Assessing whether isoniazid is essential during the first 14 days of tuberculosis therapy: a phase 2a, open-label, randomised controlled trial

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Summary

Background—Clinical studies suggest that isoniazid contributes rapid bacterial killing during the initial two days of tuberculosis treatment but that isoniazid’s activity declines significantly after day three. We conducted a 14-day phase IIa open label, randomized trial to assess the essentiality of isoniazid in standard tuberculosis therapy.

Methods—A total of 69 adults with newly diagnosed sputum-positive tuberculosis from the South African Western Cape region were enrolled and randomized to a four-arm parallel assignment model. Participants were followed for 14 days as inpatients at either the University of Cape Town Lung Institute or at the TASK Applied Science clinical research organization. All arms...
received standard daily rifampicin, ethambutol, and pyrazinamide but differed as follows: isoniazid only on days one and two (n=17), isoniazid on days one and two then moxifloxacin on days three through 14 (n=16), no isoniazid (n=18), and a control group that received isoniazid for all 14 days (standard therapy, n=18). The primary endpoint was the rate of colony forming unit (CFU) decline during the first 14 days of treatment.

Results—For 62 participants analyzed, the initial 14-day mean daily fall in log$_{10}$ CFU (95% CI) was 0·14 (0·11, 0·18) for participants receiving isoniazid for two days only; 0·13 (0·09, 0·17) for participants receiving isoniazid for two days followed by moxifloxacin; 0·12 (0·08, 0·15) for those not receiving isoniazid; and 0·13 (0·09, 0·16) for the standard therapy group.

Conclusions—The 14 day EBA for the combination rifampicin, ethambutol, and pyrazinamide was not significantly changed by the addition of isoniazid for the first two days or for the first 14 days of treatment. In a post hoc analysis, significantly higher day-two EBAs were observed for all groups among participants with higher baseline sputum CFUs. Our finding that INH does not contribute to EBA suggests that INH could be replaced with another drug during standard treatment to improve efficacy and decrease rates of resistance to first-line drugs. (Funded by the NIH AIDS Clinical Trial Groups and NIH; A5307 ClinicalTrials.gov number, NCT01589497).

Introduction

Isoniazid has been a mainstay in chemotherapy for active tuberculosis (TB) since the 1950s when it was first discovered. Isoniazid is rapidly bactericidal for Mycobacterium tuberculosis both in vitro and in vivo. In patients with smear-positive pulmonary tuberculosis, the use of isoniazid was associated with an early bactericidal activity (EBA) of approximately 0·72 log$_{10}$ CFU per day during the first two days of treatment, but its activity typically decreased during days three −14 with a stable average daily EBA of only 0·11 log$_{10}$CFU. Animal studies have demonstrated antagonism between isoniazid and the combination of rifampicin and pyrazinamide, and similar antagonistic activity has been observed in humans. Finally, a mouse study evaluating the effect of several three - four drug regimens found that omitting isoniazid after the first two days improved the efficacy of four-drug standard therapy. These studies suggest that the main benefit of isoniazid therapy is rapid killing of bacilli during the first two days of treatment, which reduces patient infectivity, and that further use may have a limited or even antagonistic role when accompanied as part of standard treatment with rifampicin, pyrazinamide, and ethambutol.

The rate of isoniazid mono-resistance in M. tuberculosis has risen to between 3% and 4% in South Africa and is estimated to be higher in some regions. Mounting genomic evidence indicates that isoniazid resistance precedes rifampicin resistance and thus is a main driver for the development of multidrug resistant tuberculosis. Moreover, rapid molecular tests such as the Xpert® MTB/RIF (Cepheid, CA, USA) do not detect isoniazid mono-resistance, so patients with isoniazid mono-resistance could receive standard therapy in which only isoniazid and rifampicin are used during the last four months of treatment. These patients would be exposed to effective rifampicin monotherapy and a gateway combination for acquired rifampicin resistance. Lastly, clinical trials have shown treatment success with rifampicin, pyrazinamide, and ethambutol treatment for six months in patients diagnosed with isoniazid mono-resistant tuberculosis.
The unclear role of isoniazid after the first two days of treatment, concerns that high isoniazid mono-resistance levels promote acquired rifampicin resistance, and the finding that rifampicin, pyrazinamide, ethambutol given for six months is an acceptable regimen call into question the routine use of isoniazid in TB treatment.

In this study we evaluated the contribution of isoniazid to standard TB therapy in a four arm trial where isoniazid was eliminated from standard therapy during days three – 14 of treatment, substituted with moxifloxacin during days three – 14, or eliminated altogether. We hypothesized that isoniazid contributes the greatest bacillary killing effect during the first two days of treatment, and that substitution with moxifloxacin in one study arm provides an important comparison by adding additional killing power during treatment days three through 14.

**Methods**

**Trial design**

From June 2015 to February 2016 we conducted a phase IIa open-label, clinical trial with balanced randomization (1:1:1:1), using a parallel assignment model to assess the essentiality of isoniazid in TB therapy by comparing the first 14 day EBA of four treatment regimens. The study was conducted at two clinical sites (TASK Applied Science Tuberculosis Clinical Research Centre, Bellville, or the University of Cape Town Lung Institute, Mowbray) in the South African Western Cape region.

**Participants**

Study participants were recruited from the South African Western Cape region. Written informed consent was obtained in either English or Zulu before screening. Eligible individuals were adult (≥18 years of age) men and women with newly diagnosed, untreated, sputum smear positive pulmonary TB (at least 1+ on the WHO/IUATLD scale). Blood draws and spot sputum were collected during screening. HIV infected persons were included if their CD4 count was above 200 cells/mm³ and if they were not currently on anti-retroviral therapy. Participation required confirmed isoniazid and rifampicin sensitivity through sputum molecular testing (Genotype MTBDRplus, Hain Lifescience GmbH, Nehren, Germany) and the ability to provide at least 10 mL of sputum during a 16-hour overnight collection period. Patients who were in poor general condition; uncontrolled diabetes mellitus or hypertension, pregnant, or who had evidence of significant extra-pulmonary TB (Karnofsky Score ≤70) were excluded.

**Interventions**

All interventions occurred as variations of TB chemotherapy consisting of rifampicin (R), isoniazid (H), pyrazinamide (Z), ethambutol (E), and moxifloxacin (M) administered during study days one – 14 while participants were followed as inpatients. Four treatment arms were devised. The HRZE₁–₂-RZE₃–₁₄ arms received isoniazid only on days one and two. The HRZE₁–₂-MRZE₃–₁₄ arm received isoniazid on days one and two followed by the substitution of isoniazid for moxifloxacin on days three – 14. The RZE₁–₁₄ arm received no isoniazid. Finally, the HRZE₁–₁₄ arm received standard therapy. Study drugs were given as...
directly observed therapy according to the standard weight bands as per the South African National Tuberculosis Programme guidelines and according to current recommendations for moxifloxacin (400 mg daily). Following the 14-day intervention period, all participants were referred for the continuation of standard TB treatment. Fourteen days after the last dose of study treatment was administered (day 28) participants underwent follow-up at the trial sites for a final clinical assessment and sputum collection.

Sample Size

For sample size determination, study power was set to 90% in anticipation of a 2-sided t-test with a significance level of 0.10 on each side. Standard deviation within treatment groups was estimated from similar EBA studies: 0.10 for rifampicin treatment and 0.06 for standard treatment. The study team agreed on 0.125 log_{10} CFU/mL per day as the minimum detectable difference of mean EBAs allowed between arms. These parameters were applied to a power calculation that requiring total sample size of 45 individuals (15 individuals per group). Screening and enrolment continued for six months until these participation thresholds were reached.

Outcomes

Microbiology: The primary endpoint was the daily decrease in log_{10} transformed CFU counts per mL of sputum measured between baseline and day 14. Sputum was collected for 16 consecutive hours overnight on two occasions during the pre-treatment period (day −1, and day 0), and on days one, two, three, five, seven, nine, 11, and 14. Samples were homogenized by magnetic stirring and combine with dithiothreitol (1:20 dilution; Sputasol; Oxoid, Cambridge, UK) to a maximum of 10 mL total volume, vortexed for 20 seconds, and left to digest at room temperature for 20 minutes. This mixture was plated in 10-fold dilutions onto 7H11S agar plates containing polymyxin B, amphotericin B, and trimethoprim. Numbers of CFU were counted after three to four weeks.

Time until culture positivity (TTP) was also measured as a secondary endpoint. Homogenized sputum was decontaminated with NaOH-NALC (AlphaTec NAC-PAC Red; AlphaTec, Vancouver, WA, USA), centrifuged, resuspended, and 0.5 ml of the resulting 2 mL used for incubation in duplicate in a standardized liquid culture system (Bactec MGIT 960; BD). Culture positivity was confirmed by Ziehl-Neelsen staining and purity by incubation on blood agar plates. TTP was reported in hours.

Pharmacology: Pharmacokinetic (PK) studies were conducted at days one and 14 as a secondary endpoint to determine the first-dose and steady-state PK parameters of each drug in the four different regimens. Concentrations were determined with validated, liquid chromatography-tandem mass spectrometry methods as published. The primary PK parameters were maximum concentration (C_{max}), area under the concentration-time curve from time 0 to 24 (AUC_{0–24}), and apparent oral clearance (CL/F). PK parameters were determined by noncompartmental methods.
Randomization
Candidates who met the study eligibility criteria were enrolled, and permuted block randomization was used to assign participants to 1 of the 4 treatment groups via the ACTG Data Management Center.

Statistical methods
In case of missing data, CFU counts on any day were imputed by a multiple imputation method stratified by arm using every observed CFU count. Mean EBA_{0–14} by arm was estimated using a linear regression model adjusted for the baseline CFU.

For split time calculations the daily decrease was calculated as follows:

$$EBA_{X–Y} = \frac{\text{mean of log}_{10}\text{CFU/mL sputum at Day Y} – \text{mean of log}_{10}\text{CFU/mL at Day X}}{Y–X}.$$ 

Secondary endpoints included the daily decrease in log_{10} transformed CFU counts per mL sputum between baseline and day two [EBA_{0–2}] or between day two and day 14 [EBA_{2–14}], the daily increase in time till culture positivity (TTP) in hours between baseline and day 14 [EBA_{0–14} (based on TTP)], between baseline and day two [EBA_{0–2} (based on TTP)], and between day two and day 14 [EBA_{2–14} (based on TTP)]. The means of these secondary endpoints were estimated using the same method as the primary endpoint.

A post hoc analysis was conducted to investigate the individual characteristics of participants showing early CFU sharp-drop responses compared to those without early CFU sharp-drops, 49 participants (of the 62 participants used for the multiple imputation method) were selected for individual arithmetic analysis based on having valid baseline and day two CFU data.

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Results
Study participants
Of 89 patients screened, 69 were enrolled. Figure 1 lists the reasons for screen failure and early withdrawal in each of the four treatment arms. Of the 69 enrolled participants, 83% were male with a mean age of 33 years (SD 10·7). Ninety-four percent were HIV negative. Baseline characteristics, displayed in Table 1, were similar between treatment arms. Six participants discontinued the study prematurely. Of the 63 participants that completed the study, 62 had at least one valid CFU count and were eligible for the primary endpoint analysis. In total, 16 missing EBA_{0–14} were estimated based on the multiple imputation method because two participants had missing CFU counts at baseline and Day 14, one
participant had missing CFU at baseline, and ten participants had missing CFU counts on Day 14. Missing CFU counts were distributed evenly over all study arms.

**Early Bactericidal Activity: no advantage of standard therapy HRZE\(_{1–14}\) over RZE\(_{1–14}\)**

EBA was analysed by both log-transformed CFU counts (Figure 2) and TTP in hours (Supplementary Figure 1) and the values are summarized in Table 2 (also see Supplementary Tables 1 and 2). In all arms, the addition of isoniazid to RZE showed no antagonistic effects. More rapid killing during the first two days of treatment was seen with all three H-containing regimens, but by day 14 the mean EBAs of HRZE\(_{1–14}\) and RZE\(_{1–14}\) were virtually identical: 0·13 log\(_{10}\) units (95% CI: 0·091, 0·169) versus 0·12 (95% CI: 0·081, 0·159). Similarly, when isoniazid was dropped after the first two days of therapy (HRZE\(_{1–2}\)-RZE\(_{3–14}\)), the EBA over 14 days remained virtually the same at 0·14 log\(_{10}\) units (95% CI: 0·101, 0·179) compared with HRZE given for all 14 days (0·13 log\(_{10}\) units) (Supplementary Table 2). EBA by TTP was also similar between treatment arms with the mean EBAs of HRZE\(_{1–14}\), HRZE\(_{1–2}\)-RZE\(_{3–14}\), and RZE\(_{1–14}\) being essentially superimposable and HRZE\(_{1–2}\)-MRZE\(_{3–14}\) showing a slight, not statistically significant advantage.

**Early Bactericidal Activity during the first two days of therapy: RZE shows poor EBA\(_{0–2}\) but then catches up to the isoniazid-containing regimens by day 14**

At the outset of this study we sought to test whether isoniazid use during the first two days was a main driver of early rapid bacterial killing. Thus, we hypothesized that the HRZE regimens would result in sharp EBA\(_{0–2}\) drops while RZE would not.

As expected the mean EBA\(_{0–2}\) for HRZE\(_{1–2}\) recipients was higher at 0·22 log\(_{10}\) units (95% CI: 0·102, 0·338) than for RZE recipients at 0·03 log\(_{10}\) units (95% CI: −0·166, 0·226) though the difference was not statistically significant. Thus, while there was a 0·18 log\(_{10}\) unit bacterial killing advantage among participants receiving isoniazid (HRZE\(_{0–2}\) arms) compared with RZE alone for the first two days of therapy, by day 14 the gap narrowed to only 0·01 or 0·02 log\(_{10}\) units difference between all four regimens and was not substantial (Table 2 and Figure 2).

**Mono versus biphasic EBA responses: sharp-drop EBA response is associated with high initial CFU count.**

A surprising result of this study was that we did not observe the pronounced average biphasic EBA response with a sharp decline in CFU during the first two days of therapy followed by a more modest decline on days three - 14 (Figure 2). Such biphasic patterns have been observed with isoniazid-containing regimens in most EBA studies conducted between 1975 and 2005, and EBA\(_{0–2}\) values as high as 0·72 have been reported with isoniazid treatment.\(^4\)\(^5\) However, in our study the mean EBA\(_{0–2}\) for HRZE recipients was only 0·22 (SE: 0·06) (Table 2). The mean baseline log\(_{10}\) CFU count for participants in all arms of the study was 5·80 log\(_{10}\) CFUs (Table 1), whereas baseline log\(_{10}\) CFU counts in older EBA studies of 6·5 - 7·5 log\(_{10}\) CFUs have been reported.\(^4\)\(^23\) To determine whether the sharp drop observed in these older studies may be related to the higher initial CFU counts, we assessed the relationship between baseline log\(_{10}\) CFU count and EBA\(_{0–2}\) in our population. As shown in Figure 3, a linear correlation was found between baseline log\(_{10}\)
CFU count and EBA$_{0–2}$ across all study arms, indicating that high initial baseline log$_{10}$ CFU count is a likely driver of the observed sharp-drop EBA responses.

**Sharp-drop responses are not dependent on the use of isoniazid during the first two days.**

Even though we did not observe a classic biphasic response among isoniazid arm-specific EBAs calculated by the multiple imputation methods, we wondered whether biphasic responders still existed in the study population. Though the study was not originally powered to investigate response patterns within study arms, we devised and performed a post hoc subgroup analysis which identified sharp-drop responders, defined as individuals who had an EBA$_{0–2}$ of greater than or equal to 0·4 log$_{10}$ units (Table 3). Among 49 participants with arithmetic primary endpoints, 17 were sharp-drop responders demonstrating a mean (sd) EBA$_{0–2}$ of 0·63 (0·22) log$_{10}$ units as compared with −0·09 (0·36) log$_{10}$ units among the no-sharp-drop responders (Table 3). Sharp-drop responders had a statistically significantly higher initial CFU count than monophasic responders with a mean (sd) baseline count of 6·20 (1·07) versus 5·51 (0·94), respectively (p = 0·02; Wilcoxon test) (Table 3). Despite the fact that sharp drop responders tended to have higher baseline log$_{10}$ CFU counts, they experienced a greater overall clearance of bacilli over the course of our study with day 14 log$_{10}$ CFU counts of 3·81 (sd) compared to 3·90 among those without a sharp drop (Table 3).

Because of the apparent benefit of the sharp-drop response we sought to identify variables that might be predictive of this pattern. To our surprise the sharp drop response was independent of whether participants received isoniazid (Table 3). Sharp-drop responders were equally distributed among participants who received isoniazid-containing regimens in the initial two days of therapy (13/37, 35%) and those who received RZE only in the first two days (4/12, 33%) (p = 1·00; Fisher’s exact test). The mean (sd) EBA$_{0–14}$ for sharp-drop responders who received any isoniazid was 0·20 (0·12) while it was 0·16 (0·11) among RZE$_{1–14}$ recipients, both values being well above the mean EBA$_{0–14}$ observed in our study which was 0·13 (Table 3).

We considered other possible variables that might contribute to the sharp drop response. No significant differences were observed between age, BMI, sex, or HIV status with regard to sharp-drop responses (Table 3). We also assessed plasma drug concentrations of isoniazid or rifampicin on day one of therapy and found no statistically significant differences between sharp-drop responders and those with no sharp drop (Supplementary Table 3).

The classic biphasic “sharp-drop” EBA response occurred in only about one-third of our participants. We found that the sharp-drop response is significantly associated with having a high initial CFU count, but that isoniazid administration is not required for this rapid killing as the effect was seen in an equal proportion of participants who received RZE only.

**Pharmacology**

The pharmacokinetic (PK) parameters by drug at day 14 are shown in Supplementary Table 4. On Day 14, no differences were observed for each drug across the arms in the primary PK parameters ($C_{\text{max}}$, AUC$_{0–24}$ and CL/F). Also, the PK parameters by drug were not different across arms at day one. The discontinuation of isoniazid had no significant effect on the
primary PK parameters for the co-administered drugs. The only effect on the primary PK parameters from the addition of moxifloxacin was an approximate 13% slower CL/F of pyrazinamide in the HRZE\textsubscript{1–2}-MRZE\textsubscript{3–14} arm compared with the HRZE\textsubscript{1–2}-RZE\textsubscript{3–14} arm. The C\textsubscript{max} of rifampicin and the C\textsubscript{last} of ethambutol were associated with TTP at Day 14 and EBA\textsubscript{0–2} outcomes, respectively.

Safety

Of 69 enrolled participants, 25% (17) experienced a Grade 2 or higher adverse event during the study period (Supplementary Table 5). One participant on the HRZE\textsubscript{1–14} arm died on day three of treatment shortly after experiencing a sudden loss of consciousness. A definitive cause of death could not be confirmed posthumously, and this event was considered possibly related to study treatment by the site investigator. Three participants experienced Grade 3 adverse events, 1 (respiratory system dysfunction and abnormal rapid heart rate) in the HRZE\textsubscript{1–2}-MRZE\textsubscript{3–14} arm, considered related to the study treatment, 1 in the HRZE\textsubscript{1–2}-RZE\textsubscript{3–14} arm (haemoptysis), which was not related to the study treatment, and 1 in the HRZE\textsubscript{1–14} arm (Grade 3 alanine amino transferase (ALT) elevation), which was considered possibly related to study treatment. Six participants reported one or more new diagnoses during the study (Supplementary Table 6).

Discussion

This trial evaluated the essentiality of isoniazid in lowering sputum bacterial burden over the first fourteen days of therapy for pulmonary tuberculosis. We did not detect significant differences among RZE\textsubscript{1–14} and the three alternative regimens including standard therapy (HRZE\textsubscript{1–14}), a regimen in which the isoniazid was given for only the first two days (HRZE\textsubscript{1–2}-RZE\textsubscript{3–14}), or a regimen in which isoniazid was given for two days and then replaced by moxifloxacin for the remaining 12 doses (HRZE\textsubscript{1–2}-MRZE\textsubscript{3–14}). The results were consistent whether we evaluated the expectorated sputum by colony forming unit (CFU) counts or liquid culture time to positivity (TTP).

While EBA studies cannot predict long term clinical outcomes, a large body of previous work including human EBA studies with isoniazid monotherapy, as well as microbiologic and animal model research, indicate that isoniazid has potent killing activity against rapidly dividing bacteria and contributes significantly to killing *M. tuberculosis* during the initial days of therapy\textsuperscript{9,18,24,25}. Thus, the lack of any deleterious effect of omitting isoniazid from standard multidrug therapy for the first 14 days was surprising. Because isoniazid kills rapidly dividing bacteria (predominant in the early phases of treatment) by inhibiting mycolic acid biosynthesis and has little activity against persisters (predominant in the later phases of treatment), our study raises questions about whether isoniazid adds any benefit during 6-month short course therapy (2HRZE-4HR) other than to protect rifampicin (which inhibits RNA synthesis) against the development of resistance during the four-month continuation phase. In support of the notion that isoniazid may not be required, it is worth noting that the effectiveness of six-month, three-drug combination therapy of rifampicin, pyrazinamide, and ethambutol (6RZE) for isoniazid monoresistant *M. tuberculosis*\textsuperscript{26} has been demonstrated by several studies.\textsuperscript{27,28} Furthermore, a recent meta-analysis has shown
comparable treatment failure, relapse, and acquired drug resistant rates for patients with INH monoresistant TB who are treated with RZE for six - nine months to those of patients with pan-susceptible TB treated with standard therapy.²⁹

In addition to concerns regarding isoniazid as a driver for MDR-TB development, isoniazid-rifampicin antagonistic activity has recently been identified in certain settings during treatment.⁶⁻⁸ Our results are discordant with these studies and show that isoniazid is not antagonistic to the standard RZE drug combination during the first 14 days of treatment. However, researchers and clinicians should be cautious when generalizing the results of this EBA study to regimen efficacy over longer durations of treatment since Chigutsa et al. characterized two distinct slopes that form the sputum bacillary curve over time.³¹,³² Chigutsa et al. further hypothesize that the early and steep α-slope corresponds to bactericidal activity while the second late and shallow β-slope corresponds to sterilizing activity against non-replicating bacteria. Since our study might have taken place entirely within the α-slope time-window, it is possible that isoniazid may become antagonistic to regimens after the conclusion of the initial bactericidal period when isoniazid has less activity due to lower concentrations of actively replicating bacteria.

A second major finding in this study was that we did not consistently observe the classic sharp drop in CFU counts over the first two days seen with isoniazid monotherapy in EBA studies conducted from 1975–2005. However, the presence of two distinct slopes within the sputum bacillary curve may also help explain the absence of the characteristic sharp-drop response observed in other studies but absent in our findings. Our ad hoc analysis revealed a group of 17 participants (35%) who did display a sharp CFU drop. These participants did not associate with any particular treatment arm but did tend to have a high baseline CFU count (6·20 log₁₀ CFU units compared with 5·51 in non-sharp drop responders). These participants may be those individuals with higher concentrations of rapidly dividing bacteria (e.g. those with cavities) and whose sputum curves project a more defined early steep α-slope. A regimen with strong 2-day EBA may be important for certain high-risk populations such as those with M. tuberculosis septicaemia or cavitary disease where a rapid reduction in bacterial burden could dramatically increase survival and reduce transmission events.

The lower CFU counts at diagnosis and a concomitant loss of the classic early sharp drop response curve may indicate an important shift in the epidemiology of newly-diagnosed pulmonary tuberculosis at least in our population—an urban, metropolis in South Africa. Earlier EBA studies showing a sharp CFU count drop with isoniazid monotherapy were largely performed several decades ago and the initial CFU counts in these studies were considerably higher than we observed: 6·5 – 7·5 log₁₀ CFU units per mL sputum⁴,²³ versus 5·80 in our study. Drivers for this decline in initial bacterial burden remain uncertain, but may reflect several factors that each would lead to earlier diagnosis of TB: improved diagnostics including the recently introduced GeneXpert test, better patient education, and the increased availability of internet access.³⁰

In summary, our findings indicate that isoniazid was not essential for early bactericidal killing of bacilli over the first 14 days of therapy. Isoniazid, while a mainstay in TB chemotherapy for more than six decades, is associated with a number of liabilities. Foremost
among concerns is the high rate of isoniazid monoresistance that is now between 3% and 4% in South Africa and is associated with poor treatment outcomes.\textsuperscript{11,12} Since the Xpert MTB/RIF and MTB/RIF Ultra platforms are unable to detect isoniazid monoresistance and several public health systems have become increasingly reliant on these Xpert MTB/RIF tests, there is growing opportunity for patients with isoniazid monoresistant \textit{M. tuberculosis} to be treated with standard therapy (2HRZE-4HR). This regimen exposes isoniazid monoresistant bacilli to four months of isoniazid plus rifampicin alone—essentially monotherapy—setting the stage for loss of rifampicin susceptibility and the development of MDR-TB. Indeed, a major risk factor for MDR-TB is previous treatment for TB\textsuperscript{33}. Additionally, genome sequencing studies of global collections of \textit{M. tuberculosis} isolates applying molecular clock approaches to date the acquisition of isoniazid- versus rifampicin-resistance have identified isoniazid resistance as the initial drug-resistance marker in ~98% of isolates.\textsuperscript{13,34} Our study, which demonstrates that isoniazid adds no measurable benefit during the first two weeks of treatment, heightens the need to develop modernized standard therapy regimens which no longer include isoniazid.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Evidence before this study

Isoniazid (INH) is one of four drugs used for first-line treatment of tuberculosis (TB). Inclusion of INH in combination therapy contributes enhanced early (first two weeks) bactericidal activity to the regimen, but this benefit may come at a cost. A 2017 systematic meta-analysis published in *The Lancet Infectious Diseases* found that treatment of INH mono-resistant TB with the standard four-drug regimen produced higher rates of treatment failure, relapse, and acquired drug resistance. Therefore, isoniazid might also be a liability to the integrity of first-line TB treatment and the efficacy of first-line therapy might benefit from substitution of isoniazid with a different drug. We searched PubMed for original research studies investigating the role of isoniazid in TB treatment using the search terms (isoniazid AND tuberculosis). The returned studies reported that acquired isoniazid mono-resistance often precedes multidrug resistance to other first-line drugs and that rates of isoniazid mono-resistance appear to be rising in TB endemic regions. However, these results showed a less clear consensus about the advantages of INH in standard therapy. Standard therapy with rifampicin, pyrazinamide, and ethambutol, but without isoniazid, appears to have similar efficacy to the four-drug combination in cases of INH mono-resistance and reports further conflict on the magnitude of the benefit that INH contributes to early bacterial killing.

Added value of this study

We conducted a four-arm, 14-day, randomized clinical trial to study the contribution of INH to early bactericidal activity (EBA) with the goal of resolving the relative importance of INH to the EBA of standard therapy. Our results reveal that when INH is added to the base regimen rifampicin-pyrazinamide-ethambutol, no additional EBA is observed, and thus INH cannot be demonstrated to augment bacillary killing during the first two weeks of treatment. Rates of INH mono-resistance are estimated to exceed 10% in many parts of the world; hence during the continuation phase of standard TB therapy (INH and rifampicin alone for four months) rifampicin may go unprotected in 10% of cases or more. Our work shows that isoniazid is not essential for early bacterial killing, and replacing it with alternative agents that better protect rifampicin and contribute to therapeutic efficacy may be reasonable.

Implications of all the available evidence

Our study casts doubt on the long-held belief that INH is essential for early killing of *M. tuberculosis* during standard therapy. Genomic studies have shown that in the majority of cases of MDR-TB, resistance to INH precedes rifampicin resistance, and it is known that rifampicin-pyrazinamide-ethambutol triple therapy for 6 months is effective. These observations alongside the high-rates of INH mono-resistance implicate INH as a “weak link” in preventing the ongoing emergence of MDR-TB.
Figure 1: Enrollments and Outcomes.
Cohort chart showing the numbers of participants screened, enrolled, and analysis eligible.
Criteria for exclusion at each level are shown.
Figure 2. Early bactericidal activities based on log10 transformed CFU counts.
Normalized bilinear piece-wise regression modelling showing the log_{10} CFU counts in sputum of participants by day of collection from the four study arms. The baseline log_{10} CFU counts for each study group are shown in Table 1.
Figure 3. Correlation between baseline CFU count and magnitude of EBA$_{0-2}$.
Linear regression fit for baseline log$_{10}$CFU counts versus EBA$_{0-2}$. The 95% confidence limits and 95% prediction limits are shown.
Table 1:
Baseline characteristics of randomized participants

| Characteristic                        | HRZE 1–14 (n=18) | RZE 1–2 (n=17) | HRZE 1–2– RZE 3–14 (n=16) | MRZE 3–14 (n=16) | All Participants (n=69) |
|--------------------------------------|------------------|----------------|---------------------------|------------------|------------------------|
| Male – no. (%)                       | 13 (72)          | 16 (94)        | 13 (81)                   | 15 (83)          | 57 (83)                |
| Race – no. (%)                       |                  |                |                           |                  |                        |
| Black African                        | 13 (72)          | 10 (59)        | 7 (44)                    | 11 (61)          | 41 (59)                |
| Coloured                             | 5 (28)           | 7 (41)         | 9 (56)                    | 7 (39)           | 28 (41)                |
| Age (years) – Mean (s.d.)            | 32 (12)          | 32 (8)         | 35 (12)                   | 33 (11)          | 33 (11)                |
| Karnofsky Score – Mean (s.d.)        | 89 (4)           | 89 (2)         | 88 (4)                    | 89 (3)           | 89 (4)                 |
| Body Mass Index (kg/m²) – Mean (s.d.)| 19.9 (3.5)       | 19.3 (2.5)     | 20.6 (4.2)                | 18.5 (2.4)       | 19.6 (3.2)             |
| HIV Positive – no. (%)               | 2 (11)           | 1 (6)          | 1 (6)                     | 0 (0)            | 4 (6)                  |
| Baseline Log10 Transformed CFU per ml Sputum – Mean (s.d.) | 5.63 (0.92) | 5.55 (1.26) | 6.15 (0.88) | 5.90 (0.84) | 5.80 (0.98) |
| Baseline TTP (hours) – Mean (s.d.)   | 113 (29)         | 117 (41)       | 104 (27)                  | 102 (16)         | 109 (29)               |
| Bilateral Disease by CXR – no. (%)   | 13 (72)          | 10 (59)        | 12 (75)                   | 14 (78)          | 49 (71)                |
| Cavities by CXR – no. (%)            | 14 (78)          | 16 (94)        | 14 (88)                   | 12 (67)          | 56 (81)                |
| AFB Smear Result – no. (%)           |                  |                |                           |                  |                        |
| (+1)                                 | 4 (22)           | 1 (6)          | 0 (0)                     | 1 (6)            | 6 (9)                  |
| (+2)                                 | 6 (33)           | 10 (59)        | 10 (63)                   | 6 (33)           | 32 (46)                |
| (+3)                                 | 8 (44)           | 6 (35)         | 6 (38)                    | 11 (61)          | 31 (45)                |
| Site – no. (%)                       |                  |                |                           |                  |                        |
| TASK Applied Science                 | 13 (30)          | 7 (16)         | 12 (28)                   | 11 (26)          | 43 (62)                |
| Lung Institute                       | 5 (19)           | 10 (38)        | 4 (15)                    | 7 (27)           | 26 (38)                |
Table 2:
Early bactericidal activities based on Log10 transformed CFU counts and TTP in hours by arm

| Arm No. | Treatment Arm | Primary Endpoint, CFU (n=62*) | Secondary Endpoint, TTP (n=63) |
|---------|---------------|-------------------------------|--------------------------------|
|         |               | EBA_{0-2} Mean (95% CI)       | EBA_{2-14} Mean (95% CI)       | EBA_{0-14} Mean (95% CI) | EBA_{0-2} Mean (95% CI) | EBA_{2-14} Mean (95% CI) | EBA_{0-14} Mean (95% CI) |
| 1       | HRZE_{1-14}   | 0.22 (0.02, 0.42)             | 0.11 (0.06, 0.16)              | 0.13 (0.09, 0.16)       | 34 (26, 42)              | 9 (6, 12)                | 13 (10, 15)               |
| 2       | HRZE_{1-2-}   | 0.30 (0.09, 0.51)             | 0.12 (0.07, 0.17)              | 0.14 (0.11, 0.18)       | 29 (21, 38)              | 9 (7, 12)                | 12 (9, 15)               |
| 3       | RZE_{1-14}    | 0.13 (−0.09, 0.35)            | 0.14 (0.08, 0.19)              | 0.13 (0.09, 0.17)       | 29 (20, 37)              | 13 (10, 16)              | 15 (12, 18)              |
| 4       | HRZE_{1-2-}   | 0.22 (0.10, 0.34)             | -                              | -                       | 31 (26, 35)              | -                        | -                        |
|         | MRZE_{3-14}   | -                             | -                              | -                       | -                        | -                        | -                        |

* Although 62 participants were eligible for the primary endpoint analysis.
### Table 3:
Characteristics of Participants with a Sharp Drop in EBA on Days 1–2

|                          | Not Sharp (N=32) | Sharp (N=17) | Total (N=49) | P-Value     |
|--------------------------|------------------|--------------|--------------|-------------|
| **Patients**             |                  |              |              |             |
|                          | 32 (65%)         | 17 (35%)     | 49 (100%)    |             |
| **Regimen**              |                  |              |              |             |
| HRZE days 1–2            | 24 (65%)         | 13 (35%)     | 37           | 1.00*       |
| RZE days 1–2             | 8 (67%)          | 4 (33%)      | 12           |             |
| **EBA₀–₂ Mean (s.d)**    |                  |              |              |             |
| All                      | -0.09 (0.36)     | 0.63 (0.22)  | 0.16 (0.47)  | <0.01       |
| HRZE days 1–2            | -0.02 (0.31)     | 0.61 (0.18)  | 0.20 (0.41)  | <0.01       |
| RZE days 1–2             | -0.28 (0.45)     | 0.69 (0.34)  | 0.04 (0.62)  | <0.01       |
| **EBA₀–₁₄ Mean (s.d)**   |                  |              |              |             |
| All                      | 0.11 (0.07)      | 0.19 (0.11)  | 0.13 (0.09)  | 0.03        |
| HRZE days 1–2            | 0.10 (0.07)      | 0.20 (0.12)  | 0.13 (0.10)  | 0.04        |
| RZE days 1–2             | 0.12 (0.08)      | 0.16 (0.11)  | 0.13 (0.09)  | 0.54        |
| **Baseline Log₁₀ CFU, Mean (s.d.)** |          |              |              |             |
| All                      | 5.51 (0.94)      | 6.20 (1.07)  | 5.75 (1.03)  | 0.03        |
| HRZE days 1–2            | 5.45 (0.94)      | 6.24 (1.20)  | 5.72 (1.09)  | 0.05        |
| RZE days 1–2             | 5.69 (0.96)      | 6.08 (0.58)  | 5.82 (0.85)  | 0.41        |
| **Log₁₀ CFU on day 2, Mean (s.d.)** |          |              |              |             |
| All                      | 5.68 (0.86)      | 4.93 (1.12)  | 5.42 (1.02)  | 0.02        |
| HRZE days 1–2            | 5.49 (0.73)      | 5.01 (1.27)  | 5.32 (0.97)  | 0.22        |
| RZE days 1–2             | 6.26 (1.02)      | 4.69 (0.46)  | 5.74 (1.14)  | <0.01       |
| **Log₁₀ CFU on day 14, Mean (s.d.)** |          |              |              |             |
| All                      | 3.90 (0.80)      | 3.81 (1.22)  | 3.87 (0.93)  | 0.80        |
| HRZE days 1–2            | 3.92 (0.80)      | 3.79 (1.32)  | 3.88 (0.95)  | 0.79        |
| RZE days 1–2             | 3.84 (0.85)      | 3.83 (1.15)  | 3.84 (0.91)  | 1.00        |
| **Sex**                  |                  |              |              |             |
| Male                     | 26 (81%)         | 15 (88%)     | 41 (84%)     | 0.70*       |
| Female                   | 6 (19%)          | 2 (12%)      | 8 (16%)      |             |
| **Age (years), Mean (s.d.)** |          |              |              |             |
|                          | 32.5 (10.7)      | 30.0 (8.0)   | 31.6 (9.8)   | 0.36        |
| **BMI (kg/m²), Mean (s.d.)** |          |              |              |             |
|                          | 19.6 (3.3)       | 19.4 (2.7)   | 19.6 (3.0)   | 0.82        |
| **HIV Status**           |                  |              |              |             |
| HIV negative             | 30 (94%)         | 15 (88%)     | 45 (92%)     | 0.60*       |
| HIV positive             | 2 (6%)           | 2 (12%)      | 4 (8%)       |             |

* F-test with Unequal Variance unless otherwise noted
* Fisher’s Exact Test