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CLINICAL THERAPEUTICS

Early Bactericidal Activity Trial of Nitazoxanide for Pulmonary Tuberculosis

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ABSTRACT This study was conducted in treatment-naive adults with drug-susceptible pulmonary tuberculosis in Port-au-Prince, Haiti, to assess the safety, bactericidal activity, and pharmacokinetics of nitazoxanide (NTZ). This was a prospective phase II clinical trial in 30 adults with pulmonary tuberculosis. Twenty participants received 1 g of NTZ orally twice daily for 14 days. A control group of 10 participants received standard therapy over 14 days. The primary outcome was the change in time to culture positivity (TTP) in an automated liquid culture system. The most common adverse events seen in the NTZ group were gastrointestinal complaints and headache. The mean change in TTP in sputum over 14 days in the NTZ group was 3.2 h ± 22.6 h and was not statistically significant ($P = 0.56$). The mean change in TTP in the standard therapy group was significantly increased, at 134 h ± 45.2 h ($P < 0.0001$). The mean NTZ MIC for *Mycobacterium tuberculosis* isolates was 12.3 μg/ml; the mean NTZ maximum concentration (C_{max}) in plasma was 10.2 μg/ml. Negligible NTZ levels were measured in sputum. At the doses used, NTZ did not show bactericidal activity against *M. tuberculosis*. Plasma concentrations of NTZ were below the MIC, and its negligible accumulation in pulmonary sites may explain the lack of bactericidal activity. (This study has been registered at ClinicalTrials.gov under identifier NCT02684240.)

KEYWORDS tuberculosis, nitazoxanide, bactericidal activity

Nitazoxanide (NTZ) is a safe, widely available anti-infective agent developed in the 1970s but has only recently been shown to be active against *Mycobacterium tuberculosis in vitro* (1–3). When tested against clinical isolates of drug-susceptible and drug-resistant strains of *M. tuberculosis*, NTZ was effective, with a median MIC of 16 μg/ml (2, 4). Even when 10^{12} CFU of *M. tuberculosis* was exposed to NTZ, no resistance was detected (2). NTZ appears to work via numerous mechanistic pathways, including affecting the cellular host response to *M. tuberculosis* and disrupting intrabacterial homeostasis (5, 6). NTZ's activity against *M. tuberculosis* has not been studied in clinical trials.

Early bactericidal activity (EBA) trials are a standard method for evaluating the safety, antimicrobial activity, and pharmacokinetics (PK) of new antituberculous drugs (7). Over the first 14 days of treatment, drug safety is monitored, sputum is collected overnight, and the amount of *M. tuberculosis* in each overnight sample is quantified. Therefore, we conducted an EBA trial of NTZ in treatment-naive adults with drug-susceptible pulmo-

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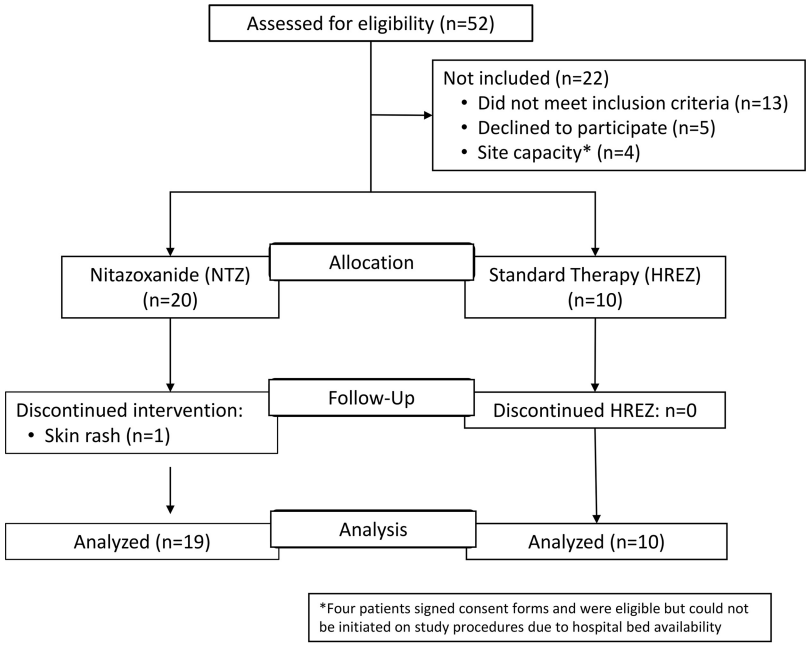

FIG 1 Participant flow chart.

nary tuberculosis (TB) in Port-au-Prince, Haiti. A dose of 1 g of NTZ twice daily was studied because previous reports indicated that this dose was sufficient to achieve a plasma level near the MIC of NTZ and that this was the maximum dose tolerated without a high proportion of people having treatment-limiting side effects (8–10). The main treatment-limiting side effects of NTZ are gastrointestinal complaints (abdominal pain, diarrhea, nausea, and vomiting) and headache.

In addition to monitoring safety and quantifying *M. tuberculosis* in sputum, we also systematically collected plasma and sputum samples for pharmacokinetic studies. The objective of measuring drug levels in sputum was to determine if NTZ accumulates in pulmonary secretions. Prior studies demonstrated that NTZ is highly plasma protein bound and therefore may not leave the blood compartment (10, 11). To our knowledge, this is the first time that sputum sampling for pharmacokinetic data has been included in an EBA trial.

RESULTS

Enrollment and participant characteristics. This study was conducted from February 2016 to April 2018. Fifty-two patients were assessed for eligibility. Thirty participants were enrolled, with 20 allocated to the NTZ treatment arm and 10 to standard four-drug therapy (isoniazid, rifampin, ethambutol, and pyrazinamide [HREZ]) (Fig. 1).

Thirteen participants (68%) in the NTZ arm and 7 (70%) in the standard therapy arm were male (Table 1). Median age in the NTZ group was younger than the median age in the standard therapy group. Extent and burden of disease as measured by presence of cavities and mycobacterial load were similar between the groups. All baseline cultures were susceptible to first-line antituberculous drugs based on phenotypic drug susceptibility testing.

Safety and tolerability. No grade 4 or 5 events occurred during this trial. One participant receiving NTZ developed a grade 3 skin rash on day 3, which was determined to be related to NTZ. NTZ was discontinued, and the participant was monitored in the hospital until the rash resolved. The participant was started on standard therapy. This was the only drug-related grade 3 event. All grade 1 and 2 adverse events related to NTZ or related to the standard four-drug therapy are listed in Table 2. The most common adverse events seen in both groups were gastrointestinal complaints and headache. The incidence rate of abdominal pain was 1.72 times larger in the NTZ group

TABLE 1 Demographic and clinical characteristics

Parameter[b]	Value for the group	
	NTZ ($n = 20$)	Standard therapy ($n = 10$)[a]
No. (%) of male subjects	13 (65)	7 (70)
Median age (yr [IQR])	24.5 (21, 27)	35.5 (25, 41)
Median weight (kg [IQR])	56 (48.5, 60.1)	53.8 (50, 57.5)
Median hemoglobin at baseline (g/dl [IQR])	10.7 (9.7, 11.5)	10.95 (9.5, 12.2)
Median ALT at baseline (U/liter [IQR])	27.5 (20.5, 46.5)	19.5 (18, 23)
Median AST at baseline (U/liter [IQR])	28 (20, 37.5)	22.5 (18, 29)
Median alkaline phosphatase at baseline (U/liter [IQR])	106.5 (92.5, 141.5)	94.5 (71, 115)
Median creatinine at baseline (mg/dl [IQR])	0.8 (0.7, 0.9)	0.7 (0.6, 0.9)
Bilateral disease present on chest radiography	11 (55)	6 (60)
Cavities present on chest radiography	14 (70)	6 (60)
AFB smear grade (no. [%] of subjects)		
0	5 (25)	1 (10)
1+	0 (0)	1 (10)
2+	9 (45)	6 (20)
3+	6 (30)	2 (20)
GeneXpert semiquantitative level (no. [%] of subjects)		
Very low	0 (0)	0 (0)
Low	1 (5)	1 (10)
Medium	5 (25)	3 (30
High	14 (70)	6 (60)
Baseline time (h) to positivity (mean ± SD)	90.6 ± 23.2	85.9 ± 9.2
Baseline $\log_{10}$ CFU/ml (mean ± SD)	6.5 ± 0.8	6.5 ± 0.4

[a]Standard therapy refers to rifampin, isoniazid, ethambutol, and pyrazinamide therapy.
[b]Standard therapy patients were older than NTZ patients ($P = 0.029$, by two-sample t test). All other characteristics did not differ between the two groups. ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFB, acid-fast bacillus.

than in the standard four-drug group ($P = 0.084$). The rate of other adverse events was comparable in those receiving NTZ single-drug therapy to that in those receiving standard four-drug therapy.

TTP and CFU counts. The mean change in time to positivity (TTP) in sputum over 14 days in the NTZ group was 3.2 h ± 22.6 h and was not statistically significant ($P = 0.56$). The mean change in TTP in the standard therapy group was significantly

TABLE 2 Grade 1 and 2 adverse events, stratified by treatment arm[a]

Event[d]	Value for the group			
	NTZ single-drug therapy ($n = 20$)		Standard four-drug therapy ($n = 10$)[b]	
	No. (%) of participants	No. of events[c]	No. (%) of participants	No. of events[c]
Abdominal pain[e]	17 (85)	43	7 (70)	13
Nausea	15 (75)	30	8 (80)	21
Vomiting	14 (70)	33	6 (60)	11
Diarrhea	8 (40)	19	4 (40)	7
Headache	9 (45)	17	5 (50)	13
Skin pruritus	1 (5)	1	0 (0)	0
Elevated AST	2 (10)	2	1 (10)	1
Elevated ALT	3 (15)	3	0 (0)	0
Elevated alkaline phosphatase	5 (25)	6	0 (0)	0
Other[f]	9 (45)	10	1 (10)	1
Total	20 (100)	164	10 (100)	67

[a]There was one grade 3 adverse event (rash) related to NTZ. There were no grade 4 events related to NTZ. There were no grade 3 or 4 adverse events related to standard therapy.
[b]Therapy with isoniazid, rifampin, ethambutol, and pyrazinamide (HREZ).
[c]Events are unique episodes of the described symptom. Participants may have had more than one event of the same symptom.
[d]AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[e]The incidence rate of abdominal pain in the NTZ single-drug treatment group was 1.72 times greater than that in the standard four-drug treatment group using Poisson regression ($P = 0.084$).
[f]Arthralgia, anorexia, hypoglycemia, hyperglycemia, loose stool, myalgia, palpitation, polyarthralgia, or scleral icterus.

TABLE 3 Time to positivity and change over time in CFU counts

Therapy and time period[a]	Mean (±SD) change in:	
	Time (h) to positivity (*P*)	Log_{10} CFU/ml (*P*)[b]
NTZ (*n*)		
Days 0–14 (18)	3.2 ± 22.6 (0.56)	0.3 ± 0.7 (0.104)
Days 0–2 (15)	4.8 ± 12.1 (0.15)	
Days 2–14 (14)	−1.3 ± 16.8 (0.79)	
HREZ (*n*)		
Days 0–14 (9)	134.0 ± 45.2 (<0.0001)	3.0 ± 1.3 (0.0001)
Days 0–2 (10)	61.3 ± 20.3 (<0.0001)	
Days 2–14 (9)	73.4 ± 37.8 (0.0004)	

[a]NTZ, nitazoxanide; HREZ is isoniazid, rifampin, ethambutol, and pyrazinamide (standard therapy).
[b]For NTZ, $n = 19$; for HREZ, $n = 10$.

increased at 134 h ± 45.2 h ($P < 0.0001$). The mean change in CFU counts over 14 days did not significantly decrease in the NTZ group but did significantly decrease in the standard HREZ therapy group (Table 3).

In a mixed-effects model there was no significant change in TTP values over 14 days in the NTZ group ($P = 0.672$). There was a significant change in the standard HREZ therapy group ($P < 0.0001$). Figure 2 shows the mean TTP values for each group over the 14 days, with an increase in the standard therapy group and no significant increase in the NTZ group.

Pharmacokinetics and MIC. Eighteen participants in the NTZ group completed the PK study. Three participants had two missing time points, and one sample arrived in the laboratory with an insufficient quantity of plasma. Therefore, the pharmacokinetic model used 137 metabolite data points from 18 participants. Tizoxanide (TZ) pharmacokinetics were best described by a one-compartment model with first-order absorption. TZ clearance and volume of distribution were estimated to be 14.13 liters/h (relative standard error [RSE], 7%) and 42 liters (RSE, 28%), respectively. Average maximum concentration (C_{max}) for NTZ participants was 10.2 μg/ml (6.5 and 18.4 μg/ml for the 5th and 95th percentiles, respectively). Individual participant pharmacokinetic profiles are shown in Table S2 in the supplemental material.

The MIC of NTZ was 12.3 μg/ml (interquartile range [IQR], 12.3, 12.3). The MIC was determined for *M. tuberculosis* laboratory strain H37Rv as a control and was also 12.3 μg/ml. Model simulations showed that TZ levels were rarely above the MIC. The

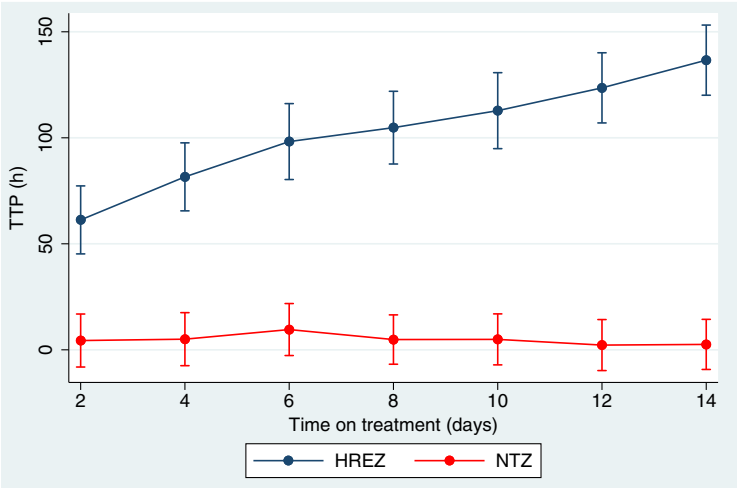

FIG 2 Time to positivity, in hours, over 14 days. Mean TTP (h) values and predictive margins with 95% confidence are plotted over time by the treatment arm after fitting of a mixed-effects model (HREZ group, $n = 10$; NTZ group $n = 19$).

TABLE 4 Sputum and plasma drug concentrations at 4 h postadministration on days 5 and 14

Drug[a]	Day	Mean (SD) concn (ng/ml) in:		Mean (SD) ratio of all the individuals (sputum/plasma)[b]
		Sputum	Plasma	
INH	5	1.8 (0.6)	1.8 (0.6)	1.0 (0.4)
	14	1.5 (0.7)	1.7 (0.8)	0.9 (0.2)
RIF	5	1.20 (1.7)	4.1 (1.2)	1.0 (0.4)
	14	0.7 (0.2)	3.9 (0.5)	0.9 (0.2)
ETM	5	2.5 (2.3)	1.6 (0.4)	1.0 (0.4)
	14	2.0 (0.8)	1.6 (0.3)	0.9 (0.2)
PZA	5	24.1 (6.9)	29.1 (4.9)	1.0 (0.4)
	14	13.7 (6.3)	25.8 (3.1)	0.9 (0.2)
TZ	5	0.3 (0.3)	11.3 (4.6)	0.03 (0.03)
	14	0.2 (0.2)	9.0 (4.1)	0.03 (0.03)

[a]INH, isoniazid; RIF, rifampin; ETM, ethambutol; PZA, pyrazinamide; TZ, tizoxanide (nitazoxanide's active metabolite).
[b]For all but TZ, data were available for $n = 10$ participants on days 5 and 14. For TZ, $n = 18$ participants at day 5, and $n = 16$ participants at day 14.

C_{max} for approximately 70% of the patients never reached the average MIC of 12 μg/ml. Three participants had C_{max}/MIC ratios above 1, indicating that the TZ concentration exceeded the necessary MIC. The change in TTP values over 14 days in these individuals was not statistically significant.

The sputum/plasma ratios for standard drugs (HREZ) were similar, ranging from 0.9 to 1.0, indicating that these drugs enter pulmonary secretions (Table 4). In contrast, the sputum/plasma ratio for TZ was 0.03, suggesting that TZ does not enter pulmonary secretions.

DISCUSSION

In this phase II trial, NTZ had no bactericidal activity against *M. tuberculosis* over 14 days of treatment. Negligible concentrations of NTZ in pulmonary secretions may explain this lack of bactericidal activity.

We did not detect significant bactericidal activity of NTZ as measured by either a change in TTP or a change in CFU counts over 14 days. Standard therapy (HREZ) showed a significant effect, consistent with that reported in the literature (7, 12), indicating that the laboratory methods used to quantify *M. tuberculosis* were valid. The sample size was chosen to detect a change in the TTP of 40 h, and so we cannot rule out that NTZ may have a smaller effect.

The average plasma C_{max} of TZ in participants receiving NTZ was 10.2 μg/ml, lower than the 12.3-μg/ml MIC required to inhibit growth of *M. tuberculosis*. The levels were also lower than those measured in a study of healthy males in Belgium who received 1 g of NTZ every 12 h with food (8). In our study, NTZ was given at 9 a.m. and 6 p.m. to align with hospital meals. Further differences in race, sex, health status, and diet may have resulted in lower drug concentrations than expected. A previous study demonstrated a 43% reduction in plasma NTZ concentrations when NTZ was taken without food (9). As our Haitian participants were ill, with 85% reporting stomach pain and 70% reporting vomiting, it is very possible that their food intake differed from that of the healthy Belgian subjects. The adverse event data from our study and data from prior reports suggest that a higher dose would not be well tolerated (8–11).

Compared to the sputum concentrations seen with standard therapy medications, the sputum concentration of NTZ was low, suggesting that it did not penetrate pulmonary lesions to a sufficient degree. TZ is highly bound to plasma proteins, which may have impacted its distribution and militated against a bactericidal effect against *M.*

tuberculosis in the lungs. Even if the plasma C_{max} of NTZ had exceeded the necessary MIC, the drug would likely still have not shown any effect on *M. tuberculosis* quantity in sputum, given that the concentration of NTZ in pulmonary secretions was almost nil.

To our knowledge, this is the first time that plasma and sputum concentrations of antituberculous drugs have been measured systematically in an EBA trial. This design provided critical understanding into the penetration of drugs into pulmonary secretions and an explanation for the absence of NTZ activity against *M. tuberculosis* despite highly suggestive *in vitro* studies. Further studies are needed to determine if the sputum C_{max} may be a good predictor of a drug's antibactericidal activity in pulmonary diseases. It would be informative to include sputum drug levels in future EBA studies.

Conclusions. The study did not show bactericidal activity of NTZ against *M. tuberculosis*. The low concentrations of TZ in plasma and in the lesions that were the source of the sputum samples in which *M. tuberculosis* was quantified likely limited the drug's effect against *M. tuberculosis*.

MATERIALS AND METHODS

Study setting and population. This study was conducted in an inpatient facility for patients with drug-susceptible tuberculosis at Groupe Haïtien d'Étude du Sarcome de Kaposi et des Infectieuses Opportuniste (GHESKIO), the largest HIV and tuberculosis treatment center in Haiti.

Participants were HIV-negative adult men and women presenting with symptoms suggestive of tuberculosis, chest radiography consistent with tuberculosis, and sputum smear positive score of at least 2+ or positive for *M. tuberculosis* by GeneXpert MTB/RIF test at the medium or high level without evidence of rifampin (RIF) resistance. Exclusion criteria included serious comorbidities, hemoptysis, hypoxia, extrapulmonary tuberculosis, history of tuberculosis treatment, use of antituberculous drugs within the past 30 days, pregnancy, or breastfeeding.

Trial design and registration. This was a phase II bactericidal activity trial of NTZ at a dose of 1 g administered orally twice daily with meals. The primary outcome of the current trial was the change in time in hours to *M. tuberculosis* culture positivity (TTP) from day 0 to day 14 in participants with pulmonary tuberculosis who received NTZ. We randomly assigned 10 additional participants to receive standard therapy (isoniazid [H], rifampin [R], ethambutol [E], and pyrazinamide [Z]) to assess the quality of assays used to quantify *M. tuberculosis* in sputum.

Ethical approval was granted by Weill Cornell and GHESKIO institutional review boards. Participants provided written informed consent prior to study participation. The trial was registered with ClinicalTrials.gov under identifier NCT02684240.

Screening for tuberculosis and enrollment. Patients with suspected TB were screened with spot and morning sputum samples. Spot sputum samples were graded according to U.S. Centers for Disease Control and Prevention (CDC) standards (13). Morning samples were used for the GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) molecular assay. All patients had chest radiography.

Randomization and treatment. Participants were randomized prior to the first dose of medication in a 2:1 fashion (NTZ/HREZ) using a computer-generated block allocation scheme. Laboratory staff were blinded to drug allocation, but hospital staff were not. Participants allocated to NTZ received nitazoxanide (Uniplus; Industria Farmaceutica del Caribe, Santo Domingo, Dominican Republic) at 1 g orally with meals at 9 a.m. and 6 p.m. daily. Prior studies demonstrated that NTZ must be taken with food for optimal absorption, with a 45% decrease in C_{max} between the fasting and fed states (9). Participants allocated to standard therapy received fixed-dose combination pills of isoniazid, rifampin, pyrazinamide, and ethambutol, dosed according to weight. Standard therapy was given with food at 9 a.m. daily. Medication administration was directly observed by hospital staff.

Follow-up of participants. Participants were hospitalized for the duration of the study. Overnight sputum samples were collected at bedside over 16 h (5 p.m. until 9 a.m. the following morning) in a single temperature-controlled box (2 to 8°C) starting at admission (baseline). Participants started either NTZ or HREZ, depending on treatment allotment after baseline overnight sputum samples were obtained. Subsequent overnight sputum samples were collected after 2, 4, 6, 8, 10, 12, and 14 days of therapy. Upon completion of 14 days of treatment, participants were discharged home on HREZ in accordance with World Health Organization and Haitian national guidelines to complete 6 months of therapy.

Assessment of safety. Participants were assessed for adverse events daily throughout hospitalization. Physicians asked about the commonly associated side effects of NTZ (gastrointestinal symptoms and headaches) as well as any additional symptoms using a structured questionnaire each morning. Participants were further assessed by hospital staff every 4 h for the occurrence of new symptoms. Adverse events were graded according to the grading system of the National Institutes of Health, Division of AIDS, and assessed for their relationship to NTZ (14, 15). A safety monitoring committee reviewed all safety issues after the first 10 participants completed the study and when a grade 3 or higher adverse event occurred in participants receiving NTZ.

***M. tuberculosis* quantification: TTP.** Quantification of time to positivity (TTP) utilizes an automated system and is comparable with determination of CFU counts (16–20). *M. tuberculosis* was quantified by the number of hours to positive signal in an automated liquid culture system. The minimum amount of

overnight sputum sample required for *M. tuberculosis* quantification was 5 ml, and all overnight sputum samples collected during this study exceeded that amount. Sputum samples were decontaminated via a 20-min incubation, with vortexing, with 5 ml of NALC-NaOH (3% sodium hydroxide, 0.5 to 0.6% N-acetyl-L-cysteine, 1.47% sodium citrate) (21). Automated liquid culture was performed in duplicate using a Bactec 960 instrument (Becton and Dickenson [BD], Franklin Lakes, NJ) according to the manufacturer's instructions. The endpoint was positive fluorescent signal indicating microbial growth. The reported TTP is the average of the two individual liquid cultures.

All positive liquid cultures were subcultured onto blood agar to monitor for contamination; contaminated cultures were excluded from analysis. Additionally, the presence of *M. tuberculosis* was verified using a TB Ag MPT64 Rapid (Standard Diagnostics, Republic of Korea) immunochromatography assay.

***M. tuberculosis* quantification: CFU count.** CFU assays were performed in duplicate on overnight sputum samples collected prior to treatment initiation and after 14 days of treatment as described previously (21). Briefly, decontaminated sputa were serially diluted (1:10) 10 times in liquid medium supplemented with an antimicrobial cocktail (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA; BD]), and dilutions were spread on Middlebrook 7H10 agar plates. Plates were incubated at 37°C and read after 3, 5, 7, and 9 weeks. Individual assays were terminated when no change in CFU count was observed over a 2-week interval (after 5, 7, or 9 weeks). The number of CFU per milliliter was calculated from dilutions from plates with 10 to 150 CFU. The reported CFU count is the average of the two dilution series.

Phenotypic drug susceptibility testing. Drug susceptibility testing for first-line antituberculous drugs was performed using Bactec MGIT 960 SIRE and PZA kits (BD, Franklin Lakes, NJ) on *M. tuberculosis* isolated from day 0. The NTZ MIC was determined for isolates from day 0 and day 14 overnight sputum samples. NTZ was serially diluted in dimethyl sulfoxide (DMSO) and incubated in triplicate with *M. tuberculosis* at an optical density at 600 nm (OD_{600}) of 0.01 in microtiter plates (range, 0.23 to 49.17 μg/ml) at 37°C until cells in DMSO-exposed control wells reached exponential phase. The MIC was defined as the minimum concentration of NTZ required to inhibit 90% of *M. tuberculosis* growth (IC_{90}). A control strain (H37Rv, ATCC 27294) was processed with each MIC batch.

Pharmacokinetics. Blood was collected in K_2EDTA-coated tubes 30 min prior to and 2, 4, and 6 h after drug administration on days 5 and 14 and centrifuged to obtain plasma. Sputum samples were systematically collected 4 h after drug administration on the same days. Samples were stored at −80°C and shipped on dry ice for analysis.

LC/MS-MS bioanalysis. NTZ is metabolized into its active form, tizoxanide (TZ), within 6 min of ingestion; therefore, only TZ was quantified (11). TZ (Cayman Chemical, Ann Arbor, MI) stock solutions were diluted in drug-free K_2EDTA human plasma (BioIVT, Westbury, NY) or gamma-irradiated pretreated sputum (provided by Laura Via, NIH, NIAID) to create standards and quality controls (QCs). Sputum samples were pretreated by dilution with 1 part phosphate-buffered saline (PBS) 7.4 buffer weight to volume and homogenized on a bead beater for 1 min to ease pipetting. Standards, QCs, controls, and study samples were extracted with acetonitrile-methanol (50/50) containing 100 ng/ml of the labeled internal standard tizoxanide-tetradeuterated tizoxanide (TZ-d4) (Toronto Research Chemicals, North York, ON, Canada). Liquid chromatography-tandem mass spectrometry (LC/MS-MS) was performed on an AB Sciex Qtrap 6500+ MS (Sciex, Concord, ON, Canada) coupled to a Shimadzu 30ACMP high-performance liquid chromatograph (HPLC) (Shimadzu, Columbia, MD). Chromatography was performed on an Agilent Zorbax SB-C_8 column (4.6 by 75 mm; 3.5-μm particle size) (Agilent, Santa Clara, CA). Multiple-reaction monitoring (MRM) of transitions 263.90/216.80 and 267.90/220.80 were used for TZ and TZ-d4, respectively. Analysis of HRZE was performed as previously published (22, 23).

Pharmacokinetic modeling. Data were analyzed using a nonlinear mixed-effects approach with NONMEN software, version 7.4 (Icon, Dublin, Ireland). An initial base model structure was established using the full pharmacokinetic profile data from TZ at day 5. Absorption delay was modeled using a lag time or chain-of-transit compartment model. Parameters were assumed to be log-normally distributed. Diagonal and full variance-covariance blocks of the parameter distributions were investigated. Additive, proportional, and combined error models were evaluated for residual variability. The first-order conditional estimation with interaction method was used for population pharmacokinetic parameter estimation. Individual *post hoc* Bayesian estimates of pharmacokinetic parameters were derived from the model. The model was evaluated using visual predictive checks and residual plots.

Sample size and data analysis. The primary analysis was to compare TTPs in each individual from day 0 to day 14 using a paired *t* test. We also compared TTPs in each individual from day 0 and day 2. To take advantage of the entire array of TTP measurements over time, a mixed-effects model was also fitted, and TTP means and 95% predictive margins were plotted over time. For CFU counts, bactericidal activity was calculated for days 0 and 14, and the difference between the two results was tested with a paired *t* test. Data analysis was performed using STATA, version 14 (Stata Corp., College Station, TX). With 20 participants receiving NTZ and an estimated loss to follow-up rate of 20%, we had 80% power to achieve an effect size of >1 with 0.05 significance. This translates to a difference in TTP of 40 h between day 0 and day 14 or a 0.5-log difference in CFU counts.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.03 MB.

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REFERENCES

1. Shakya A, Bhat HR, Ghosh SK. 2018. Update on nitazoxanide: a multifunctional chemotherapeutic agent. Curr Drug Discov Technol 15:201–213. https://doi.org/10.2174/1570163814666170727130003.
2. de Carvalho LP, Lin G, Jiang X, Nathan C. 2009. Nitazoxanide kills replicating and nonreplicating Mycobacterium tuberculosis and evades resistance. J Med Chem 52:5789–5792. https://doi.org/10.1021/jm9010719.
3. Piccaro G, Giannoni F, Filippini P, Mustazzolu A, Fattorini L. 2013. Activities of drug combinations against Mycobacterium tuberculosis grown in aerobic and hypoxic acidic conditions. Antimicrob Agents Chemother 57:1428–1433. https://doi.org/10.1128/AAC.02154-12.
4. Shigyo K, Ocheretina O, Merveille YM, Johnson WD, Pape JW, Nathan CF, Fitzgerald DW. 2013. Efficacy of nitazoxanide against clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother 57:2834–2837. https://doi.org/10.1128/AAC.02542-12.
5. Lam KK, Zheng X, Forestieri R, Balgi AD, Nodwell M, Vollett S, Anderson HJ, Andersen RJ, Av-Gay Y, Roberge M. 2012. Nitazoxanide stimulates autophagy and inhibits mTORC1 signaling and intracellular proliferation of Mycobacterium tuberculosis. PLoS Pathog 8:e1002691. https://doi.org/10.1371/journal.ppat.1002691.
6. de Carvalho LP, Darby CM, Rhee KY, Nathan C. 2011. Nitazoxanide disrupts membrane potential and intrabacterial pH homeostasis of Mycobacterium tuberculosis. ACS Med Chem Lett 2:849–854. https://doi.org/10.1021/ml200157f.
7. Jindani A, Aber VR, Edwards EA, Mitchison DA. 1980. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am Rev Respir Dis 121:939–949. https://doi.org/10.1164/arrd.1980.121.6.939.
8. Stockis A, De Bruyn S, Gengler C, Rosillon D. 2002. Nitazoxanide pharmacokinetics and tolerability in man during 7 days dosing with 0.5 g and 1 g b.i.d. Int J Clin Pharmacol Ther 40:221–227. https://doi.org/10.5414/cpp40221.
9. Stockis A, Allemon AM, De Bruyn S, Gengler C. 2002. Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. Int J Clin Pharmacol Ther 40:213–220. https://doi.org/10.5414/cpp40213.
10. Stockis A, Deroubaix X, Lins R, Jeanbaptiste B, Calderon P, Rossignol JF. 1996. Pharmacokinetics of nitazoxanide after single oral dose administration in 6 healthy volunteers. Int J Clin Pharmacol Ther 34:349–351.
11. Broekhuysen J, Stockis A, Lins RL, De Graeve J, Rossignol JF. 2000. Nitazoxanide: pharmacokinetics and metabolism in man. Int J Clin Pharmacol Ther 38:387–394. https://doi.org/10.5414/cpp38387.
12. Diacon AH, Dawson R, Hanekom M, Narunsky K, Venter A, Hittel N, Geiter LJ, Wells CD, Paccaly AJ, Donald PR. 2011. Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. Int J Tuber Lung Dis 15:949–954. https://doi.org/10.5588/ijtld.10.0616.
13. Kent PTK, George P. 1985. Public health mycobacteriology: a guide for the level III laboratory. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA.
14. National Institute of Allergy and Infectious Diseases. 2014. Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events. Version 2.0. National Institutes of Health, Bethesda, MD.
15. National Institute of Allergy and Infectious Diseases. 2010. Manual for expedited reporting of adverse events to DAIDS, version 2.0. National Institutes of Health, Bethesda, MD.
16. Diacon AH, Dawson R, Du Bois J, Narunsky K, Venter A, Donald PR, van Niekerk C, Erondu N, Ginsberg AM, Becker P, Spigelman MK. 2012. Phase II dose-ranging trial of the early bactericidal activity of PA-824. Antimicrob Agents Chemother 56:3027–3031. https://doi.org/10.1128/AAC.06125-11.
17. Diacon AH, van der Merwe L, Demers AM, von Groote-Bidlingmaier F, Venter A, Donald PR. 2014. Time to positivity in liquid culture predicts colony forming unit counts of Mycobacterium tuberculosis in sputum specimens. Tuberculosis (Edinb) 94:148–151. https://doi.org/10.1016/j.tube.2013.12.002.
18. Pheiffer C, Carroll NM, Beyers N, Donald P, Duncan K, Uys P, van Helden P. 2008. Time to detection of Mycobacterium tuberculosis in BACTEC systems as a viable alternative to colony counting. Int J Tuber Lung Dis 12:792–798.
19. Diacon AH, Maritz JS, Venter A, van Helden PD, Andries K, McNeeley DF, Donald PR. 2010. Time to detection of the growth of Mycobacterium tuberculosis in MGIT 960 for determining the early bactericidal activity of antituberculosis agents. Eur J Clin Microbiol Infect Dis 29:1561–1565. https://doi.org/10.1007/s10096-010-1043-7.
20. Bark CM, Gitta P, Ogwang S, Nsereko M, Thiel BA, Boom WH, Eisenach KD, Joloba ML, Johnson JL. 2013. Comparison of time to positive and colony counting in an early bactericidal activity study of antituberculosis treatment. Int J Tuber Lung Dis 17:1448–1451. https://doi.org/10.5588/ijtld.13.0063.
21. McAulay K, Saito K, Warrier T, Walsh KF, Mathurin LD, Royal-Mardi G, Lee MH, Ocheretina O, Pape JW, Fitzgerald DW, Nathan CF. 2018. Differentially detectable Mycobacterium tuberculosis in sputum from treatment-naïve subjects in Haiti and their proportionate increase after initiation of treatment. mBio 9:e02192-18. https://doi.org/10.1128/mBio.02192-18.
22. Prideaux B, Via LE, Zimmerman MD, Eum S, Sarathy J, O'Brien P, Chen C, Kaya F, Weiner DM, Chen P-Y, Song T, Lee M, Shim TS, Cho JS, Kim W, Cho SN, Olivier KN, Barry CE, Dartois V. 2015. The association between sterilizing activity and drug distribution into tuberculosis lesions. Nat Med 21:1223–1227. https://doi.org/10.1038/nm.3937.
23. Zimmerman M, Lestner J, Prideaux B, O'Brien P, Dias-Freedman I, Chen C, Dietzold J, Daudelin I, Kaya F, Blanc L, Chen PY, Park S, Salgame P, Sarathy J, Dartois V. 2017. Ethambutol partitioning in tuberculous pulmonary lesions explains its clinical efficacy. Antimicrob Agents Chemother 61:e00924-17. https://doi.org/10.1128/AAC.00924-17.