazid after that initial interval needs more research.

Funding

This work was supported by the U.S. Centers for Disease Control and Prevention (CDC) internal funding. U.D.R., S.B., K.D., D.N., B.D.D., R. Srinivasan, M.B., D.RK., D.S., G.R., G.M., S.T. and S.S. were supported by their Indian government (NIRT) salaries. S.E.S.-J. and J.P.C. were supported by their US government (CDC) salaries. J.L., K.M., S.K. and R. Sridhar were supported by their Tamil Nadu state salaries. K.D.E. was supported by the US CDC through an Intergovernmental Procurement Act (IPA) transfer to the University of Arkansas for Medical Sciences.

Transparency declarations

All authors confirm they have no conflicts of interest.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention (CDC).

Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

References

1 WHO. Global tuberculosis report 2021. https://www.who.int/publications/digital/global-tuberculosis-report-2021

2 WHO. Global tuberculosis report 2020. https://www.who.int/publications/i/item/9789240013131

3 Donald PR, Sirgel FA, Botha FJ *et al.* The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. *Am J Respir Crit Care Med* 1997; **156**: 895–900. https://doi.org/10.1164/ajrccm.156.3.9609132

4 Jindani A, Aber VR, Edwards EA *et al.* The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1980; **121**: 939–49. https://doi.org/10.1164/arrd.1980.121.6.939

5 Institute of Medicine, Forum on Drug Discovery, Development, and Translation. *The New Profile of Drug-Resistant Tuberculosis in Russia: a Global and Local Perspective. Summary of a Joint Workshop by the Institute of Medicine and the Russian Academy of Medical Science*. Institute of Medicine, The National Academies Press, 2011. https://doi.org/10.17226/13033

6 Cegielski JP. Second-line drug resistance among MDR TB patients starting treatment in 8 countries [oral presentation]. In: *The New Profile of Drug-Resistant Tuberculosis in Russia: a Global and Local Perspective*. Sponsored by U.S. National Academy of Sciences; Russian Academy of Medical Sciences; International Science & Technology Center, Moscow, Russian Federation, 26 May 2010.

7 Rieder HL, Van Deun A. Rationale for high-dose isoniazid in the treatment of multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2017; **21**: 123–4. https://doi.org/10.5588/ijtld.16.0619

8 Bonnett LJ, Ken-Dror G, Koh GCKW *et al.* Comparing the efficacy of drug regimens for pulmonary tuberculosis: meta-analysis of endpoints in early-phase clinical trials. *Clin Infect Dis* 2017; **65**: 46–54. https://doi.org/10.1093/cid/cix247

9 CLSI. *Laboratory Detection and Identification of Mycobacteria—Second Edition: M48*. 2018.

10 Donald PR, Diacon AH. The early bactericidal activity of antituberculosis drugs: a literature review. *Tuberculosis (Edinb)* 2008; **88** Suppl 1: S75–83. https://doi.org/10.1016/S1472-9792(08)70038-6

11 Diacon AH, Donald PR. The early bactericidal activity of antituberculosis drugs. *Expert Rev Anti Infect Ther* 2014; **12**: 223–37. https://doi.org/10.1586/14787210.2014.870884

12 Dooley KE, Miyahara S, von Groote-Bidlingmaier F *et al.* Early bactericidal activity of different isoniazid doses for drug-resistant tuberculosis (INHindsight): a randomized, open-label clinical trial. *Am J Respir Crit Care Med* 2020; **201**: 1416–24. https://doi.org/10.1164/rccm.201910-1960OC

13 Diacon AH, Pym A, Grobusch M *et al.* The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 2009; **360**: 2397–405. https://doi.org/10.1056/NEJMoa0808427

14 Gler MT, Skripconoka V, Sanchez-Garavito E *et al.* Delamanid for multidrug-resistant pulmonary tuberculosis. *N Engl J Med* 2012; **366**: 2151–60. https://doi.org/10.1056/NEJMoa1112433

15 Diacon AH, van der Merwe L, Demers AM *et al.* Pre-treatment mycobacterial sputum load influences individual on-treatment measurements. *Tuberculosis (Edinb)* 2014; **94**: 690–4. https://doi.org/10.1016/j.tube.2014.08.015

16 De Jager V, van der Merwe L, Venter A *et al.* Time trends in sputum mycobacterial load and two-day bactericidal activity of isoniazid-containing antituberculosis therapies. *Antimicrob Agents Chemother* 2017; **61**: e02088-16. https://doi.org/10.1128/AAC.02088-16

17 Donald PR, Sirgel FA, Venter A *et al.* Early bactericidal activity of antituberculosis agents. *Expert Rev Anti Infect Ther* 2003; **1**: 141–55. https://doi.org/10.1586/14787210.1.1.141

18 Lempens P, Meehan CJ, Vandelannoote K *et al.* Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations. *Sci Rep* 2018; **8**: 3246. https://doi.org/10.1038/s41598-018-21378-x

19 Rivière E, Whitfield MG, Nelen J *et al.* Identifying isoniazid resistance markers to guide inclusion of high-dose isoniazid in tuberculosis treatment regimens. *Clin Microbiol Infect* 2020; **26**: 1332–7. https://doi.org/10.1016/j.cmi.2020.07.004

20 World Health Organization. Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance. 2021. https://www.who.int/publications/i/item/9789240028173

21 Sirgel FA, Botha FJ, Parkin DP *et al.* 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**: 867–75. https://doi.org/10.1093/jac/32.6.867

22 Toman K. How many bacilli are present in a sputum specimen found positive by smear microscopy? In: Frieden T, ed. *Toman's Tuberculosis:*

Case Detection, Treatment and Monitoring, 2nd edn. World Health Organization, 2004; 11–3. Ch. 4.

23 Toman K. How reliable is smear microscopy? In: Frieden T, ed. *Toman's Tuberculosis: Case Detection, Treatment and Monitoring*, 2nd edn. World Health Organization, 2004; 14–22. Ch. 5.

24 Rockwood N, Pasipanodya JG, Denti P *et al*. Concentration-dependent antagonism and culture conversion in pulmonary tuberculosis. *Clin Infect Dis* 2017; **64**: 1350–9. https://doi.org/10.1093/cid/cix158

25 Almeida D, Nuermberger E, Tasneen R *et al*. Paradoxical effect of isoniazid on the activity of rifampin-pyrazinamide combination in a mouse model of tuberculosis. *Antimicrob Agents Chemother* 2009; **53**: 4178–84. https://doi.org/10.1128/AAC.00830-09

26 Grosset J, Truffot-Pernot C, Lacroix C *et al*. Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. *Antimicrob Agents Chemother* 1992; **36**: 548–51. https://doi.org/10.1128/AAC.36.3.548

27 Dalton T, Cegielski P, Akksilp S *et al*. Prevalence of and risk factors for resistance to second-line drugs in people with multidrug-resistant tuberculosis in eight countries: a prospective cohort study. *Lancet* 2012; **380**: 1406–17. https://doi.org/10.1016/S0140-6736(12)60734-X