A pharmacometric approach to optimal use of second line drugs for multidrug-resistant tuberculosis

Richard Gray Court

MBChB, DipHIVMan (SA), FCP (SA), MMED (UCT)

CRTRIC005

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Supervisor: Professor Helen McIlleron

Co-supervisor: Professor Gary Maartens

DECLARATIONS

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- Court R, Wiesner L, Stewart A, de Vries N, Harding J, Gumbo T, Maartens G, McIlleron H. **Steady state pharmacokinetics of cycloserine in patients on terizidone for multidrug-resistant tuberculosis.** Int J Tuberc Lung Dis; 2018; 22(1): 30-33. PMID: 29297422

- Court R, Centner C, Chirehwa MT, Wiesner L, Denti P, de Vries N, Harding J, Gumbo T, Maartens G, McIlleron H. **Neuropsychiatric toxicity and cycloserine concentrations during treatment for multidrug-resistant tuberculosis.** Int J Infect Dis. 2021 Apr; 105:688-694. Epub 2021 Mar 5. PubMed PMID: 33684562

- Court R, Wiesner L, Chirehwa MT, Stewart A, De Vries N, Harding J, Gumbo T, McIlleron H, Maartens G. **Effect of lidocaine on kanamycin injection-site pain in patients with multidrug-resistant tuberculosis.** Int J Tuberc Lung Dis; 2018; 22(8): 926-930. PMID: 29991403

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Signature: Date: 26 May 2023

Student Name: Richard Gray Court Student Number: CRTRIC005

ABSTRACT

Background

Until the recent introduction of short course regimens, treatment regimens for multidrug-resistant TB (MDR-TB) were long and toxic. Consequently, only approximately half of MDR-TB patients completed their treatment. TB dosing guidelines have historically been unrefined with little consideration for pharmacokinetic/pharmacodynamic relationships. Large knowledge gaps therefore exist in the understanding of pharmacokinetic/pharmacodynamic relationships for both efficacy and toxicity in MDR-TB. My PhD used clinical pharmacology approaches to improve the understanding of drug exposures, toxicity, and exposure-toxicity relationships during the first 12 weeks of therapy in a cohort of MDR-TB patients at a TB hospital in South Africa.

Aims and methods

1. Using non-compartmental analyses, describe the pharmacokinetics of cycloserine and, using regression modelling, explore the association of covariates with cycloserine exposure.
2. Using validated screening tools, describe the incidence of neuropsychiatric toxicity in MDR-TB patients, and explore associations with cycloserine pharmacokinetics.
3. Using a validated pain-rating scale in a crossover study design, investigate whether the addition of a local anaesthetic reduces kanamycin-related injection pain, and explore effects on kanamycin pharmacokinetics.
4. Using geometric mean ratios, compare the exposures of crushed versus whole formulations of pyrazinamide, moxifloxacin, ethionamide, ethambutol, cycloserine, and isoniazid.

Results and conclusions

We found no measurable terizidone in plasma supporting the hypothesis that terizidone is hydrolysed pre-systemically to cycloserine. The cycloserine time-concentration profile supports once daily dosing of terizidone. We describe a high incidence of peripheral neuropathy in MDR-TB patients, with lower cycloserine clearance and high-dose pyridoxine significantly associated with a higher incidence of neuropathy. The addition of a local anaesthetic reduced the pain experienced by MDR-TB patients in the first 15 minutes post intramuscular administration of kanamycin, which could improve adherence to MDR-TB treatment. We also found the bioavailability of crushed

isoniazid to be approximately 42% less than the whole tablet formulation, and therefore recommend that the crushing of isoniazid be avoided. Although some recent treatment advances have improved MDR-TB outcomes, enhancing the understanding of drugs used to treat MDR-TB, which continues to have an unacceptably high mortality and treatment-related morbidity, is a public health priority. This thesis comprises four peer-reviewed publications, all of which made a pragmatic contribution to the fight against MDR-TB.

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This PhD journey, which began in 2015, would not have been possible without the love and support of my darling wife, Danielle. During this time, we have had two children, Zachary and Joshua. As a practicing physician during four relentless Covid-19 waves in South Africa, when the thought of continuing with academic work outside of the practicalities of confronting the pandemic seemed frivolous at times, Danielle's unwavering encouragement has seen me through thick and thin. This PhD is a celebration of both Danielle and my sacrifice – I look forward to one day sharing this story with our children.

No student can succeed without mentorship. My supervisors, Prof Helen McIlleron and Prof Gary Maartens have patiently and practically supported me in every way required to complete this thesis. Through the supervision of my MMed, Gary was an early inspiration for me in research; Helen's internationally respected expertise in TB drug pharmacokinetics was the foundation of my learning in this PhD. After some early disappointments in academics, I clearly remember leaving my undergraduate medical training never wanting to see, let alone study at, a university ever again. Completing my PhD as a specialist physician therefore feels surreal and is a direct reflection of Helen and Gary's mentorship and the division of Clinical Pharmacology at the University of Cape Town, which invests in people before investing in science.

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PREFACE

The PODrTB study (Pharmacometric Optimisation of second line Drugs in the treatment of multidrug-resistant TB) was an observational study exploring pharmacokinetic (PK)/pharmacodynamic relationships in patients during the intensive phase of therapy for MDR-TB. The overall aim of PODrTB was to identify doses and drug combinations to treat MDR-TB that were better than the standard of care at the time of study. PODrTB was funded by the National Institutes of Health, R01AI116155 (principal investigators: Helen McIlleron/Tawanda Gumbo). I was appointed as the medical officer for PODrTB at the time of the study initiation in 2015, responsible for driving recruitment and participant follow-up, supervising the study nurse, overseeing study logistics and maintaining the relationship with the study sites. PODrTB laid the platform for me to generate the research questions, which formed the aims of this PhD thesis.

The opportunity to work towards this PhD was timeous following the completion of my specialist training in Internal Medicine at the University of Cape Town (2011-2015), which included a three-month rotation at Brooklyn Chest and DP Marais TB Hospitals. Whilst working at these TB hospitals (which would later become the study sites for PODrTB), I was witness not only to the unacceptably poor outcomes of patients treated for drug-resistant TB, but also to other treatment-related challenges experienced by patients, including a high pill burden and painful daily injections needed for the administration of intramuscular kanamycin, which until 2019, was included in WHO MDR-TB treatment regimens. PODrTB presented a unique opportunity for me to contribute to the understanding and improvement of drug dosing in MDR-TB, and to enhance my research career. On a personal note, my passion for understanding and improving TB treatment was birthed when I became ill with TB as an undergraduate medical student in 2003. Although my infection was fortunately sensitive to first line anti-TB drugs, I experienced first-hand the challenge of completing a chronic TB treatment regimen with related adverse effects.

The research questions were planned with four separate analyses (see chapters 2-5), two of which were sub-studies related to the parent PODrTB protocol (1,2). Under the supervision of Professors Helen McIlleron and Gary Maartens, I designed the protocols for both of these sub-studies and submitted them for institutional ethics review. All four studies have been published as original articles in peer-reviewed international journals (1–4). Three of the manuscripts were also presented

at international conferences. Several other manuscripts, which I co-authored, were published using the PODrTB data, and although not formally included as thesis manuscripts, were relevant both to the learning and outputs derived from this PhD (5–7).

With the help of a research nurse, I successfully recruited the PODrTB cohort to target, supervised clinical follow-up of participants, and managed the collection and transport of PK and other biological samples. I also engaged with and provided input to the creation of the electronic database, (RedCap®), which we used for data capture, and assisted with data cleaning and preparation. Under Helen and Gary's supervision and mentorship, I learned and performed the statistical work required for the four manuscripts including (but not limited to): descriptive statistics, non-compartmental PK analysis, non-parametric tests, geometric mean ratios, linear/logistic regressions, and Kaplan Meier analyses. I wrote the first draft and was the corresponding author through the editorial process of all four manuscripts. The first three manuscripts were all published in the International Journal of TB and Lung Disease (IJTLD), which has a wide readership including clinicians, pharmacologists and researchers, and therefore was an appropriate platform for dissemination of the data. The cycloserine-neurotoxicity manuscript (chapter 3), which was the most complex of the four manuscripts from an analytical point of view, was published in 2021 in a specialist infectious disease journal (International Journal of Infectious Diseases).

Although management guidelines for MDR-TB have evolved considerably since the initiation of PODrTB with the rollout of bedaquiline and other drugs repurposed for the treatment of TB, several drugs which we studied are still key inclusions in WHO MDR-TB treatment guidelines, and therefore remain highly relevant in current practice.

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CHAPTER 1: INTRODUCTION

Optimising approaches to treat multidrug-resistant tuberculosis (MDR-TB) has become a public health priority. Approximately 157,903 people were infected with RR-TB tuberculosis (RR-TB) in 2020, including pre-XDR and XDR-TB (extensively drug-resistant TB) (1). Multidrug-resistant TB is defined as mycobacterial TB infection (M-TB), which is resistant to both rifampicin and isoniazid; Pre-XDR TB, which was previously defined as MDR-TB infection with additional resistant to fluoroquinolones or injectables, was updated by the WHO in 2022 to be M-TB infection fulfilling the criteria for MDR-TB plus resistance to fluoroquinolones. XDR-TB, which was previously defined as MDR-TB infection plus resistance to fluoroquinolones and injectables, now refers to MDR-TB infection with resistance to fluoroquinolones plus any one of the WHO Group A drugs (2). The WHO group classification stage of MDR-TB drugs, which is used to construct long treatment regimens for patients who do not qualify for the standardised shortened MDR-TB treatment regimen, includes three groups (A, B, and C) based on evidence-supported effectiveness. Group A includes the fluoroquinolones (levofloxacin and moxifloxacin), bedaquiline and linezolid for inclusion in all regimens unless contraindicated, Group B includes clofazimine and cycloserine (or terizidone) as conditional recommendations as second-choice TB drugs, and Group C included a list of substitute drugs when agents from Group A or B cannot be used. Group C drugs are ranked sequentially by the relative drug-specific balance of benefit to harms (3).

Approximately 15% of patients with RR-TB or MDR-TB die from their disease, of whom 26% die from XDR-TB (3). Until the recent introduction of a short course regimen for select patients (4), drug regimens for MDR-TB have been long (18-24 months) with a high burden of treatment-related adverse effects (5,6). Consequently, only approximately half of MDR-TB patients have been reported to complete their treatment (1). Dosing guidelines in the management of TB have traditionally been unrefined with little consideration for population-specific co-variates or local resistance profiles. Understanding drug exposure in the context of pharmacokinetic/pharmacodynamic (PK/PD) relationships is key to dose adjustment recommendations. As supratherapeutic drug exposures may increase a participant's risk of treatment-related adverse effects, low drug exposure may also increase the risk of poor clinical outcomes, including the development of drug resistance (7–9). Few studies have prospectively collected outcomes data in MDR-TB. Studies describing the high burden of adverse events in MDR-

TB patients have typically been retrospective, and seldom included an analysis of drug exposure (6,10–12). Large knowledge gaps therefore exist in the understanding of PK/PD relationships of second line TB drugs.

Since the design of the studies comprising this thesis, the landscape of MDR-TB management guidelines has changed considerably. At the time of study initiation, the South African national MDR-TB treatment guidelines included a six-month intensive phase regimen (kanamycin, moxifloxacin, ethionamide, terizidone, and pyrazinamide) with a 12-18 month continuation phase of the same drugs, excluding kanamycin (13). Local guidelines in the city of Cape Town (where the study recruitment sites were situated) also recommended the addition of ethambutol and isoniazid for patients diagnosed with rifampicin-resistant (RR-TB) using GeneXpert® PCR testing (14). Either ethionamide or isoniazid were discontinued depending on the results of the baseline line-probe assay for *katG* and *inhA* mycobacterium tuberculosis mutations identified in pretreatment sputum cultures, indicating high-level resistance to isoniazid or ethionamide, respectively (15). Bedaquiline is a novel TB drug approved by the United States Food and Drug Administration, which was made available to TB patients in South Africa through a clinical access programme in 2012 (16). Initially, bedaquiline was reserved for the treatment of pre-XDR or XDR-TB, or as a substitute drug for treatment-related toxicities in MDR-TB patients (17). In 2018, a shorter 9-12 month treatment regimen containing bedaquiline and other repurposed drugs including clofazimine and levofloxacin, was made available for select MDR-TB patients in South Africa where the risk of resistance to second line drugs was considered to be low (4). A longer regimen of 18-20 months is still used for MDR-TB patients not eligible for the short-course regimen, with a minimum of four drugs recommended at treatment start, including all three drugs in group A (levofloxaxcin/moxifloxacin, bedaquiline and linezolid) plus one of the drugs in group B (clofazimine and cycloserine/terizidone). A group C drug (ethambutol, delamanid, pyrazinamide, imipenem-cilastatin/meropenem, amikacin/streptomycin, ethionamide/prothionamide or para-aminosalycylic acid) may be used as a substitute drug when one of the group A or group B drugs cannot be used (4). In 2018, which to my knowledge, is the most recent cohort for which data is available, global treatment success rates for MDR-TB have improved to 59% (1). It is likely that the use of new and repurposed drugs including bedaquiline, as well as advances in earlier detection of rifampicin-resistance with the widespread rollout of GeneXpert® PCR testing have contributed to the observed improvement in outcomes (18). However, some “older” MDR-TB drugs are still relevant in current practice including

cycloserine/terizidone which, tolerability permitting, is currently a priority inclusion in long WHO MDR-TB treatment regimens (Group B), while others including ethionamide and aminoglycosides, excluding kanamycin, are considered substitute drugs (Group C) (3).

For some second line TB drugs e.g., cycloserine, an enhanced understanding of pharmacokinetics has been urgently required; guidelines on dosing frequency to achieve therapeutic concentrations have also been unclear. Prior to the initiation of my PhD studies, data on the PK of terizidone, which is a structural analogue of cycloserine, was scarce. Zitkova et al. previously reported the PK of cycloserine dosed as terizidone in a small single dose study in TB patients in 1974, but the method used to measure the drug concentrations i.e., calorimetry is no longer used (19). The recommended therapeutic target plasma cycloserine concentration has historically been 20-35 μL/mL (20), but exposure thresholds for the drug's toxicity have not been well defined. Cycloserine or "psych-serine" as it has previously been referred to (21), is associated with severe neuropsychiatric adverse effects, including depression and psychosis, which has limited widespread use of the drug in TB treatment programmes. The presence of these neuropsychiatric adverse events commonly leads to the withdrawal of cycloserine from MDR-TB treatment regimens (5,11). Peripheral neuropathy is another neurotoxic adverse event which has been observed, albeit uncommonly, in early reports of patients treated with cycloserine (22,23). More recently, cycloserine dosed as terizidone was shown to increase the incidence of neuropathy in MDR-TB patients, although the increase was not statistically significant (24). Few studies have prospectively and systematically collected neuropsychiatric toxicity data in patients treated with cycloserine/terizidone for MDR-TB. Many studies have reported data on specific neuropsychiatric adverse events in MDR-TB patients retrospectively, and often via patient self-report (5,6,10–12,25) - information on drug exposure in adverse event reports in MDR-TB patients is also usually lacking. Several small case studies/series have indicated that symptoms of psychosis are more likely to occur with cycloserine concentrations >35 μL/mL (26,27), but well-powered PK studies analysing factors, which affect cycloserine exposure, are required to appropriately explore these toxicity thresholds. It was previously considered that terizidone, which consists of two molecules of cycloserine (28), may be less toxic than cycloserine but a recent report demonstrated no significant safety difference between the two formulations (29).

Kanamycin was considered a key second line TB drug before an important meta-analysis, published in 2018, showed the efficacy of kanamycin to be poor (30). Consequently, kanamycin is no longer recommended by the WHO for inclusion in MDR-TB treatment regimens but is still used in some settings where newer TB drugs may not yet be available (31). The toxicity associated with the use of kanamycin, including irreversible hearing loss, is well known (32). The activity of kanamycin is concentration dependent (33); the relationship between the area under the concentration-time curve and audio-toxicity has also been well described (34). However, the pain caused by the intramuscular administration of kanamycin, which is often not included in reports describing adverse effects in patients treated for MDR-TB, may also be debilitating with painful lumps developing at the injection site (35). It is reasonable to assume that the pain caused by the administration of injectables, has contributed to poor treatment completion rates in MDR-TB (36). Lidocaine, a local anaesthetic, has been used with success to limit the pain caused by the intramuscular administration of some drugs in other clinical settings, including ceftriaxone and penicillin G (37,38). At the time of study initiation, it was unknown whether lidocaine could reduce the pain experienced by participants treated with injectables for MDR-TB, and if the addition of lidocaine could affect the pharmacokinetics of kanamycin. Considering the burdensome toxicity profile of aminoglycosides, the recent transition of MDR-TB treatment to injectable-free regimens has come as a relief to clinicians and patients alike. Although kanamycin is now seldom used, other aminoglycosides (i.e., amikacin or streptomycin) are still recommended as substitute drugs (group C) where aminoglycoside sensitivity can be demonstrated, and facilities are in place to monitor for hearing loss (3). It is therefore important to know whether lidocaine reduces injection-related pain when co-administered with an aminoglycoside, potentially improving adherence to the drug in the long-term. By reducing injection-related pain, an opportunity therefore exists to reduce pain and suffering in patients treated with injectables for MDR-TB.

The heavy pill burden in the multi-drug MDR-TB treatment regimen has historically been another challenge for MDR-TB patients. The high number of tablets/capsules consumed daily for long durations is a particular challenge for patients unable to swallow whole tablets (e.g., children or in patients with a depressed level of consciousness). In an attempt to improve tolerability of the treatment regimen, TB drugs are therefore regularly crushed by hospital nursing staff or home carers and mixed with water prior to dosing. However, tablet crushing has been shown to affect the bioavailability of some important TB drugs e.g., rifapentine (39), but has no effect on other drugs

commonly used in TB treatment centres, including lopinavir (40). At the time of this study, it was unknown whether crushing affects the bioavailability of the drugs used to treat MDR-TB, which is important considering that low drug concentrations may affect treatment outcomes, including the development of drug resistance (8,9,41). A recent report by Winckler et al, published after the outputs from this PhD, also described low isoniazid exposures in children treated for MDR-TB, many of which were treated with a crushed formulation (42).

The aims of this thesis are therefore as follows:

1. Describe the pharmacokinetics of cycloserine in patients treated for MDR-TB.
    1.1. Use non-compartmental analyses to describe key pharmacokinetic measures including peak concentration, area under the concentration-time curve to 10 hours (AUC_{0-10}), and half-life in the first subset of recruited participants in PODrTB.
    1.2. Use multivariate logistic regression modelling to explore the association of selected covariates with cycloserine AUC_{0-10}.

2. Describe the incidence of neuropsychiatric toxicity and the association of cycloserine exposure with specific treatment-related adverse effects in MDR-TB patients.
    2.1. Use validated screening tools to prospectively monitor the incidence of psychosis, depression, and peripheral neuropathy in the PODrTB cohort.
    2.2. Explore whether key cycloserine pharmacokinetic measures are associated with these specific neuropsychiatric adverse events.

3. Explore whether the addition of a local anaesthetic reduces the pain caused by the injection of kanamycin in MDR-TB patients.
    3.1. Using a validated pain-rating scale in a crossover study design, investigate whether the addition of lidocaine reduces the pain experienced by participants treated with intramuscular kanamycin.
    3.2. Assess whether the addition of lidocaine to the kanamycin solution affects the pharmacokinetics of kanamycin post administration.

4. Explore whether crushing affects the bioavailability of drugs used to treat MDR-TB.
    4.1. Using geometric mean ratios with a sequential study design, compare the AUC_{0-10} and peak concentration of crushed versus whole formulations of pyrazinamide, moxifloxacin, ethionamide, ethambutol, terizidone, and isoniazid.

The PODrTB study (referred to in the preface) was the platform used to complete the objectives of this thesis. PODrTB was an observational PK study of patients on standard treatment (18-24 months) for RR-TB which, during the time of study, included: kanamycin, moxifloxacin, ethionamide, cycloserine dosed as terizidone, isoniazid, ethambutol, and pyrazinamide. PODrTB included a prospective collection of toxicity data using validated collection tools, and serial MGIT sputum samples to monitor treatment response (see Annexure: PODrTB study design details and outcomes).

The specific questions generated to be answered in this thesis were designed to make a pragmatic contribution to the management of patients with MDR-TB, the outcome of which has historically been poor. Although the four studies can be considered stand-alone, they are closely linked in focussing on PK knowledge gaps for key drugs in particular cycloserine, which is studied in three of the four manuscripts. The four studies, which constitute this thesis, laid the platform for further important collaborative PK/PD analyses of second line TB drugs - collectively another step towards improving the safety, tolerability and ultimately treatment success in the fight against MDR-TB.

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CHAPTER 2

Steady state pharmacokinetics of cycloserine in patients on terizidone for multidrug-resistant tuberculosis.

R. Court,* L. Wiesner,* A. Stewart,† N. de Vries,‡ J. Harding,§ G. Maartens,* T. Gumbo,¶ H. McIlleron*

*Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, †Clinical Research Centre, Health Sciences Faculty, University of Cape Town, Cape Town, ‡Brooklyn Chest Hospital, Cape Town, §D P Marais Hospital, Cape Town, South Africa; ¶Center for Infectious Diseases Research and Experimental Therapeutics, Baylor Research Institute, Baylor University Medical Center, Dallas, Texas, USA

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Cycloserine and its structural analogue terizidone, were reclassified from a group C to a group B drug by the WHO in 2019, after a recent meta-analysis showed cycloserine to more efficacious than some anti-TB drugs (1). Tolerability permitting, cycloserine/terizidone are therefore now indicated for inclusion in long treatment regimens for MDR-TB (2). Prior to our work, little was known regarding the pharmacokinetics of cycloserine dosed as terizidone. In 1974, Zitkova et al. described cycloserine and terizidone pharmacokinetics in a small cohort of patients, but the method used to measure the drug concentrations (calorimetry) is no longer used (3). Other studies describing cycloserine exposure were either single-dose or small studies, which measured cycloserine pharmacokinetics after a limited treatment duration. Cycloserine is rapidly absorbed under fasting conditions; absorption is moderately decreased after eating particularly with a high fat meal (4). The C_{max} of cycloserine is reached approximately two hours post-dose; the half-life of cycloserine is approximately seven hours – plasma sampling in PK studies should therefore be scheduled three to four days post treatment initiation to allow exposures to reach steady-state (5). Target cycloserine concentrations have historically been 20-35 μg/mL, but there is limited data supporting this, and exposure thresholds for efficacy and toxicity have not been well defined (5). Cycloserine is eliminated renally (3), requiring dose adjustment in patients with renal impairment.

Using non-compartmental analysis, we described the pharmacokinetics of the first 35 participants enrolled in the PODrTB cohort, whose treatment regimens included cycloserine dosed as terizidone. To our knowledge at the time of publication, our report was the largest description of cycloserine pharmacokinetics measured at steady-state in MDR-TB patients. We found no measurable terizidone in plasma by liquid chromatography mass spectrometry, supporting the

hypothesis that terizidone is completely hydrolysed pre-systemically to cycloserine (6). The time-concentration profile of cycloserine at steady state shown in figure 1 suggests possible accumulation and, together with its long half-life, indicate that once daily dosing of terizidone, as per current WHO guidelines, is appropriate.

I presented our cycloserine non-compartmental analysis data at the 48th World Union meeting on TB and Lung health in Guadalajara, Mexico in October 2017 (Abstract no: SOA-419-13), and at the 10th International Conference on TB pharmacology, October 2017, Atlanta, US. (Abstract no: 20). Prof Helen McIlleron also presented this data at a TB pharmacokinetic/pharmacodynamic technical meeting convened by the WHO in 2018, which made a significant contribution to current terizidone dosing guidelines (7).

2.1 ABSTRACT

Setting

Terizidone/cycloserine is included in standard treatment regimens for multidrug-resistant tuberculosis (MDR-TB) treatment regimens in many countries. The steady state pharmacokinetics of cycloserine after terizidone administration are unknown.

Objectives and design

We recruited in-patients treated with 250-750 mg oral terizidone daily as part of standard treatment regimens for pulmonary MDR-TB in Cape Town, South Africa. Plasma cycloserine assays were performed in samples taken pre-dose and at 2, 4, 6, 8 and 10 hours post dose. Cycloserine concentrations were measured using a validated liquid chromatography tandem mass spectrometry method. Non-compartmental pharmacokinetic analyses were performed.

Results

Of 35 participants enrolled: 22 were males and 20 (57%) were infected with the human immunodeficiency virus, the median age was 37 years. The median duration on terizidone at the time of sampling was 33 (IQR: 28-39) days. The area under the concentration-time curve at 0-10 hours (AUC_{0-10}) was 319 (IQR: 267.5-378.7) µg.hr/mL, and peak concentration was 38.1 (IQR: 32.6-47.2) µg/mL. On multiple regression, dose (mg/kg) was the only factor independently associated with $AUC_{0-10.}$

Conclusion

Steady state concentrations of cycloserine in patients treated with terizidone for MDR-TB were higher than those reported with cycloserine formulations. Our findings support once-daily dosing.

Key words: dose frequency, dose duration, HIV

2.2. INTRODUCTION

Terizidone is a pro-drug of cycloserine and consists of two linked molecules of cycloserine (8). Cycloserine is categorized as a group C anti-tuberculosis drug by the World Health Organization (WHO) and is widely used for the treatment of drug resistant tuberculosis (TB) (9). Terizidone has been used as a source of cycloserine in some countries, including South Africa. A meta-analysis of retrospective data reported that terizidone may be better tolerated than cycloserine, however the difference was not statistically significant (8). A literature search revealed one single dose study published in 1974 by Zitkova and Tousek of 25 patients with TB comparing cycloserine and terizidone pharmacokinetics in patients given both drugs at different doses (3). However, the method used to measure drug concentrations (calorimetry) is non-specific and is no longer used to measure drug concentrations. Furthermore, single dose studies give limited data on drug exposure. An enhanced understanding of the pharmacokinetics and pharmacodynamics of cycloserine administered as terizidone is important for dose optimization. Terizidone hydrolyses pre-systemically to cycloserine (6). We performed a prospective pharmacokinetic study of cycloserine at steady state in patients treated with terizidone for pulmonary MDR-TB at two TB hospitals in Cape Town, South Africa.

2.3. STUDY POPULATION AND METHODS

Study design

We recruited patients ≥18 years initiated on treatment for pulmonary MDR-TB within the previous month. Patients were enrolled between July 2015 and January 2016 at two TB hospitals in the Western Cape province of South Africa, the Brooklyn Chest Hospital and DP Marais Hospital. During the study period, the standard regimen used to treat MDR-TB in Cape Town consisted of daily pyrazinamide, moxifloxacin, kanamycin, terizidone, and either ethionamide or isoniazid depending on the results of the line probe assay for katG and inhA Mycobacterium tuberculosis mutations identified in the pretreatment sputum cultures, which would indicate high level isoniazid or ethionamide resistance respective (10). Ethambutol was added if a patient had not been treated with ethambutol for ≥ one month before starting MDR treatment, and if suspicion for ethambutol resistance was low. After at least two weeks of treatment, patients received a dose of either 250,

500 or 750 mg of terizidone in accordance with national guidelines during the study period (11). However, dosing was modified for weight and creatinine clearance at the discretion of the treating clinician. Dosing was directly observed and performed under fasting conditions. Blood samples for cycloserine assays were collected pre-dose and at 2, 4, 6, 8 and 10 hours post dose. The blood samples were centrifuged, and the plasma was frozen within 30 minutes of sampling and stored at -80°C.

Cycloserine assays were performed in the Division of Clinical Pharmacology of the University of Cape Town, South Africa. The method was validated for the quantification of cycloserine in plasma and consisted of a protein precipitation extraction and derivatization of protein, followed by high performance liquid chromatography with tandem mass spectrometry detection. An AB Sciex API 3000 mass spectrometer (GenTech Scientific, Arcade, NY, USA) at unit resolution in the multiple reaction monitoring (MRM) mode was used to monitor the transition of the protonated precursor ion m/z 335.9 to the product ion m/z 157.2. Electro Spray Ionization (ESI) was used for ion production. The assay was validated over the concentration range of 0.313 – 40.0 µg/mL. The accuracy of the combined low (0.783 µg/mL), medium (16.0 µg/mL) and high (32.0 µg/mL) quality controls was between 98.6 and 102.0%, and the coefficient of variation was < 11.5%.

Statistical analyses

Stata version 13.1 (StataCorp; College Station, Texas, USA) was used for non-compartmental pharmacokinetic analysis and to perform the statistical analyses. Peak concentrations, area under the concentration-time curve at 0-10hr (AUC_{0-10}) and half-life for each patient were assessed. As AUC_{0-10} displayed a skewed distribution, it was log-transformed before using linear regression to identify factors associated with plasma cycloserine exposure. The following variables were identified a priori and analysed using single and multiple regression analyses: sex, age at study enrolment, human immunodeficiency virus (HIV) status, body mass index, duration on terizidone, creatinine clearance, and dose in mg/kg.

Ethics

This study protocol was approved by Human Research Ethics Committee of the University of Cape Town (HREC 065/2015). Written informed written consent was provided by all participants in their language of choice.

2.4. RESULTS

Thirty-five participants were enrolled (Table 1) The median time from start of treatment commencement until blood draw for cycloserine assays was 33 (range: 18-56) days. On the day of blood sampling, 28 participants received 750mg terizidone, 6 participants received 500mg terizidone and one participant, who had significantly reduced creatinine clearance, received 250mg terizidone (see cycloserine concentration-time curves in the Figure).

Table 1. Characteristics of 35 participants at steady state intensive pharmacokinetic sampling on treatment for multidrug-resistant tuberculosis

No. of participants		Male:22; Female:13
Median age (years)		37 (IQR: 28-46)
Median BMI (kg/m^2)		17.8 (IQR: 15.6-20.1)
HIV status		Positive: 20; Negative:15
On ART at time of pharmacokinetic sampling	Efavirenz*	n=10
	Lopinavir**	n=1
Median creatinine clearance (mL/min)		76.1 (IQR: 49.3-101.9)
No. with creatinine clearance <50 mL/min		10 (28.6%)
Median dose (mg/kg)		14.4 (IQR: 13.4-16.0)
Dose per weight range (mg)	750; n=28	39-73kg
	500; n=6	33-38kg
	250; n=1	43kg

BMI: Body Mass Index; HIV: Human immunodeficiency virus; IQR: Interquartile Range; ART: Antiretroviral therapy; *including 2 nucleoside/tide reverse transcriptase inhibitors; ** Boosted with ritonavir

Table 2 gives the steady state pharmacokinetic measures of cycloserine. The half-life of cycloserine for eight participants could not be calculated, as the cycloserine concentration was either increasing or not decreasing at the final time of sampling.

Table 2. Median pharmacokinetic measures in 35 participants at steady state on multi-drug resistant tuberculosis therapy

T_{max}	4 hours
C_{max}	38.1 (IQR: 32.6-47.2) µg/mL
AUC_{0-10}	319 (IQR: 267.5-378.7) µg.hr/mL
Half-life (n=27)	14.7 (IQR: 9.5-14.8) hours

Tmax: time to maximum concentration, C_{max}: maximum concentration, AUC_{0-10}: 10 hours area under the concentration-time curve

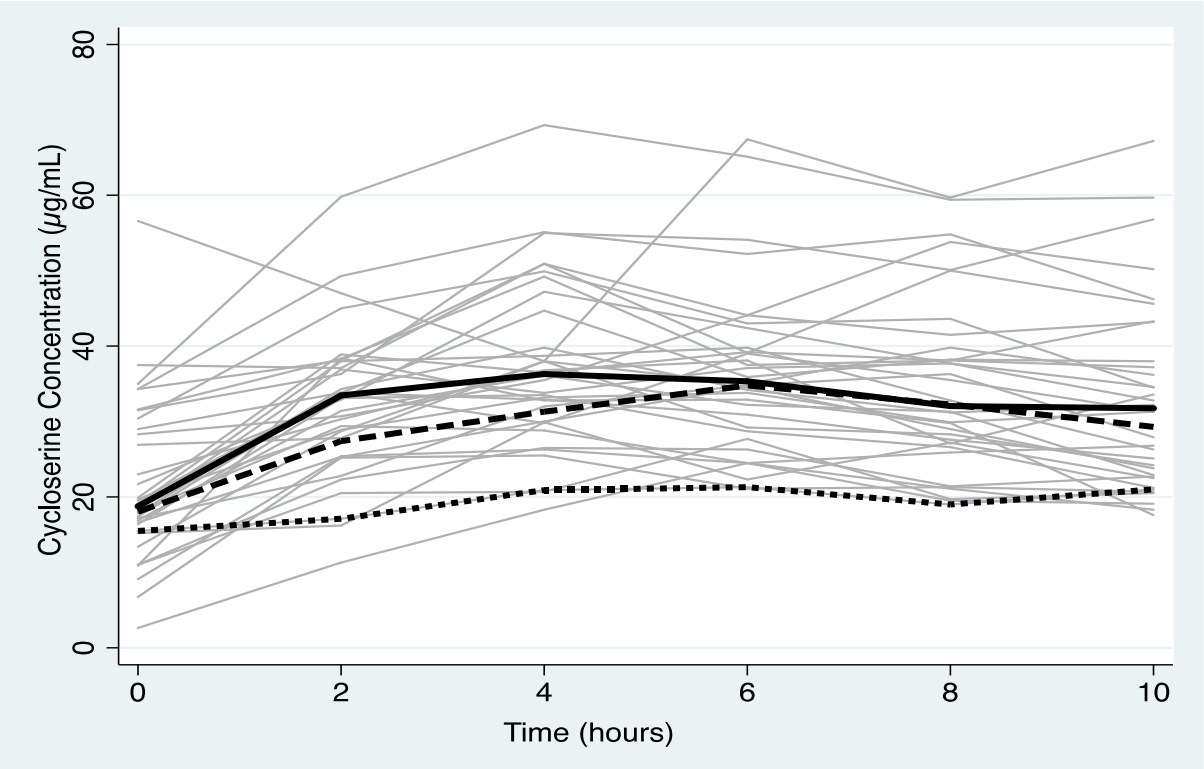
Covariates associated with cycloserine exposure are shown in Table 3. On single and multiple regression analyses, the only factor independently associated with AUC_{0-10} was dose (mg/kg). There was a non-significant trend towards a higher AUC_{0-10} in patients who were HIV infected.

Table 3. Mean change in steady state area under the concentration-time curve (AUC_{0-10}) of cycloserine associated with covariates in patients with multi-drug resistance tuberculosis.

	Single			Multiple		
	AUC[†] Change	95% CI	P	AUC Change	95% CI	P
Sex	-9%	-26% to 13%	0.377	-5%	-25% to 21%	0.669
Age, years	0%	-1% to 1%	0.790	0%	-1% to 1%	0.961
HIV-infected	18%	-3% to 45%	0.097	16%	-9% to 47%	0.216
BMI, kg/m^2	-1%	-5% to 2%	0.452	1%	-4% to 6%	0.72
Duration on terizidone, days	0%	-1% to 1%	0.945	0%	-1% to 1%	0.951
Creatinine clearance, mL/min	0%	-1% to 0%	0.325	0%	-1% to 0%	0.68
Dose (mg/kg)	5%	1% to 10%	0.012	6%	1% to 12%	0.028

The change in AUC is based on estimates from the linear regression model of the covariates on the log-transformed AUC and expressed as percent change in AUC for each unit change in covariate.
AUC_{0-10} area under the concentration-time curve at 0–10 h; HIV: human immunodeficiency virus; BMI: body mass index.

Figure 1. Cycloserine concentration-time profiles in 35 participants at steady state receiving treatment for multi-drug resistant tuberculosis, including median concentrations per dose



Solid grey lines: time-concentration profile of each participant (n=35)
Solid black line: Median time-concentration profile dosed at 750mg (n=28)
Dashed black line: Median time-concentration profile dosed at 500mg (n=6)
Dotted black line: Median time-concentration profile dosed at 250mg (n=1)

2.5. DISCUSSION

To our knowledge, this is the largest report of the pharmacokinetics of cycloserine at steady state, irrespective of whether it was administered as terizidone or cycloserine. Considering its association with neurotoxicity, and that efficacy is likely to be related to the serum concentrations achieved, characterising the pharmacokinetics of cycloserine is important for dose optimisation (8). Due to the paucity of pharmacokinetic data on terizidone, the dose equivalent of cycloserine is not known.

We found that maximum serum concentration (C_{max}) and $AUC_{0\text{-}10}$ to be higher than that reported previously reported for cycloserine dosed at 250-500mg daily (12). Cycloserine pharmacokinetics have been reported in small, single dose studies in healthy volunteers (12,13), or in patients after a limited duration of treatment (12,4). We performed intensive pharmacokinetic sampling after a

longer treatment duration in patients treated given the prodrug terizidone, and accumulation could account for the higher C_{max} and AUC_{0-10} we observed. An alternative explanation for the high exposures of cycloserine we found is a drug-drug interaction. A recent report of cycloserine pharmacokinetics in two groups of healthy volunteers given different MDR-TB treatment regimens reported a significantly higher cycloserine AUC in participants in whom moxifloxacin was co-administered than in those who were treated with levofloxacin, even after accounting for weight differences between the two groups, suggesting the possibility of a drug-drug interaction between cycloserine and moxifloxacin (12). Those findings need to be confirmed, but it is possible that moxifloxacin interferes with cycloserine elimination. Unanticipated drug-drug interactions should also be contemplated for other second line anti-tuberculosis drugs about which there is incomplete knowledge of disposition pathways.

The long half-life we observed suggests that once daily dosing should be recommended for terizidone. However, the pharmacokinetic-pharmacodynamic relationship associated with cycloserine efficacy and toxicity is as yet not known. Definitive recommendations on optimal terizidone dosing await further studies.

Dose (mg/kg) was the strongest determinant of AUC. This observation is in keeping with the findings of a recent pharmacokinetic report of cycloserine in a smaller cohort without HIV infection in Taiwan (4). We found a non-significant trend towards a higher AUC in patients with HIV after adjusting for dose/weight differences between patients who were non-HIV infected. However, our sample size was not sufficiently powered to analyse the effect of HIV on cycloserine pharmacokinetics.

Our study was limited in that pharmacokinetic sampling was only done for 10 hours post dose. As some participants had a rising cycloserine concentration at 10 hours post dose, it was not possible to extrapolate the AUC to infinity or accurately calculate the half-life of cycloserine for all participants. A study with a longer sampling interval is needed to adequately characterize the pharmacokinetics of cycloserine administered as terizidone. Second, although to our knowledge this is the largest report of cycloserine pharmacokinetics at steady state, a study with a larger cohort would be better powered to analyse factors associated with AUC and C_{max}. Finally, our study

was not designed to assess whether drug-drug interactions affected the pharmacokinetics of cycloserine.

This is the largest study to describe the steady state pharmacokinetics of cycloserine and the first to describe the steady state pharmacokinetics of cycloserine dosed as terizidone in patients treated for TB. The steady state concentrations of cycloserine in patients treated with terizidone for MDR-TB were higher than those reported with cycloserine formulations. Our findings suggest the accumulation of cycloserine and support the notion of once-daily dosing.

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CHAPTER 3

Neuropsychiatric toxicity and cycloserine concentrations during treatment for multidrug-resistant tuberculosis.

Richard Court[a],* Chad M. Centner[b], Maxwell Chirehwa[a], Lubbe Wiesner[a], Paolo Denti[a], Nihal de Vries[c], Joseph Harding[d], Tawanda Gumbo[e], Gary Maartens[a,f], Helen McIlleron[a,f] .

[a] Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa [b] Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa [c] Brooklyn Chest Hospital, Cape Town, South Africa [d] DP Marais Hospital, Cape Town, South Africa [e] Quantitative Preclinical and Clinical Sciences Department, Praedicare, Dallas, TX, USA [f] Wellcome Centre for Infectious Diseases Research in Africa (CIDRI-Africa), Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

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The cycloserine time-concentration data (chapter 2) was later modelled by my friend and colleague, Dr Maxwell Chirehwa using NONMEM® software. (1) Using simulations, Chirehwa et al. showed that, with daily doses of terizidone (750 mg and 1000 mg for patients weighing ≤45 kg and >45 kg, respectively), the probability of maintaining the plasma cycloserine concentration above the minimum inhibitory concentration (MIC) for more than 30% of the dosing interval (30% T>MIC) (which is associated with a 1.0-log10-colony-forming units/ml kill in vitro) exceeded 90% at MIC values of ≤16 mg/litre. The proportion of patients achieving 100% T>MIC (which is associated with the prevention of drug resistance) was more than 90% only at MICs of ≤8 mg/litre. The manuscript therefore concluded that current WHO-recommended doses of terizidone are effective for cycloserine MICs of ≤8 mg/litre, but that higher doses are required to prevent the development of resistance.

The concern with higher recommended doses of cycloserine is that concentration thresholds for cycloserine-related neuropsychiatric toxicity (depression, psychosis, and peripheral neuropathy), which has impeded widespread use of the drug (2–5), have not been well defined. We therefore prospectively evaluated the incidence of neuropsychiatric toxicity at serial timepoints in the PODrTB cohort of patients treated for MDR-TB with cycloserine dosed as terizidone, using validated data collection tools. Using Cox Proportional Hazards modelling, we subsequently explored associations of selected covariates with neuropsychiatric toxicity including individual estimates for cycloserine exposure generated by Chirehwa et al. (1): area under the concentration-time curve to 10 hours ($AUC_{0\text{-}10}$), peak concentration (C_{max}), and clearance (CL). We also included categorized

pyridoxine doses as a covariate in our model. High-dose pyridoxine, which was previously recommended by some treatment centres for inclusion in MDR-TB treatment regimens as prophylaxis for peripheral neuropathy, has paradoxically been reported to be toxic to peripheral nerves (6–9). In this manuscript, we describe a high incidence of peripheral neuropathy, and found cycloserine $AUC_{0\text{-}10}$, C_{max}, CL, and high-dose pyridoxine to be significantly associated with peripheral neuropathy on univariate analysis. Cycloserine clearance and high-dose pyridoxine maintained significance on multivariate analysis. None of the factors we explored were associated with psychosis or depression.

The data in this manuscript contributes to the growing evidence indicating that high-dose pyridoxine is toxic to peripheral nerves, and therefore supports adjustment of dosing schedules for pyridoxine in patients treated for MDR-TB. Cycloserine/terizidone should also be considered as a cause of unexplained neuropathy in patients on treatment for MDR-TB. I presented this neuropsychiatric toxicity PK/PD data at the 51st Union World Conference on lung Health, October 2020. Abstract Number: 1439.

3.1. ABSTRACT

Background

Cycloserine, or its structural analogue terizidone, has been associated with neuropsychiatric toxicity (psychosis, depression, and neuropathy). Prospective clinical data of the incidence of and risk factors for neuropsychiatric toxicity in TB patients treated with cycloserine are limited.

Methods

A prospective evaluation of neuropsychiatric toxicity was performed using validated screening tools in patients with multidrug-resistant tuberculosis treated with terizidone. Cox proportional hazard modelling was performed to explore the effects of clinical variables and measures of cycloserine pharmacokinetics in plasma.

Results

A total 144 participants were recruited: 86 were male and 58 were female; their median age was 35.7 years and 91 (63%) were HIV-infected. Fifty-five (38%) participants developed at least one neuropsychiatric event (30 cases per 100 person-months): 50 (35%) neuropathy, 14 (10%) depression, and 11 (8%) psychosis. Neuropathy was independently associated with cycloserine clearance ((adjusted hazard ratio 0.34 (aHR), P = 0.03)) and high-dose pyridoxine (200 mg vs 150 mg daily, aHR: 2.79, P=0.01).

Conclusions

A high incidence of early neuropsychiatric toxicity was observed in this cohort of patients treated with terizidone. Cycloserine clearance and higher doses of pyridoxine are associated with incident or worsening peripheral neuropathy.

Key words: Cycloserine; Terizidone; Pyridoxine; Pharmacokinetics; Neuropathy; Neuropsychiatric

3.2. INTRODUCTION

D-4-amino-3-isoxazolidinone, or D-cycloserine (cycloserine), was first discovered and synthesized by Hidy et al. almost 70 years ago (10). Neuropsychiatric toxicity, including depression and psychosis, was first reported with the earliest known use of cycloserine and in subsequent early treatment reports (2,3).

Cycloserine-associated peripheral neuropathy has been reported less frequently (2,4,11). Neuropsychiatric side-effects led to patients and pharmacologists giving cycloserine the moniker "psych-serine" (8). The popular press has called cycloserine a "cure that also kills" (12). Historically, this has impeded widespread inclusion of cycloserine, and its structural analogue terizidone, in treatment regimens for drug-resistant tuberculosis. The World Health Organization (WHO) has recently included cycloserine or terizidone as a group B drug for long multidrug-resistant tuberculosis (MDR-TB) treatment regimens, (13) after an individual participant data meta-analysis showed cycloserine to be more efficacious than some commonly used anti-TB drugs, including kanamycin and ethionamide (14). Terizidone, which is hydrolysed pre-systematically to two molecules of cycloserine (15)(16), was previously considered to be associated with less neurotoxicity than cycloserine, but a recent review demonstrated no significant safety difference between the two drugs (5).

There are limited systematically collected prospective data describing neuropsychiatric toxicity, including peripheral neuropathy, in patients treated for MDR-TB. Further, there are no existing data describing the association of cycloserine concentrations with incident or worsening neuropsychiatric events in TB patients. Studies reporting the effect of cycloserine concentrations on both microbial kill and resistance suppression have recently been published, including penetration of the drug into TB cavities and resistance arising therein (17,18). Defining the relationship between cycloserine concentrations and neuropsychiatric toxicity, identifying exposure thresholds associated with specific neuropsychiatric events, and then comparing these thresholds to those associated with microbial kill and resistance suppression, will significantly contribute to dose optimisation in the management of patients treated for MDR-TB.

A prospective observational study was conducted amongst hospitalized patients treated with a terizidone-containing regimen for rifampicin-resistant TB or MDR-TB, to determine risk factors, including cycloserine pharmacokinetic parameters, for neuropsychiatric toxicity). Serial measurements were performed using standardized tools to detect treatment-emergent (or worsening on treatment) psychiatric disorders and peripheral neuropathy.

3.3 METHODS

Study design and patient recruitment

A prospective observational study was performed in patients treated for MDR-TB at Brooklyn Chest Hospital and DP Marais Hospital in Cape Town. Patients who were diagnosed with pulmonary rifampicin-resistant TB or MDR-TB were recruited between July 2015 and September 2017. Inclusion criteria included adults ≥18 years of age with confirmed pulmonary MDR-TB, initiated on standard MDR-TB treatment within the previous month. Critically ill patients and those unable to provide informed consent were excluded.

Treatment administered

During the study period, the standard regimen for MDR-TB in South Africa consisted of pyrazinamide, moxifloxacin, kanamycin, terizidone, and either ethionamide or isoniazid (depending on the presence of katG and inhA mutations identified by line-probe assay in the pre-treatment sputum culture, indicating high-level resistance to isoniazid or low-level resistance to isoniazid and resistance to ethionamide, respectively) (19). Ethambutol was added if the risk of ethambutol resistance was considered low. High-dose pyridoxine (150 or 200 mg daily) was included as prophylaxis for terizidone-related pyridoxine deficiency, and the dose of terizidone was adjusted for weight as per national guidelines during the study period (20). The dose of terizidone administered to participants is the same terizidone dose currently recommended by the WHO (13). Dosing was adjusted for renal dysfunction at the discretion of the treating physician.

Clinical follow-up for adverse events monitoring

Neuropsychiatric toxicity was evaluated at recruitment and monthly to 12 weeks using validated tools. The Brief Peripheral Neuropathy Rating Screen (BPNS) for peripheral neuropathy (21) was used to rate neuropathic symptoms (pain, paraesthesia, and numbness) on a numeral rating scale

from 0 to 11 points. Neuropathy (incident or worsening) was defined as an increase in BPNS symptom score after recruitment of ≥2 points for pain, numbness, or pins and needles (21). A minimum of two serial neuropathy assessments per participant were therefore required for inclusion in the analysis. The BPNS objective clinical scores (i.e., ankle jerks and vibration sense) were not included in the analysis, as these assessments could not be reliably standardized due to a high turnover of study staff during the recruitment period.

The Kessler 10 scale (K10) and the Brief Psychiatric Rating Scale (BPRS) tool were also administered at recruitment and monthly to 12 weeks to screen for depression and psychosis, respectively (22)(23). Participants who had a K10 or BPRS score on treatment of ≥20 or ≥32 were defined as having probable depression or psychosis, respectively; these cut-offs have previously been validated as predictive of these psychiatric events (24,25). Participants who had K10 and BPRS scores above the identified threshold at the time of recruitment were considered to have treatment-related depression or psychosis if their score had increased by ≥1 point on treatment. Where appropriate, adverse event severity was graded according to the Division of AIDS Classification (26).

Pharmacokinetics

Blood was drawn after a minimum of 1 week of therapy at six time-points over 10 h (pre- dose, and 2, 4, 6, 8, and 10 h post-dose). A subset of patients had three additional blood samples drawn at 12, 24, and 26 h post-dose. An additional subset of patients had two pharmacokinetic sampling occasions. Liquid chromatography tandem mass spectrometry was used to obtain the cycloserine concentrations in plasma using a validated assay at the Division of Clinical Pharmacology, University of Cape Town (15). The lower limit of quantification for plasma cycloserine was 0.313 µg/mL and the top of the validated range was 40.0 µg/mL (15). Cycloserine concentration–time data were interpreted using non-linear mixed-effects modelling, as described previously for this cohort dosed with terizidone (1). The final model was used to generate the steady-state 24-hour cycloserine area under the concentration time curve ($AUC_{0–24}$), trough concentration (C_{24}), peak concentration (C_{max}), and clearance.

Statistical analyses

Stata v.15 (StataCorp, TX, USA) was used to perform the statistical analysis. Factors associated with key neuropsychiatric adverse events were explored, including psychosis and/or depression and/or peripheral neuropathy, using a Cox proportional hazards regression model. The following potential covariates were selected a priori and evaluated in univariate models: sex, age, HIV status, previous exposure to anti-TB drugs, history of alcohol and recreational drug use respectively, and key cycloserine measures including $AUC_{0–24}$, C_{max}, C_{max} >35 μg/mL, C_{24}, and drug clearance. For the analysis of peripheral neuropathy, the following additional factors were included, which were also identified a priori as potential causes of, or predisposing factors for neuropathy: height, presence of diabetes, pyridoxine dose (150 vs 200 mg daily), and history of isoniazid and/or ethionamide use since treatment initiation of the current MDR-TB episode. Isoniazid and efavirenz use were added in the models exploring factors associated with psychosis and/or depression. Covariates with a P-value of <0.2 in the univariate analysis were included in multivariate analyses for psychosis, depression, and neuropathy, respectively, and combined events, i.e., any psychiatric event (depression and/or psychosis) or any neuropsychiatric event (depression and/or psychosis and/or neuropathy). If more than one cycloserine pharmacokinetic measure had a P-value of <0.2 on univariate analysis, the pharmacokinetic measure with the strongest univariate association was included in the multivariate analysis. Incident or worsening neuropathy and depression and/or psychosis were evaluated over time using Kaplan–Meier failure analyses, and the two-sample Wilcoxon rank sum (Mann–Whitney) test was used to compare cycloserine $AUC_{0–24}$, C_{max}, C_{24}, and clearance between participants who developed new or worsening peripheral neuropathy and those who did not. A P-value of <0.05 was considered as significant.

3.4. RESULTS

The clinical characteristics of the 144 participants recruited into the study are shown in Table 4. Cycloserine pharmacokinetic data were available for 132 (92%) participants, of whom 20 had two pharmacokinetic sampling occasions and eight had three additional blood draws to 26 hours post-dose. For one participant, only the pre-dose cycloserine concentration was available. The pharmacokinetic results have been reported before (1). A one-compartment disposition model with absorption described by a transit compartment model and first-order elimination described the

data well. Elimination of cycloserine was described using two pathways: renal and non-renal (1). The renal pathway was driven by creatinine clearance, while the non-renal pathway included the effects of fat-free mass and smoking status. Other pharmacokinetic covariates included the effects of tablet crushing on the duration of absorption delay and fat-free mass on the volume of distribution (included via allometric scaling). Clearance varied between individuals, while bioavailability, absorption rate constant, and transit time varied between occasions.

Table 4. Clinical and demographic Characteristics of 144 patients on treatment with terizidone for multidrug-resistant tuberculosis

Characteristic	N (%) or median (IQR)
N	144
Males	86 (59.7%)
Age, yrs.	35.7 (29.7 to 43.8)
BMI, kg/m^2	17.2 (15.6 to 18.9)
HIV status	Positive: 91 (63.2%)
	Negative: 52 (36.1%)
	Unknown: 1 (0.7%)
Diabetes	10 (6.9%)
Terizidone dose	750mg: 108 (81.8%) 500mg: 22 (16.7%) 250mg: 2 (1.5%)
Pyridoxine dose	200mg: 12 (8.3%) 150mg: 121 (84.0%) Unknown: 11 (7.6%)
Creatinine clearance, mL/min	99.2 (78.8 to 119.6)
Alcohol	Use prior to recruitment: 98 (68.1%)
	Never used: 46 (31.9%)
Recreational drugs	Use prior to recruitment: 74 (51.4%)
	Never used: 70 (48.6%)
Cycloserine AUC_{0-24}	597.2 (425.7 to 762.7) μg·mL/hr
Cycloserine C_{max}	33.5 (24.6 to 40.4) μg /mL
	$C_{max} > 35$ μg/mL: 79
	$C_{max} \leq 35$ μg/mL : 65
Cycloserine trough concentration (C_{24})	16.8 (11.0 to 24.2) μg/mL
Cycloserine clearance	0.8 (0.6 to 1.1) L/hr

The classification of key adverse events is shown in Table 5. Fifty-five of the 144 participants (38.2%) developed at least one new or worsening neuropsychiatric event (30 cases per 100 person-months), with peripheral neuropathy being the major contributor, affecting 50 (34.7%) participants (25 cases per 100 person-months). As a change in the BPNS score was used to define treatment-related peripheral neuropathy, it was not possible to grade neuropathy severity using the Division of AIDS Classification instrument (26). Fourteen participants who had only one neuropathy assessment at recruitment were excluded from the neuropathy time-to-event analysis. The median time to neuropathy was 42.5 days (interquartile range (IQR) 21–63 days); in comparison, the median time to depression and/or psychosis was 49.5 days (IQR 14–63 days). The median time to neuropathy in participants who were HIV-positive was 42 days (IQR 21–62 days) vs 48 days (21–70 days) in those who were HIV-negative.

Figure 2 shows the time in days to neuropathy stratified by HIV status and Table 6 reports the covariates associated with neuropathy. Figure 3 shows the time to neuropathy in participants who were dosed with 200 mg pyridoxine daily as prophylaxis versus those who received 150 mg daily. The median time to neuropathy in participants dosed with pyridoxine 200 mg daily was 38.5 days (IQR 21–55.5 days) versus 43 days (IQR 22–63 days) in participants dosed with 150 mg daily. Table 6 shows that increasing cycloserine AUC_{0-24} was associated with the development of incident/worsening neuropathy in the univariate analysis, as were C_{max} >35 µg/l, trough concentration, and total clearance. The significance of the association of cycloserine clearance with neuropathy remained significant (adjusted hazard ratio 0.31, P = 0.026) after adjusting for the effect of HIV and age. Pyridoxine dose was associated with incident or worsening neuropathy in both univariate and multivariate analyses (see Table 6). An analysis was performed to determine whether any of the covariates included in the final multivariate model modified the effect of cycloserine clearance on peripheral neuropathy, but no such modification was found. A comparison of the key cycloserine pharmacokinetic parameters in those who developed peripheral neuropathy versus those who did not is shown in Table 7.

Table 5. Number of participants with neuropsychiatric adverse events out of 144 patients on treatment with terizidone

	Any grade	Grade 3 or higher
Psychosis	11 (7.6%)	0
Depression	14 (9.7%)	0
Any neuropsychiatric event (depression or psychosis)	21 (14.6%)	0
Peripheral neuropathy	50 (34.7%)	na
Seizures	3 (2.1%)	1

Grading by DIVAIDS classification unless otherwise indicated
Psychosis: BPRS score ≥32 any grade; ≥55: grade 3 or higher
Depression: K10 score >20: any grade; >29: grade 3 or higher
Peripheral neuropathy: ≥ 2 BPNS symptom point increase

Table 6. Covariates associated with incident or worsening peripheral neuropathy in patients treated with terizidone for multidrug-resistant tuberculosis

		HR (95% CI)	p value	aHR (95% CI)	p value
HIV		2.12 (1.10 to 4.07)	0.025	1.67 (0.81 to 3.44)	0.161
Sex (male vs female)		1.03 (0.59 to 1.81)	0.912		
Age (per 1 yr. increase)		1.02 (1.00 to 1.05)	0.110	1.03 (1.00 to 1.07)	0.036
Diabetes		1.29 (0.47 to 3.60)	0.621		
Height (per 1cm increase)		1.00 (0.97 to 1.03)	0.949		
Previous TB treatment		1.59 (0.77 to 3.27)	0.210		
History of alcohol use		1.50 (0.81 to 2.71)	0.200		
History of recreational drug use		0.70 (0.40 to 1.23)	0.217		
Ethionamide use, n=120		1.41 (0.64 to 3.15)	0.395		
Isoniazid use, n=121		0.69 (0.33 to 1.41)	0.305		
Ethionamide and Isoniazid use, n=101		0.98 (0.54 to 1.80)	0.955		
Cycloserine $AUC_{0\text{-}24}$ (per 100 unit increase)		1.08 (1.00 to 1.17)	0.037		
Cycloserine C_{max}		1.02 (1.00 to 1.04)	0.020		
C_{max} > 35 μg/mL		2.03 (1.13 to 3.65)	0.018		
Trough concentration (C_{24})		1.02 (1.00 to 1.05)	0.044		
Clearance, L/hr		0.24 (0.09 to 0.62)	0.003	0.31 (0.11 to 0.87)	0.026
Pyridoxine dose	150mg (referent)				
	200mg	2.80 (1.29 to 6.08)	0.009	2.79 (1.26 to 6.20)	0.012
	unknown	1.26 (0.45 to 3.54)	0.658	1.92 (0.54 to 6.86)	0.314

HR: hazard ratio; aHR: adjusted hazard ratio; CI: confidence interval

Table 7. Comparison of key pharmacokinetic measures in 132 participants with and without incident or worsening peripheral neuropathy treated with terizidone for multidrug-resistant tuberculosis

	Neuropathy	No neuropathy	p value
	n=47	n=85	
$AUC_{0\text{-}24,}$ μg*mL/hr	651.7 (516.2 - 803.5)	547.1 (395.3 - 726.0)	0.010
C_{max}, μg/mL	38.1 (32.5 - 50.9)	33.5 (24.6 - 40.4)	0.006
C_{24}, μg/mL	20.0 (14.8 - 25.8)	15.6 (9.7-22.8)	0.009
Clearance, L/hr	0.7 (0.5 – 0.9)	0.9 (0.7 – 1.2)	0.002

The median value is shown with interquartile range in brackets

Figure 2. Time to incident or worsening peripheral neuropathy stratified by HIV status in patients treated with terizidone for multidrug-resistant tuberculosis

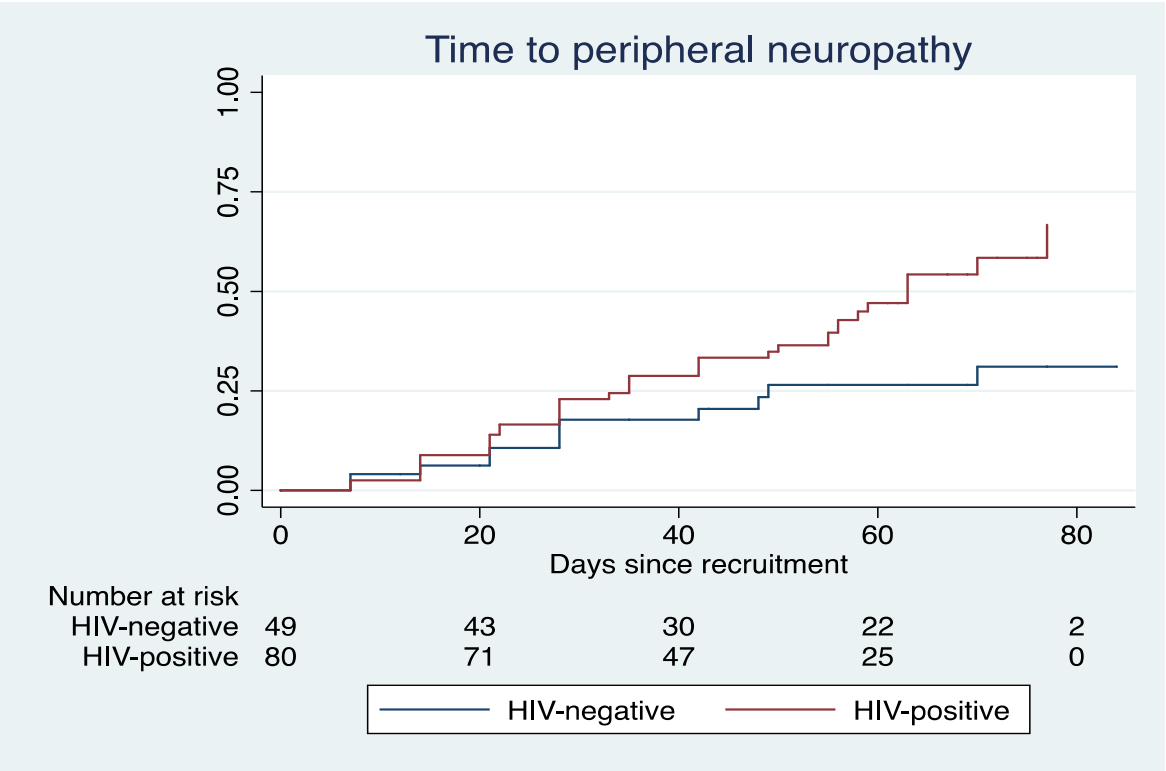
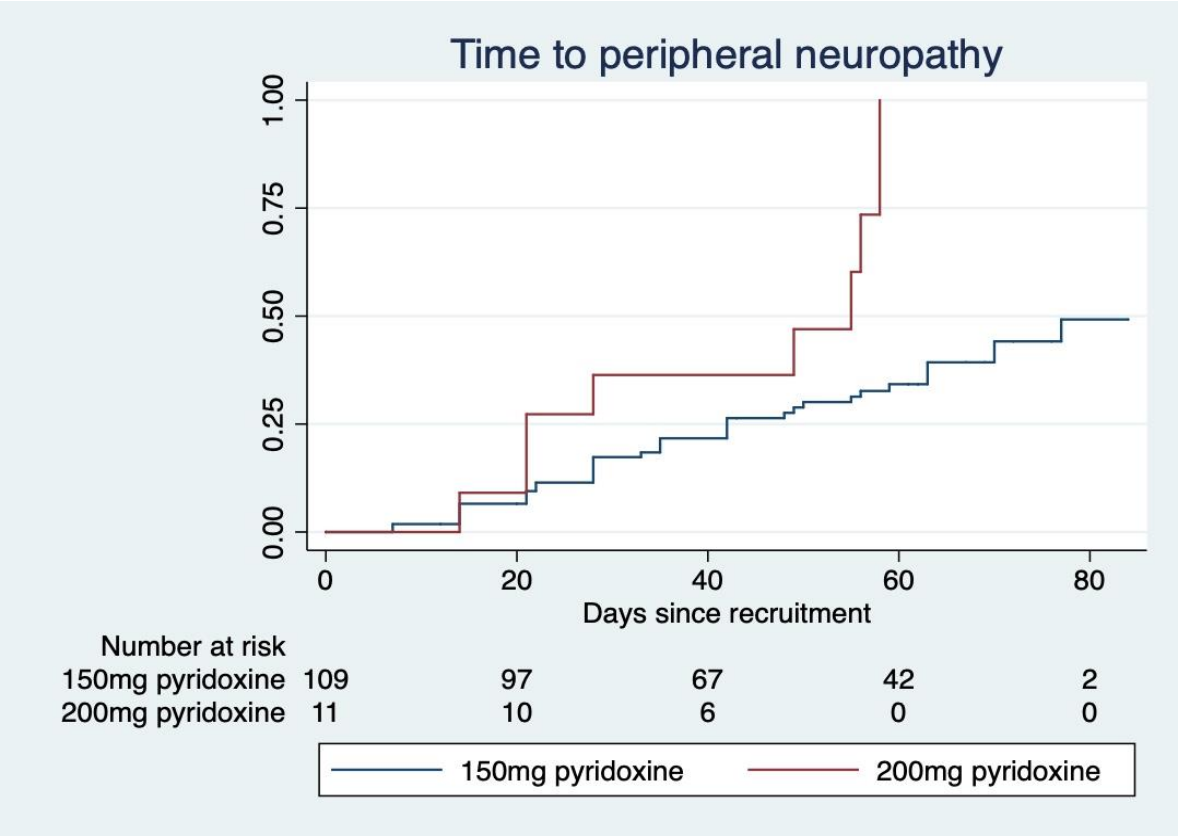


Figure 3. Time to incident or worsening peripheral neuropathy stratified by pyridoxine dose in patients treated with terizidone for multidrug-resistant tuberculosis



Eleven of the 144 participants (7.6%) developed either incident or worsening psychosis (5 cases per 100 person-months) and 14 (9.7%) developed either incident or worsening depression (7 cases per 100 person-months). Altogether 21 (14.6%) of the 144 participants developed a psychiatric event (incident or worsening depression and/or incident or worsening psychosis), i.e., 10 cases per 100 person-months. Table 8 illustrates the relationship between the selected covariates and depression and/or psychosis, respectively. When exploring associations with individual psychiatric adverse events, none of the covariates explored, including HIV infection, age, and cycloserine measures, was associated with the incidence of new or worsening depression or psychosis.

Table 8. Covariates associated with new or worsening depression and/or psychosis in 144 patients treated with terizidone for multidrug-resistant tuberculosis

	HR (95% CI)	p value
Age	1.02 (0.98 to 1.07)	0.325
Sex	1.21 (0.48 to 3.07)	0.692
HIV	1.09 (0.43 to 2.77)	0.855
Previous TB treatment	0.78 (0.29 to 2.04)	0.608
History of alcohol use	0.99 (0.38 to 2.63)	0.999
History of recreational drug use	1.12 (0.46 to 2.76)	0.804
Isoniazid use	0.78 (0.23 to 2.69)	0.699
Efavirenz use	1.06 (0.43 to 2.65)	0.895
Cycloserine AUC_{0-24} (per 100 unit increase)	0.86 (0.71 to 1.05)	0.134
Cycloserine C_{max}	0.97 (0.94 to 1.01)	0.160
C_{max} >35 μg/mL	1.04 (0.42 to 2.57)	0.924
Trough concentration (C_{24})	0.96 (0.92 to 1.01)	0.161
Clearance, L/hr	1.22 (0.38 to 3.94)	0.736

HR: hazard ratio; CI: confidence interval

3.5. DISCUSSION

High rates of different neuropsychiatric events were identified in participants treated with terizidone for MDR-TB. The neuropsychiatric incident or worsening rate of 30 cases per 100 person-months means that with the currently recommended dose of cycloserine, which is frequently co-administered with high-dose pyridoxine in MDR-TB treatment programmes to prevent neuropathy, a large proportion of patients will develop clinically important adverse events. The major contributor to the high rate of neuropsychiatric toxicity was neuropathy, with 25 cases per 100 person-months. Over a third of the participants in the study cohort developed new or worsening neuropathy during the first 12 weeks of MDR-TB treatment, which is higher than previously reported (3). It was found that cycloserine exposure was associated with an 8% increase in the risk of developing peripheral neuropathy for each

100 µg·mL/h increase in cycloserine $AUC_{0–24}$. Cycloserine C_{max} and C_{24} were also significantly associated with neuropathy on univariate analysis, while cycloserine clearance was associated with neuropathy in both univariate and multivariate analyses. That cycloserine clearance retained its association in the multivariable model suggests that cycloserine pharmacokinetics are related to an increased risk of neuropathy independent of HIV and/or age, which might be associated with changes in bioavailability or distribution more than clearance. As all patients received high-dose pyridoxine, it was not possible to identify the risks of neuropathy associated with its use. However, patients prescribed the 200 mg daily dose had 2.78 times the risk of neuropathy compared to those on 150 mg daily (P = 0.012, in the adjusted analysis).

Cycloserine has infrequently been reported as a significant peripheral nerve toxin. Early cycloserine treatment reports either do not mention or report a low incidence of neuropathy (2–4,27,28). More recently, cycloserine dosed as terizidone was shown to increase the incidence of neuropathy in MDR-TB patients, although the association was not statistically significant (11). Conversely, reports of high-dose pyridoxine as a cause of peripheral neuropathy are emerging with increasing frequency. The effect appears to be dose-related, but the duration of treatment, even at lower doses, appears to be an important risk factor (6,7,9,29,30). It is plausible that the high dose of pyridoxine intended to prevent neuropathy, rather than cycloserine, is responsible for the high incidence of peripheral neuropathy observed. The highest pyridoxine dose may have been prescribed for patients at risk of neuropathy or for patients with established neuropathic pain at baseline, thereby explaining the association observed. The dose of pyridoxine required to prevent isoniazid-related neuropathy (6–50 mg/day) is significantly lower than the 150–200 mg routinely prescribed to prevent cycloserine-related neuropathy in the study cohort (31). High-dose pyridoxine (>25 mg/day) was not found to improve overall vitamin B6 status over the standard 25 mg/kg dosage in patients treated with isoniazid for drug-sensitive TB (32).

On univariate analysis, increasing age was significantly associated with neuropathy, and HIV infection doubled the hazard for neuropathy; both are well-established risk factors for neuropathy (33,34). The exposure–toxicity relationship of cycloserine with neuropathy was enhanced by the finding of an association of cycloserine clearance with neuropathy, which

was significant on both univariate and multivariate analysis. The relationship with clearance (which has renal and non-renal components) also suggests that procedures that improve cycloserine clearance, such as haemodialysis, could be explored for roles in managing severe neuropsychiatric adverse events. The management of new or worsening peripheral neuropathy in patients treated with cycloserine should also include optimisation of the pyridoxine dose. Previous alcohol or recreational drug use was not found to be associated with neuropathy. Data were collected on alcohol use via patient self-report, specifically enquiring about the quantity of alcohol consumed in the months leading up to the MDR-TB diagnosis. Alcohol consumption quantified by patient self-report has been shown to underestimate alcohol intake (35); it is therefore possible that the lack of an observed association between alcohol use and neuropathy may have been due to under-reporting.

The mechanism of cycloserine-induced neuropathy is understood to be a combination of pyridoxine antagonism by cycloserine and increased renal elimination of pyridoxine (36). Supplemental pyridoxine is included in many programmatic MDR-TB treatment regimens. The finding that cycloserine clearance itself was associated with adverse events also suggests that direct accumulation of the drug could have a neurotoxic effect, independent of pyridoxine renal elimination. An analysis was also performed to determine whether the use of isoniazid and/or ethionamide, which cause neuropathy via a similar mechanism (7), were associated with incident neuropathy, but no such association was found.

It is currently unknown what threshold cycloserine concentration is associated with incident or worsening neuropsychiatric events in patients treated for MDR-TB. The typical peak concentration range of cycloserine in patients receiving a dose of 250 mg or 500 mg is 20 – 35 μg/mL (37). An early study of cycloserine in the management of TB described psychotic symptoms in several patients with cycloserine concentrations >40 μg/mL (38). In a more recent report, a case of psychosis in an MDR-TB patient treated with cycloserine was reported with cycloserine concentrations >35 μg/mL (39). In the present study, it was found that patients with a cycloserine C_{max} >35 μg/mL were approximately twice as likely to develop peripheral neuropathy on univariate analysis (hazard ratio 1.89, 95% confidence interval 1.04–3.44; P = 0.035).

Psychosis and/or depression were not significantly associated with cycloserine exposure or any of the other covariates in this study. Although the study found a higher incidence of depression and psychosis than a pooled estimate of drug-related neuropsychiatric events in a recent review of neurotoxicity in patients treated with cycloserine or terizidone for MDR-TB (5), the present study may have been underpowered to assess associations with cycloserine exposure. The relationship between cycloserine exposure and the incidence of any psychiatric adverse event was consequently explored by combining depression and psychosis, but no association was found (Table 8). No symptoms suggestive of possible psychosis or depression graded 3 or higher were observed (Table 5).

This study has several limitations. First, the participants in the cohort had multiple risk factors for neuropsychiatric toxicity (comorbidities, high-dose pyridoxine, and other drugs). Therefore, we cannot be certain that the neuropsychiatric toxicity observed was due to cycloserine exposure at all. Second, it was not possible to perform the neuropsychiatric assessments at treatment start, as most participants were referred to the TB hospitals from referral clinics or tertiary centres where MDR-TB treatment was initiated. Therefore, the onset of neuropathy and early psychiatric events occurring in the first weeks of treatment, before recruitment, may have been missed. The adverse event rate reported here is therefore likely to be an underestimate. Third, cycloserine exposure was assessed on one pharmacokinetic sampling occasion only, and therefore the possibility that changes in dose and/or exposure during the study period may have affected the observed incidence of neuropsychiatric toxicity cannot be excluded.

This study appears to be the first large prospective longitudinal study describing the association of cycloserine exposure with neuropsychiatric toxicity in patients treated for MDR-TB. The results of this study highlight the growing evidence that high-dose pyridoxine is toxic to peripheral nerves and, although the association should be confirmed, cycloserine should be considered as a cause of unexplained neuropathy in patients on treatment for MDR-TB. The relationship of neuropsychiatric adverse events with cycloserine concentrations and clearance provides clinicians with potential tools for use in managing patients with neuropsychiatric toxicity. Finally, the study findings support the role of

therapeutic drug monitoring to lower cycloserine doses, and adjustment of dosing schedules for pyridoxine in patients treated for MDR-TB.

3.6. ACKNOWLEDGEMENTS

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CHAPTER 4

Effect of lidocaine on kanamycin injection-site pain in patients with multidrug-resistant tuberculosis.

Court R*, Wiesner L*, Chirehwa M.T*, Stewart A†, de Vries N‡, Harding J§, Gumbo T ¶, McIlleron H*, Maartens G*

*Division of Clinical Pharmacology, Department of Medicine, and †Clinical Research Centre, Faculty of Health Sciences, University of Cape Town, Cape Town, ‡Brooklyn Chest Hospital, Cape Town, §D P Marais Hospital, Cape Town, South Africa; ¶Center for Infectious Diseases Research and Experimental Therapeutics, Baylor Research Institute, Baylor University Medical Center, Dallas, Texas, USA

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Until the rollout of shortened all-oral regimens since 2018, injectable drugs formed the backbone of many MDR-TB treatment regimens. Due to the long duration and high toxicity of the drugs used in these previous regimens, which are poorly tolerated, only approximately half of patients previously completed the 18-24 month treatment (1). One of the adverse events often not included in reports of treatment-related toxicities is the pain caused by the intramuscular administration of kanamycin, one of several choice aminoglycosides used for the treatment of MDR-TB. The pain caused by the injection of kanamycin is reported as a common reason for treatment non-adherence (2). Lidocaine, or lignocaine as it is locally referred to, is a local anaesthetic, which has been used with success to reduce the pain caused by the intramuscular injection of some drugs, including ceftriaxone and penicillin G (3,4). We therefore performed a randomized crossover study to explore whether the addition of lidocaine could reduce the pain experienced by MDR-TB patients treated with intramuscular kanamycin. As a secondary objective, we aimed to explore whether lidocaine affects the pharmacokinetics of kanamycin.

We demonstrated that the addition of lidocaine reduced the pain experienced by patients in the first 15 minutes post injection of kanamycin, which is important, as the highest pain scores were observed immediately post administration. We also found the use of lidocaine to be significantly associated with pain reduction on multivariate analysis. Lidocaine had no effect on the pharmacokinetics of kanamycin.

With a burdensome toxicity profile including irreversible hearing loss and renal failure (5–7),

and a recent meta-analysis showing little therapeutic benefit with the use of kanamycin (8), injectable-free regimens are now standard of care in many settings. Although the WHO currently does not recommend the use of kanamycin for the treatment of MDR-TB (9), injectables are still being used as a substitute in some centres where newer drugs e.g., bedaquiline are unavailable, or where priority drugs from WHO groups A or B are contraindicated. Lidocaine reduces the pain experienced by patients when treated with intramuscular kanamycin, which could improve adherence to MDR-TB therapy in settings where injectables are still used.

4.1. SUMMARY

Setting

Reducing pain from intramuscular injection of kanamycin (kanamycin) could improve the tolerability of multidrug-resistant tuberculosis (MDR-TB) treatment. Lidocaine has been shown to be an effective anaesthetic diluent for some intramuscular injections, but has not been investigated with kanamycin in the treatment of adult patients with MDR-TB.

Objective and design

We performed a randomised single-blinded crossover study to determine if lidocaine reduces kanamycin injection-site pain. We recruited patients aged >18 years on MDR-TB treatment at two TB hospitals in Cape Town, South Africa. kanamycin pharmacokinetic parameters and a validated numeric pain scale were used at intervals over 10 h following the injection of kanamycin with and without lidocaine on two separate occasions.

Results

Twenty participants completed the study: 11 were males, the median age was 36 years, 11 were HIV-infected, and the median body mass index was 17.5 kg/m^2. The highest pain scores occurred early, and the median pain score was 0 by 30 min. The use of lidocaine with kanamycin significantly reduced pain at the time of injection and 15 min post-dose. On multiple regression analysis, lidocaine halved pain scores (adjusted OR 0.5, 95%CI 0.3–0.9). The area under the curve at 0–10 h of kanamycin with and without lidocaine was respectively 147.7 and 143.6 μg·h/mL.

Conclusion

Lidocaine significantly reduces early injection-site pain and has no effect on kanamycin pharmacokinetics.

Keywords: TB; injectable; adherence; adverse effect

4.2. INTRODUCTION

Treatment completion rates in multidrug-resistant tuberculosis (MDR-TB) are poor. Only half of patients with MDR-TB are successfully treated (1,10,11). Treatment default, reported to be as high as 40% in some settings, is a significant contributor to poor treatment outcomes (12). The drugs used to treat MDR-TB have significant adverse effects, which have been described in one qualitative study to be worse than the disease itself (2), and may result in poor treatment adherence or loss to follow-up (13).

Kanamycin is a key second line drug in the intensive phase of treatment for MDR-TB, but has considerable toxicity, including irreversible deafness and renal impairment (5–7). The pain associated with the intramuscular administration of kanamycin is also significant and, with repeated dosing, a painful induration may develop at the injection site (7).

Lidocaine is a local anaesthetic which significantly reduces the pain immediately following the intramuscular injection of some drugs (3,4), but it is currently unknown whether lidocaine has a similar effect in adult patients treated with kanamycin for MDR-TB. Addressing the pain associated with kanamycin administration could enhance the tolerability of MDR-TB treatment regimens and improve long-term outcomes.

4.3. STUDY POPULATION AND METHODS

We performed a randomised single-blinded crossover study to compare injection-site pain from intramuscular kanamycin with and without lidocaine. We recruited patients who were aged ≥18 years between July 2016 and April 2017 on standard treatment for MDR-TB at Brooklyn Chest Hospital and D P Marais Hospital in Cape Town, South Africa. During the study period, the treatment regimen for MDR-TB comprised pyrazinamide, moxifloxacin, kanamycin, terizidone, and either ethionamide or isoniazid depending on the results of the line-probe assay for katG and inhA mycobacterium tuberculosis mutations identified in pretreatment sputum cultures, which would indicate high-level resistance to isoniazid or ethionamide, respectively (14). Ethambutol was added if a patient had no exposure to ethambutol in the month before treatment initiation and suspicion for ethambutol

resistance was low. (15) Randomisation was performed manually by an appointed administrator who was not involved in the study design or implementation. Twenty cards, half of which were labelled 'lidocaine' and other half labelled 'no lidocaine', were placed in separate sealed opaque envelopes before randomisation. Immediately before each participant's first pain assessment, a new envelope was opened revealing whether kanamycin was to be administered alone or mixed with lidocaine. The envelopes were opened sequentially starting with envelope no. 1 for the first participant. As some participants were unable to complete the study and recruitment therefore continued beyond the initial recruitment target, six additional envelopes were prepared in an identical manner. The kanamycin dose was adjusted for creatinine clearance at the discretion of the treating clinician and the same dose was administered on both pain assessment occasions. The Wong-Baker FACES® pain rating scale (Wong-Baker of FACES Foundation, Oklahoma City, OK, USA) was used to assess the pain caused by the kanamycin injection on two separate occasions approximately 7–14 days apart (16). The Wong-Baker Faces® pain rating scale is a validated pain assessment tool designed to assist health care providers measure pain using patient self-assessment. The scale is numbered 0 to 10 and is accompanied by a 'grimace scale' of faces to assist patients with the interpretation of pain, whereby the facial grimace increases with higher reported pain scores. We assessed pain at the following time points post-dose: immediately after the injection as well as at 15 min, 30 min, 60 min, 2 h, 6 h and 10 h. Participants were blinded to the addition of lidocaine and to the results of their previous pain assessments to avoid influence from earlier pain scores.

One mL of 2% lidocaine was mixed with a 3 mL ampule of kanamycin to create 4 mL of solution volume. Depending on the prescribed dose of kanamycin, either 2 mL of the solution (500 mg), 3 mL of solution (750 mg) or the entire 4 mL of the solution (1 g) were administered intramuscularly into the superior-lateral quadrant of the buttock using a 22-gauge needle on the opposite side to that used the previous day to prevent the influence of residual pain from the previous dose. If lidocaine was not administered together with kanamycin, participants received either a 3 mL (1 g), 2.25 mL (750 mg) or 1.5 mL (500 mg) kanamycin injection. Blood was drawn on both occasions pre-dose as well as at 2, 4, 6, 8 and 10 h post-dose to assess the effect of lidocaine on kanamycin pharmacokinetics. We used the K10 Anxiety and Depression Scale to screen for psychological distress, which we

considered to be a possible influence on participant pain thresholds (17). The K10 Anxiety and Depression Scale was specifically designed to identify psychological distress in the previous month using 10 questions. The answers to the questions are scored 1–5, with higher scores recorded with increasing symptom frequency. Patients who score under 20 are likely to be well, with higher scores associated with increasing severity of mental disorders (17).

Plasma concentrations of kanamycin A were determined using liquid chromatography-tandem mass spectrometry validated according to US Food and Drug Administration and European Medicines Agency guidelines (18,19). Samples were processed with a solid-phase extraction method using 50 μl of plasma. The extracted sample (5 μl) was injected ¼ onto the high-performance liquid chromatography column. Isocratic chromatographic separation was achieved on a Discovery C18 analytical column (5 6 μm, 50 mm 3 4.6 mm) using 4 mM heptafluorobutyric acid in 0.1% formic acid in water/acetonitrile (80:20, v/v) at a flow-rate of 500 μl/min. The mobile phase flow was split (1:1) at the source of the mass spectrometer. An AB Sciex API 3000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) was operated at unit resolution in multiple-reaction monitoring mode to monitor transition of the protonated molecular ions at m/z 485.2 to the product ions at m/z 163.2 for kanamycin A and the protonated molecular ions at m/z 494.3 to the product ions at m/z 165.3 for the kanamycin-d9 internal standard. Electrospray ionisation was used for ion production. The assay was validated over the concentration range 0.625–40 μg/mL. The combined accuracy (%Nom) and precision (%CV) statistics of the limit of quantification, low, medium and high- quality controls (three validation batches, n=18) were respectively 101.3–107.0%, and 3.0–14.3%.

Sample size

Using the report by Park et al. describing kanamycin pharmacokinetics (20), and assuming that data would be non-parametric, we estimated that a minimum of 16 patients would be required to detect a 20% change in pharmacokinetics between the two groups. Considering the paucity of data on kanamycin pharmacokinetics, we considered a target sample size of 20 participants would be sufficient to allow for any inaccuracy in power estimation.

Statistics

We used Stata v15.0 (Stata Corp, College Station, TX, USA) to compare pain scores using the Wilcoxon signed-rank test with and without lidocaine at each time point, as well as non-compartmental kanamycin pharmacokinetics with and without lidocaine. We determined the following pharmacokinetic parameters: area under the concentration–time curve at 0–10 h ($AUC_{0–10}$), AUC to infinity (AUC_{∞}), half-life, peak concentration (C_{max}) and time to C_{max}. We used the trapezoidal rule to calculate $AUC_{0–10}$ and extrapolation of the exponential decline to calculate AUC_{∞}. In a secondary analysis, we used multilevel ordered logistic regression to identify the factors associated with participant pain scores, including participants who completed the study and those who completed only one pain assessment. We identified the following variables a priori, which we analysed with single regression analyses: the use of lidocaine, time of pain assessment post-dose, human immuno-deficiency virus (HIV) status, kanamycin dose in mg/kg, volume of solution injected, sequence of randomisation, body mass index, sex, age and presence of psychological distress, defined as a score of >20 using the K10 screening tool (17). We included variables with $P < 0.2$ in the multiple regression analysis.

Ethics

The study protocol was approved by the University of Cape Town Human Research Ethics Committee, Cape Town (HREC 105/2016). Informed consent was provided by each participant in their language of choice (English, Afrikaans or Xhosa).

4.4. RESULTS

We recruited 29 participants, 20 of whom finished the study, and five completed pain assessments on only one occasion. Characteristics at each dosing occasion of the 20 participants who completed the study are given in Table 9. Twelve participants received 750 mg, five participants received 1000 mg and three participants were dosed with 500 mg of kanamycin. One participant had a reduced creatinine clearance of 30.2 mL/min on the first and 34.3 mL/min on the second dosing occasion.

Table 9. Participant characteristics of patients on treatment for MDR-TB in a single-blinded randomized crossover pharmacokinetic analysis of kanamycin with and without lidocaine

Males	11/20	
Age	36 (25-46)	
HIV-infected	11/20	
Diabetic	2/20	
Previous MDR-TB	10/20	
	Occasion 1	**Occasion 2**
Dose, mg/kg	15.8 (13.8 to 17.1)	15.8 (13.8 to 17.1)
Time on kanamycin, days	41.5 (28.5-53.5)	55 (40 to 67.5)
BMI, kg/m^2	17.5 (16.4 to 19.3)	17.1 (16.6 to 19.5)
Creatinine Clearance, mL/min*	91.4 (70.8 to 102.9	86.2 (77.2 to 104.4)
K10 score	10 (10-12)	10 (10-12)

*Cockcroft-Gault method
MDR-TB = multidrug-resistant tuberculosis; IQR = interquartile range; HIV = human immunodeficiency virus; BMI = body mass index

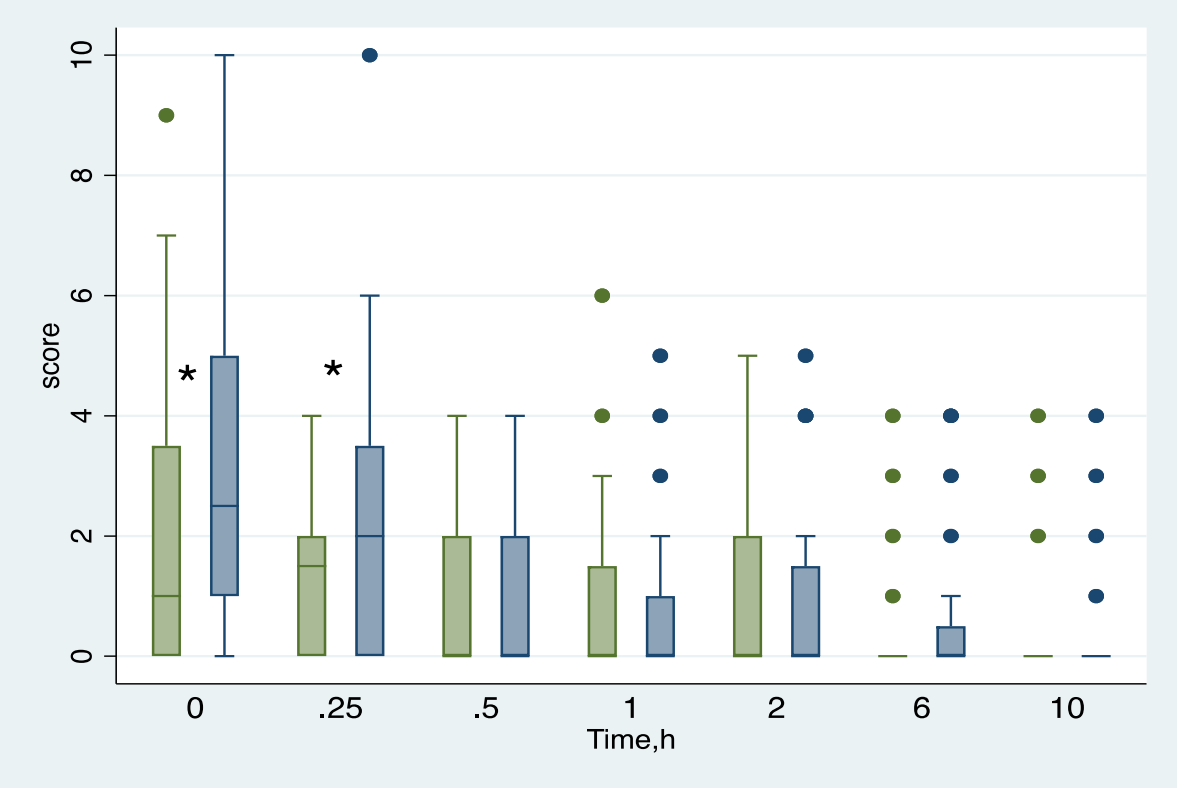
Pharmacokinetic parameters and drug concentrations of kanamycin dosed with and without lidocaine are shown in Table 10; 19 participants were included as the kanamycin concentrations for one participant were not available at the time of data analyses. Figure 4 shows the pain scores following kanamycin administration with and without lidocaine at each evaluated time point post-dose. Lidocaine dosed with kanamycin reduced the pain scores reported by participants significantly immediately following the injection (P=0.02) and at 15 min after dosing (P=0.02). On multilevel ordered logistic regression analyses (Table 11), two factors were negatively associated with differences in participant pain scores at each dosing occasion: use of lidocaine and time post-dose. HIV infection had a positive association with pain scores. We found symptoms of psychological distress, including depression, in three participants, but there was no association of psychological distress with pain.

Table 10. Pharmacokinetic parameters of kanamycin at steady state with and without lidocaine in in a crossover study of patients on treatment for multi-drug resistant tuberculosis

	Lidocaine (n=19)	**No Lidocaine (n=19)**
$AUC_{0\text{-}10}$, µg·h/mL	147.7 (85.3 to 166.6)	143.6 (101.6 to 164.2)
AUC_{∞}, µg·h/mL	155.9 (85.9 to 183.2)	148.5 (107.3 to 183.2)
C_{max}, µg/mL	33.0 (23.8 to 36.7)	33.6 (25.7 to 37.2)
T_{max}, hrs	2 (2 to 2)	2 (2 to 2)
Half-life, hrs	2.3 (2.0 to 2.7)	2.2 (1.9 to 2.7)

Interquartile range in brackets; $AUC_{0\text{-}10}$ = area under the concentration-time curve at 0-10 h;
AUC_{∞}= AUC extrapolated to infinity; C_{max} = peak concentration; T_{max} = time to Cmax

Figure 4. Median pain scores with and without lidocaine per time point following the intramuscular administration of kanamycin in 20 participants treated for multidrug resistant tuberculosis



Green Shade: Kanamycin with lidocaine; Blue shade: Kanamycin alone
*p=0.02
Median: Box midline; Interquartile range: Upper and lower bounds of boxes; 95 % range: Upper and lower bounds of whiskers

Table 11. Multilevel ordered logistic regression analysis of the factors associated with injection-site pain from intramuscular administration of kanamycin with and without lidocaine in participants on treatment for multidrug-resistant tuberculosis

	Single			Multiple		
Variable	OR	P	95% CI	aOR	P	95% CI
Lidocaine	0.6	0.046	0.4 to 1.0	0.5	0.021	0.3 to 0.9
Time post dose	0.8	0.000	0.7 to 0.8	0.8	<0.001	0.7 to 0.8
HIV infection	4.9	0.014	1.4 to 17.4	4.8	0.029	1.2 to 20.0
Dose (mg/kg)	1.0	0.120	1.0 to 1.0	1.0	0.264	1.0 to 1.0
Volume	0.7	0.274	0.4 to 1.3			
Sequence	0.5	0.380	0.1 to 2.1			
BMI	1.0	0.746	0.9 to 1.1			
Sex	0.9	0.867	0.2 to 3.7			
Age	1.0	0.237	0.9 to 1.0			
Psychological distress	0.7	0.763	0.1 to 6.9			

4.5. DISCUSSION

Our finding that lidocaine co-administered with kanamycin reduces injection-site pain in patients treated for MDR-TB could potentially improve the tolerability of MDR-TB treatment. Kanamycin is currently considered a key component of treatment regimens for MDR-TB by the World Health Organization (21). The toxicity and poor tolerability of the drugs used to treat MDR-TB are widely accepted to be significant factors affecting adherence and retention in care, resulting in poor treatment outcomes (13). Reducing the pain associated with the intramuscular injection of kanamycin, which is often not included in reports describing adverse effects in patients treated for MDR-TB, is an important step towards improving the tolerability of the intensive phase of MDR-TB treatment.
We found that use of lidocaine significantly lowered pain scores in the first 15 min following kanamycin injection, which is important considering that the highest pain scores were reported by participants immediately post-dose, and that the median time to resolution of pain was 30 min. Lidocaine has a rapid onset of action and a limited toxicity profile unless administered intravenously (22,23). Furthermore, lidocaine is inexpensive and widely available and therefore appropriate for use in low- and middle- income settings, which have the highest burden of MDR-TB.

Data on kanamycin pharmacokinetics are limited. We found that the C_{max} and AUC of kanamycin were in accordance with the expected range (20,24). A higher C_{max} was found in a small study of patients treated for MDR-TB in Korea with a higher dose of kanamycin (25). Lidocaine had no effect on kanamycin pharmacokinetics; this finding is in line with the findings of others who have assessed the effect of lidocaine on the pharmacokinetics of other drugs when administered intra-muscularly (3). In the regression analysis, we found use of lidocaine to be independently associated with pain reduction in the single and multiple variable model. As participants received a higher volume of solution when dosed with lidocaine, we explored the possibility in the regression model that a higher volume of injected solution may increase pain due to a greater stretch on pain receptors, but found this to be non-significant. We found HIV infection to be associated with a higher pain score, although this finding needs to be interpreted with

caution as the confidence intervals were wide, likely due to the small sample size. HIV infection has been associated with increased morbidity, including depression, which has been shown in several studies to lower pain thresholds (26,27). We observed symptoms of psychological distress, including anxiety and depression, in three participants who were HIV-infected, although the presence of these symptoms was not associated with higher pain scores in the regression analysis. However, our study was not powered to assess the influence of mental health or HIV infection on pain scores. Our study had three main limitations. First, for logistical reasons only the participants were blinded to whether kanamycin was dosed with lidocaine or not. However, single blinding was very unlikely to cause bias as the participants completed the pain scores without input from study staff. Second, pain assessments were done on occasion 1 and occasion 2 after a median of approximately 6 and 8 weeks of treatment, respectively. As repeated administration of kanamycin may result in a painful induration at the injection site, we cannot exclude the possibility that pain scores may have been different if pain assessments had been conducted sooner or later after treatment initiation. Third, study participants were all in-patients with a high prevalence of comorbidities and psychosocial problems. The results may therefore not be generalisable to all MDR-TB patients.

4.6. CONCLUSION

Lidocaine use reduces the pain associated with kanamycin injections. Given the toxicity and poor tolerability of the MDR-TB treatment regimen, reducing the pain caused by the injectable during the intensive phase of treatment could potentially result in improving adherence and, ultimately, treatment completion rates in MDR-TB.

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Conflicts of interest: none declared.

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CHAPTER 5

Effect of tablet crushing on drug exposure in the treatment of multidrug-resistant tuberculosis.

Court R,[1] Chirehwa MT,[1] Wiesner L[1], de Vries N[2], Harding J[3], Gumbo T[4], Maartens G[1], McIlleron H[1]

[1]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, South Africa; [2]Brooklyn Chest Hospital, Cape Town; [3]DP Marais Hospital, Cape Town, [4]Center for Infectious Diseases Research and Experimental Therapeutics, Baylor Research Institute, Baylor University Medical Center, Dallas, Texas, USA

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During the PODrTB data collection at Brooklyn Chest and DP Marais TB hospitals, I noticed that TB drugs were frequently crushed for MDR-TB patients who were unable to swallow whole tablets. Tablet crushing is also commonly used to dose patients with a depressed level of consciousness (where crushed tablets mixed with water are administered via nasogastric tube), or for children who may be unable to swallow whole tablet formulations. The process of crushing tablets has been shown to affect the bioavailability of some drugs, including rifapentine (1,2), but has no effect on other drugs (3–5). We therefore aimed to investigate whether tablet crushing affects the exposure of the drugs commonly used to treat MDR-TB.

We therefore performed a sequential pharmacokinetic study in patients on treatment for MDR-TB to compare the bioavailability of crushed and whole tablet formulations of individual drugs. Participants were sampled on two occasions spaced 7-14 days apart at six timepoints over 10 hours. Whole tablet formulations were administered on the first and crushed tablets at the second pharmacokinetic sampling occasion. We compared individual drug exposures using geometric mean ratios.

We found the bioavailability of crushed isoniazid to be approximately 42% less than the equivalent whole tablet formulation. The exposure of other drugs in the treatment regimen were also reduced with crushing, although we found the difference not to be significant. Following this study, another research group in Cape Town also described low isoniazid exposures in children treated for MDR-TB who received crushed medications (6). Terizidone

has been reported to reduce the absorption of isoniazid via a potential drug-drug interaction (6), – it is a possibility that this interaction is enhanced when the tablets are crushed and administered together with water. We also considered than an interaction with an excipient used in in the production of one of the drugs in the treatment regimen, may have accentuated the low isoniazid concentrations we observed when isoniazid was crushed. From the results of this study, we recommended that the crushing of isoniazid be avoided if possible, and that paediatric isoniazid syrup formulations be used instead if tablet crushing be clinically indicated.

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5.1. SUMMARY

Setting

Treatment outcomes in multidrug-resistant tuberculosis (MDR-TB) are poor. Due to drug toxicity and a long treatment duration, approximately half of patients are treated successfully. Medication is often crushed for patients who have difficulty swallowing whole tablets. Whether crushing tablets affects drug exposure in MDR-TB treatment is not known.

Method

We performed a sequential pharmacokinetic study in patients aged > 18 years on treatment for MDR-TB treatment at two hospitals in Cape Town, South Africa. We compared the bioavailability of pyrazinamide, moxifloxacin, isoniazid, ethambutol, and terizidone when the tablets were crushed and mixed with water before administration versus swallowed whole. We sampled blood at six time points over 10 hours under each condition separated by two weeks. Non-compartmental analysis was used to derive the key pharmacokinetic measurements.

Results

Twenty participants completed the study: 15 were men, median age 31.5 years. There was a 42% reduction in area under the concentration-time curve to 10 hours ($AUC_{0–10}$) of isoniazid when the tablets were crushed compared with whole tablets (geometric mean ratio 58%; 90% CI: 47 to 73). Crushing tablets of pyrazinamide, moxifloxacin, ethambutol, and terizidone did not affect the bioavailability significantly.

Conclusion

We recommend that crushing of isoniazid tablets in the MDR-TB treatment regimen be avoided. Paediatric isoniazid formulations may be a viable alternative if the crushing of isoniazid tablets is indicated.

Keywords: MDR-TB; crushed; pharmacokinetic; bioequivalence; bioavailability

5.2. BACKGROUND

Outcomes for multidrug-resistant tuberculosis (MDR-TB) are poor with treatment completion rates of ~54% being reported (7). A heavy pill burden together with nausea and vomiting, which has been reported to occur in $\leq$ 75% of patients contribute to poor regimen tolerability (8).

It is standard practice in some centres to crush medication, and mix the crushed tablets with water to ease ingestion in the belief that this will reduce gastrointestinal upset. Crushing of tablets before administration is also common in young children because suitable formulations are frequently not available for those unable to swallow whole tablets. One qualitative study at a paediatric hospital in Cape Town, South Africa, reported that ≤ 69% of caregivers crush, dissolve or mix TB medication with food before administration (9). Crushing tablets may also be necessary in critically ill patients with a depressed level of consciousness who cannot swallow and, therefore, require drug administration via a nasogastric tube. However, tablet crushing may alter bioavailability of the active ingredients within the drug (10). Studies comparing the bioavailability of crushed versus whole medication have shown that crushing decreases the plasma concentrations of some drugs, including rifapentine (1,2), but not of others (3,4). Combining the crushed tablets of a multidrug regimen is common, moreover the tablets may be mixed into a vehicle-containing substances that reacts with the drugs (5,11,12). Remnants may also adhere to the walls of the container in which the medication was crushed and thereby escape ingestion. Subtherapeutic plasma concentrations of some first and second line antituberculosis drugs have been associated with poor clinical outcomes, including acquisition of drug resistance (13). It is therefore important to understand whether crushing affects exposure of the drugs used to treat MDR-TB, many of which are key drugs in the recently updated World Health Organisation (WHO)-recommended management guidelines (14).

5.3. METHOD

We performed a sequential pharmacokinetic study. This involved two intensive sessions of pharmacokinetic sampling in patients aged ≥18 years on MDR-TB treatment at Brooklyn Chest Hospital and DP Marais Hospital in Cape Town. Between May 2016 and February 2017, we recruited participants with rifampicin resistant TB who qualified for MDR-TB treatment. At the time of the study, the standard MDR treatment regimen comprised pyrazinamide, moxifloxacin, kanamycin, cycloserine (dosed as terizidone), and either ethionamide or isoniazid depending on the results of the line-probe assay for katG and inhA mutations identified in the pretreatment sputum culture, which indicated high-level resistance to isoniazid or low-level resistance to isoniazid and resistance to ethionamide, respectively (15). Ethambutol was added if there had been no ethambutol exposure in the month before treatment initiation, and the possibility of ethambutol resistance was considered to be low. We considered patients eligible for recruitment who were taking whole-tablet MDR treatment, either for MDR-TB (defined as resistance to both rifampicin and isoniazid (14) or for rifampicin-monoresistant TB (resistance to rifampicin but susceptible to isoniazid).

Two sessions of pharmacokinetic sampling spaced approximately 1–3 weeks apart (to allow as little inter-session variability as possible) were completed for each participant ≥2 weeks after treatment initiation. Drug doses were in accordance with the national treatment guidelines during the study period (16), and adjusted for toxicity before the first session of pharmacokinetic sampling occasion at the discretion of the treating clinician (16).

In the case of low-level isoniazid resistance, participants were given high doses of isoniazid (10–15 mg/kg); the standard dose of isoniazid (5 mg/kg) was prescribed for participants with rifampicin-monoresistant TB. Participants were given the same drug doses on both sessions of pharmacokinetic sampling, whole tablets on the first session and crushed tablets at the second session. Participants continued with whole-tablet treatment until the second session of pharmacokinetic sampling, when they received crushed tablets; thereafter, they continued on whole tablet treatment. Dosing was performed under fasting conditions and

was strictly observed by the study physician or nurse. At the second session of pharmacokinetic sampling, tablets were crushed with a standard-size mortar and pestle, terizidone capsules were opened carefully. All contents were mixed with 200mL of water in a mixing cup. After ingestion, tablet remnants adhering to the walls of either the mortar, pestle or mixing cup were scraped off with a spatula, mixed with a small unmeasured amount of water, and swallowed by the participant. A standard breakfast was given to all participants ≥ 1 hour after dosing. We recorded all concurrent medication that could influence plasma drug concentrations via drug-drug interactions. At both pharmacokinetic sampling sessions, we sampled blood at the following time points on both pharmacokinetic sampling occasions: pre-dose and at 2, 4, 6, 8, and 10 hours post dose. After centrifugation, plasma was extracted using a pipette and stored temporarily on dry ice before being transported to the Division of Clinical Pharmacology at the University of Cape Town for storage at minus 80° Celsius. Plasma drug concentrations were determined using liquid chromatography tandem-mass spectrometry (LC-MS/MS) (17–19). LC-MS/MS was validated according to guidelines set by the US Food and Drug Administration and European Medicines Agency (20,21).

We used Stata v15.0 (Stata Corp, College Station, TX, USA) to perform non-compartmental pharmacokinetic and statistical analyses. We determined the following pharmacokinetic parameters for each drug on both dosing sessions: area under the concentration time curve at 0–10 h ($AUC_{0–10}$) using the trapezoidal rule, area under the concentration-time curve extrapolated to infinity (AUC_{∞}), half-life, peak concentration (C_{max}) and time to C_{max}. We regarded pre-dose drug concentrations below the lower level of quantification (BLQ) to be zero if all the pre-dose concentrations for a particular drug were BLQ. If any pre-dose concentrations for a drug were quantifiable, we then regarded all pre-dose BLQ concentrations for that drug to be half the lower level of quantification (LLQ). Similarly, post-dose drug concentrations within the sampling interval were unlikely to be zero, so we considered any post-dose BLQ results for any of the drugs to be half the LLQ. We used the Wilcoxon signed-rank test for paired data to compare C_{max} and $AUC_{0–10}$ at each session. Then, the log-transformed values of C_{max} and $AUC_{0–10}$ for exposure to crushed and whole tablets were compared with Student's t-tests. The geometric mean ratio (GMR) point estimates and 90% confidence intervals (CIs) of C_{max} and $AUC_{0–10}$ for crushed vs. whole

tablets were calculated for pyrazinamide, moxifloxacin, ethambutol, isoniazid and cycloserine.

Approval of our study protocol was granted by the Human Research Ethics Committee of the University of Cape Town, Cape Town, South Africa (106/2016). Written informed consent was taken from each participant in a language of his/her choice (English, Afrikaans or Xhosa). All informed consent was obtained before participant recruitment.

5.4. RESULTS

We recruited 25 participants, 20 of whom completed the study: four completed only the first pharmacokinetic sampling session and one participant was withdrawn before any pharmacokinetic sampling could be performed. The characteristics of the 20 participants who completed the study are shown in 12. A descriptive comparison of the C_{max} of each of the drugs in crushed and whole form compared with the expected range (22) is shown in Table 13. Table 14 gives a comparison of the $AUC_{0–10}$ and C_{max} of whole and crushed tablets of pyrazinamide, moxifloxacin, ethambutol, isoniazid and cycloserine. The $AUC_{0–10}$ of all drugs was reduced for crushed vs. whole-tablet formulations but the reduction was significant only for isoniazid. We did not evaluate ethionamide as too few participants (n = 8) were receiving this drug at the time of pharmacokinetic sampling. The GMRs (with 90% CIs) for crushed vs. whole tablets are shown in Table 15. Table 16 compares additional pharmacokinetic parameters of the whole and crushed forms for each of the drugs. The half-life and AUC_{∞} of some drugs could not be calculated in participants who had multiple drug concentration values reported as BLQ. Figure 5 shows the median time-concentration profiles of crushed and whole tablets of isoniazid to 10 h post-dose. The median time-concentration profiles of crushed and whole tablets of cycloserine, pyrazinamide, moxifloxacin and ethambutol are shown in Figure 6. The increased variability of isoniazid compared with the other drugs reflected the wider range of dosing, per isoniazid susceptibility, in participants with rifampicin-resistant TB (23).

Table 12. Characteristics* of 20 patients on treatment for MDR-TB in a sequential comparative pharmacokinetic analysis

Men/women		15/5	
Multidrug-resistant tuberculosis/rifampicin monoresistant tuberculosis		13/7	
Age (years)		31.5 (25.8 to 44.0)	
HIV-positive/negative		10/10	
		Occasion 1	Occasion 2
Weight (kg)		49 (44 to 54)	50 (44 to 55)
BMI (kg/m^2)		17.4 (16.0 to 19.3)	17.1 (16.6 to 19.6)
Creatinine clearance (mL/min)**		94.4 (81.1 to 105.5)	92.1 (80.7 to 103.8)
Duration on treatment at time of pharmacokinetic sampling (days)		40.5 (32 to 45)	53 (44.5 to 60)
Dose (mg/kg)	Pyrazinamide, n=20	29.8 (27.8 to 30.9)	29.4 (27.3 to 31.1)
	Isoniazid, n=17	11.8 (6.5 to 12.2)	11.4 (6.4 to 12.1)
	Moxifloxacin, n=20	8.2 (7.6 to 9.0)	8 (7.4 to 9.1)
	Ethambutol, n=19	16.7 (16 to 20)	17.0 (15.7 to 19.0)
	Terizidone, n=20	15 (13.9 to 16.3)	15 (13.8 to 16.0)

*Unless otherwise indicated summarized as median (interquartile range)
**Cockcroft-Gault method
TB = tuberculosis; HIV = human immunodeficiency virus; BMI – body mass index

Table 13. Comparison of peak concentrations of pyrazinamide, isoniazid, moxifloxacin, ethambutol and terizidone with expected ranges in patients on therapy for multidrug-resistant tuberculosis

Drug		Whole C_{max} (mg/L)	Crushed C_{max} (mg/L)	Expected C_{max} (mg/L)
Pyrazinamide		42.7 (36.85 to 46.5)	41.0 (38.25 to 46.95)	20 to 60
Isoniazid	Standard dose (5mg/kg), n=6	0.89 (0.56 to 1.22)	0.55 (0.28 to 1.09)	3 to 6
	High dose (10-15 mg/kg), n=11	4.83 (3.54 to 6.88)	2.84 (2.08 to 4.22)	
Moxifloxacin, n=20		2.44 (2.06 to 2.68)	2.27 (1.82 to 2.67)	3 to 5
Ethambutol, n=19		1.91 (1.58 to 2.27)	1.82 (1.34 to 2.87)	2 to 6
Cycloserine, n=20		33 (26.6 to 36.65)	34.45 (29.45 to 38.95)	20 to 35

C_{max}: Peak concentration
Interquartile range in brackets

41.0 [38.3–47.0]

Table 14. Geometric mean ratio (90% confidence interval) of AUC_{0-10} and C_{max} for crushed versus whole tablets in the treatment of patients with multidrug-resistant tuberculosis

	Isoniazid (n=17)	Moxifloxacin (n=20)	Pyrazinamide (n=20)	Ethambutol (n=19)	Cycloserine (n=20)
AUC_{0-10}	58% (47% to 73%)	89% (80% to 99%)	98% (93% to 103%)	100% (89% to 112%)	101% (91% to 113%)
C_{max}	54% (40% to 73%)	90% (82% to 98%)	97% (93% to 101%)	101% (85% to 121%)	102% (91% to 114%)

Interquartile range in brackets
AUC_{0-10}: Area under the concentration-time Curve from 0 to 10 hours
C_{max}: Peak concentration

Table 15. Comparison of median AUC_{0-10} and C_{max} between whole and crushed tablets in patients on treatment for multidrug-resistant tuberculosis

Drug	Whole AUC_{0-10}	Crushed AUC_{0-10}	p	Whole C_{max}	Crushed C_{max}	p
Isoniazid (n=17)	13.8 (4.6 to 24.8)	7.3 (1.8 to 12.3)	0.023	3.5 (1.2 to 5.2)	2.1 (0.3 to 3.3)	0.016
Pyrazinamide (n=20)	316.1 (256.5 to 354.6)	307.0 (281.8 to 341.4)	0.35	42.7 (36.9 to 46.5)	41 (38.3 to 47.0)	0.13
Moxifloxacin (n=20)	15.2 (10.3 to 18.7)	14.2 (9.3 to 17.6)	0.22	2.4 (2.1 to 2.7)	2.3 (1.8 to 2.7)	0.056
Ethambutol (n=19)	11.3 (9.5 to 12.8)	11.0 (8.4 to 15.2)	0.63	1.9 (1.6 to 2.3)	1.8 (1.3 to 2.9)	0.75
Cycloserine (n=17)	281.9 (227.7 to 308.7)	281.2 (259.0 to 327.3)	0.49	32.7 (26.4 to 34.8)	34.3 (29.9 to 39.2)	0.39

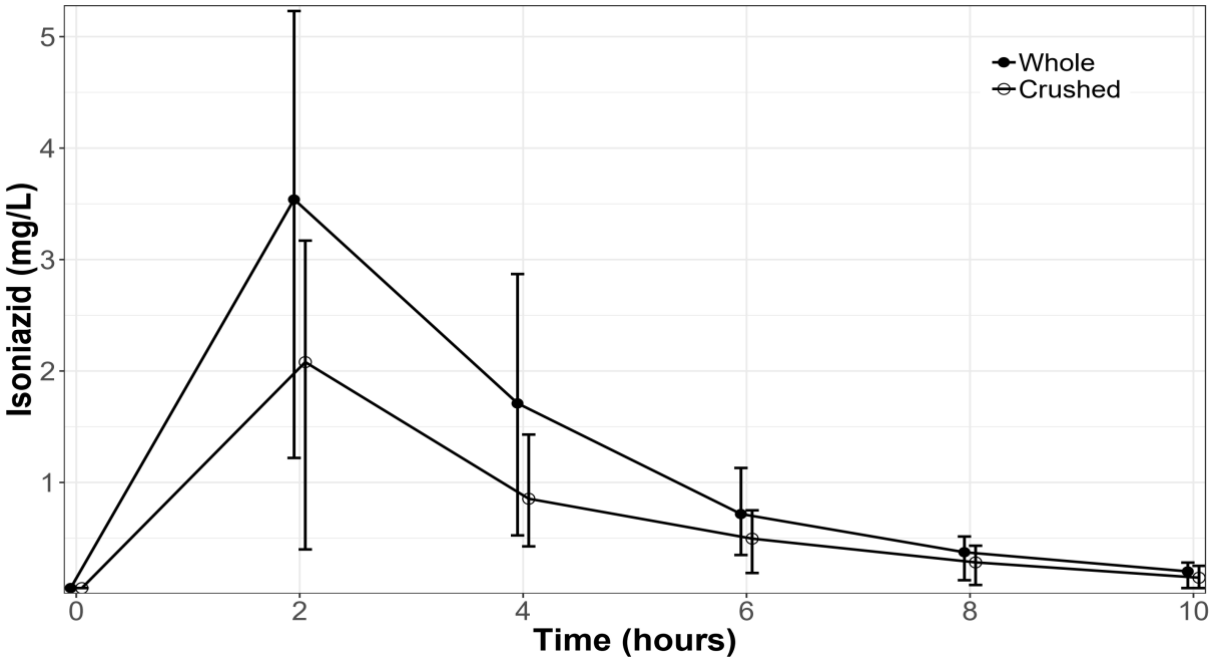
AUC_{0-10}: Area under the concentration-time curve to 10 hours
C_{max}: Peak concentration

Table 16. Additional median pharmacokinetic measures in patients* on second line drugs for multidrug-resistant tuberculosis

	AUC_{∞}** (µg·h/mL)		Half-life (hrs)		T_{max} ***(hrs)	
	Whole	Crushed	Whole	Crushed	Whole	Crushed
Isoniazid	n=16	n=13	n=16	n=13	n=17	n=17
	16.5 (6.0 to 25.8)	12.1 (7.5 to 13.3)	2.4 (1.6 to 3.6)	2.5 (1.9 to 3.3)	2 (2 to 2)	2 (2 to 2)
Pyrazinamide	619.3 (473.1 to 718.5)	517.7 (437.8 to 727.5)	9.0 (6.8 to 9.7)	7.7 (6.7 to 10.3)	2 (2 to 2)	2 (2 to 2)
Moxifloxacin	21.8 (11.9 to 27.1)	21.2 (11.2 to 26.1)	4.9 (4.0 to 6.1)	4.8 (3.1 to 6.4)	2 (2 to 2)	2 (2 to 2)
Ethambutol	n=19	n=19	n=19	n=19	n=19	n=19
	13.9 (12.0 to 16.4)	14.4 (10.9 to 19.1)	4.0 (3.3 to 4.6)	3.8 (3.4 to 5.3)	2 (2 to 2)	2 (2 to 2)
Cycloserine	n=18	n=18	n=18	n=18	4 (4 to 6)	4 (2 to 6)
	803.8 (575.0 to 1127.2)	841.8 (553.3 to 1802.9)	12.1 (11.0 to 28.4)	11.8 (8.1 to 40.2)		

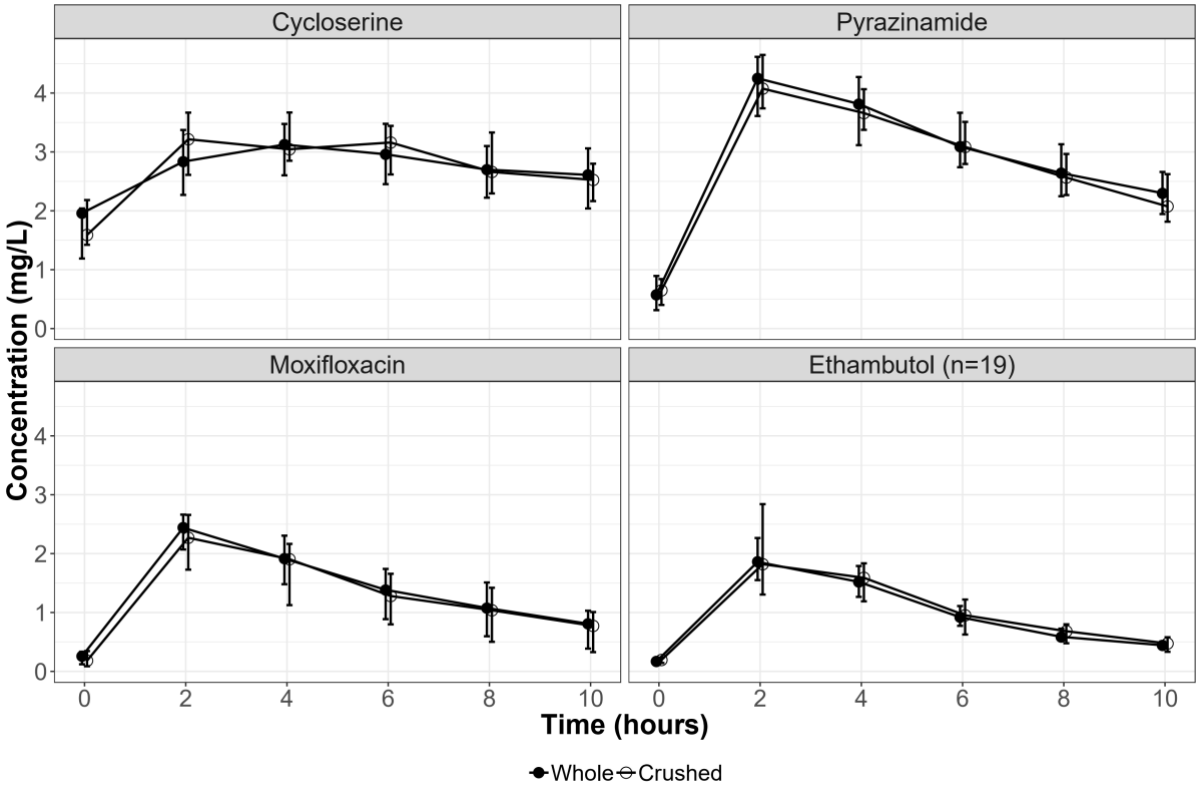
*n=20 unless otherwise indicated; interquartile range in brackets
**AUC_{∞}: Area under the concentration-curve extrapolated to infinity
***T_{max}: Time to peak concentration

Figure 5. Concentration-time profile of crushed versus whole isoniazid in 17 participants on treatment for multidrug-resistant tuberculosis



Upper and lower bound of whiskers: Upper and lower interquartile range
Median concentrations of crushed and whole tablets at each time point were offset for clarity

Figure 6. Concentration-time profiles of crushed versus whole cycloserine, pyrazinamide, moxifloxacin and ethambutol in the treatment of patients* with multidrug-resistant tuberculosis



*n=20 unless otherwise indicated
Upper and lower bound of whiskers: Upper and lower interquartile range
Median concentrations of crushed and whole tablets at each time point are offset for clarity
Cycloserine and pyrazinamide concentrations are 10-fold the concentration displayed on the y-axis

5.5. DISCUSSION

We noted significantly decreased exposure of isoniazid when the orally administered drugs in the MDR-TB treatment regimen were crushed, and mixed with water. Dosing with crushed isoniazid could affect outcomes in MDR-TB treatment, considering that low isoniazid exposure has been associated with a poor treatment response, including the development of drug resistance (13,24,25). Exposure of the crushed-tablet forms of the other drugs we assessed was also decreased compared with whole tablets at the same dose, but this difference did not reach statistical significance.

Little is known about the effect of crushing tablets on drug exposure in the treatment of MDR-TB. Recently, a study in Cape Town observed low isoniazid exposures in children on MDR treatment, most of whom were dosed with crushed isoniazid (26). Isoniazid is also a key drug in the treatment of drug-susceptible and drug-resistant TB, and is the drug of choice in TB chemoprophylaxis (27,28). These findings, therefore, have important clinical implications, particularly in MDR-TB, where the companion drugs are relatively less efficacious.

The mechanism causing poor isoniazid exposure when MDR-TB drug formulations are crushed and mixed together in water is not known. Terizidone, which consists of two molecules of cycloserine (29), has been reported to interfere with isoniazid absorption, but this effect is poorly understood (30). A drug-drug interaction whereby cycloserine degrades isoniazid before absorption or inhibits isoniazid absorption could be enhanced if the drugs are crushed and administered together in water. We observed that exposure to whole tablets of isoniazid at standard doses to be lower than that reported in the literature (see Table 13), with exposure of crushed tablets of isoniazid being significantly lower than the equivalent whole tablets. Our finding of lower-than-expected exposures of whole tablets of isoniazid supports the notion of a possible drug-drug interaction, which is enhanced if the orally administered drugs are crushed together. We also considered that an interaction with an excipient used in the production of one of the other drugs in the regimen, may be a possible cause of isoniazid degradation when the tablets were crushed together (31). Another potential reason, which could explain the reduced exposure of crushed tablets of isoniazid, is that isoniazid is considered by some scholars to be unstable if mixed with water, although there are no data to support this hypothesis. Conversely, crushed tablets of isoniazid, if included in a fixed-drug combination, have been shown to result in therapeutic concentrations in the treatment of adults with drug-susceptible TB (23). A powder formulation of isoniazid and crushed tablets of isoniazid mixed with water have also been shown to achieve target concentrations in children (31,32).

In settings in which the crushing of isoniazid tablets is indicated, there are several possible approaches to ensure optimal dosing. Paediatric isoniazid formulations or constituting the dose with smaller isoniazid tablets (e.g., 100 mg) with proven bioequivalence that are

swallowed more readily (33) should be used instead of crushing isoniazid tablets in adults on treatment for MDR-TB. The extent to which the isoniazid dose must be increased if crushed tablets of isoniazid are administered to patients is unclear and requires further study. We found a 42% reduction in the $AUC_{0–10}$ of isoniazid when the tablets were crushed compared with that using whole tablets (GMR 58%; 90% CI 47–73%), indicating that the dose of isoniazid, if crushed together with the other drugs in the MDR-TB treatment regimen, will require significant adjustment to achieve target concentrations. Exposures to pyrazinamide, moxifloxacin, ethambutol, or cycloserine were not significantly decreased by tablet crushing, indicating that tablets of these drugs may be safely crushed when necessary. Nevertheless, there was a trend towards lower exposure of crushed moxifloxacin tablets compared with the equivalent whole tablets.

Our study had three main limitations. First, when tablets were crushed, care was taken to ensure that as much of the crushed tablet remnants as possible were ingested by the participants by rinsing the mixing cup. We have observed that nursing staff in busy treatment centres often do not have time to ensure that all crushed remnants are swallowed by patients, which could result in a further reduction in drug exposure. Our study may have been more clinically relevant if we did not rinse the mortar, pestle and mixing cup after crushing the tablets on the second day of pharmacokinetic sampling. Second, the pharmacokinetic sampling sessions using crushed and whole tablets were not randomised. We, therefore, cannot exclude the possibility of a sequence effect on our bioavailability comparisons of crushed- and whole-tablet treatments. Third, the pre-dose sample on the second crushed pharmacokinetic sample may have been affected by the pre-dose sample, which may have had some effect on the AUC of the crushed tablets, as there was insufficient time for complete washout of drugs with longer half-lives, particularly cycloserine.

5.6. CONCLUSIONS

We recommend that crushing tablets of isoniazid together with the other orally administered drugs in the MDR treatment regimen be avoided. Also, paediatric isoniazid formulations should be considered for use in adults if tablet crushing is indicated.

5.7. ACKNOWLEDGEMENTS

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5.8. REFERENCES

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CHAPTER 6: DISCUSSION

The MDR-TB treatment landscape has changed considerably since the initiation of PODrTB at which time MDR-TB patients were treated for 18-24 months (1). The shortened 9-12 all-oral treatment regimen for MDR-TB, which is now available in an increasing number of settings, is indicated for patients in whom fluoroquinolone resistance has been excluded, and where previous exposure to second line drugs is limited to less than one month duration (2); patients eligible for the shortened regimen should also not have severe extrapulmonary disease (2). In May 2022, the WHO announced that an alternative shorter regimen including bedaquiline, pretomanid, moxifloxacin, and linezolid for a total duration of six months may be used for patients ≥15 years of age, where previous exposure to drugs used in the treatment regimen was limited to one month or less (3). The long regimen is recommended for MDR-TB patients with severe disease, including miliary TB and TB meningitis, and for patients who have previously failed one of the shorter regimens (3). Deciding on which of the three regimens to use is at the discretion of the treating clinician, who should consider patient preference, drug sensitivity results, the patient's treatment history, the risk of adverse events, and severity and site of disease (4).

The assembly of the long regimen should include a minimum of five effective drugs with all the three drugs from Group A (linezolid, bedaquiline, and levofloxacin/moxifloxacin), and preferably both drugs from Group B (clofazimine and cycloserine/terizidone). Recent modifications to the WHO grouping of second line TB drugs have seen some drugs e.g., kanamycin no longer considered treatment options while others, (e.g., cycloserine/terizidone) were up-classified based on the findings of a recent meta-analysis (5). Identifying exposure thresholds for individual drug-related toxicities and efficacy targets of different combination therapies is critical to optimize the dosing of TB drugs, and ultimately identify drug combinations, which will improve outcomes. The understanding of these pharmacokinetic/pharmacodynamic relationships in MDR-TB is progressing rapidly, with significant strides made in the understanding of some TB drugs from the time of protocol development to summation of this thesis.

Cycloserine, or its structural analogue terizidone, is one second line TB drug where our understanding of the drug's thresholds for efficacy and toxicity has progressed considerably. Terizidone consists of two molecules of cycloserine, which acts by interfering with peptidoglycan formation and bacterial cell wall synthesis by inhibiting two enzymes, alanine racemase and D-alanine ligase (6). Cycloserine is considered bacteriostatic against mycobacterium tuberculosis, but can be bactericidal if exposures are sufficiently high (6). There have been historical concerns regarding the efficacy of cycloserine/terizidone which, combined with the drug's neuropsychiatric toxicity (in particular, depression and psychosis), has hindered widespread use of the drug in MDR-TB treatment programmes. A recent review, however, showed cycloserine to be more efficacious than some commonly used TB drugs (5), thereby (to the surprise of many), prompting the reclassification of cycloserine from a substitute drug in Group C to become a priority inclusion as a Group B drug in long MDR-TB treatment regimens (7). Terizidone was previously considered to be safer than cycloserine, and therefore advocated for use in some programmes, but a recent review showed no significant safety difference between the two drugs (8). At the time of initiation of PODrTB, there was only one study in TB patients (published in 1974) describing the pharmacokinetics of terizidone/cycloserine, but the method used to measure the drug concentrations (calorimetry) is no longer used (9). The first publication generated from the PODrTB cohort described the pharmacokinetics of cycloserine in the first 35 recruited participants, using non-compartment analyses (NCA). Using liquid chromatography tandem mass spectrometry, we were unable to detect terizidone in plasma, in keeping with previous reports that terizidone hydrolyses pre-systematically to cycloserine (10). Although the sample size was limited, we described higher cycloserine concentrations than previously reported; our data also suggested an accumulation effect with cycloserine concentrations still rising in some participants at 10 hours post dose (11), suggesting that a once-daily dosing schedule is appropriate for terizidone. Our cycloserine NCA analysis (11) was presented at a technical meeting convened by the WHO on PK/PD relationships of second line TB drugs, and contributed significantly to current recommendations on terizidone dosing (12). Considering the long-half life and apparent accumulation effect of cycloserine, which we observed with our NCA analysis, it was apparent that population pharmacokinetic modelling would be the preferred approach to best describe the drug's pharmacokinetics. At the time of publication of our cycloserine NCA analysis, concentration thresholds for

cycloserine-related toxicity and efficacy had not been clearly defined. Using population PK modelling, a small study in Korea suggested that cycloserine dose of 500-750mg daily was required to achieve target exposures, which were recommended at the time of study (13). Using a hollow-fibre system model, which is an in-vitro system recognized by the European Medicines Agency as a method to support the selection and development of anti-TB drugs (14), Deshpande et al. observing that mycobacterial kill was related to time above minimum inhibitory concentration (MIC), with a 1.0 log10 CFU/mL mycobacterial kill achieved with 30 % exposure time above MIC (15). Deshpande et al indicated that cycloserine demonstrated good mycobacterial kill against TB, and that the previous premise that cycloserine was bacteriostatic was related more to the drug's poor penetration of lung cavities (15). Considering the drug's poor intracellular kill of TB, which constitute approximately 20% of intra-cavitary mycobacteria, the authors therefore suggested a cycloserine dose of 750mg bd(15). Shortly following the publication of Deshpande's study, Alghamadi et al. also described a population PK model of cycloserine, and showed that that the probability of target attainment increased as the dose of cycloserine was increased (16). For patients with higher cycloserine MICs of ≥ 16 mg/litre, Alghamadi also recommended a daily cycloserine dose of 500mg TDS or 750mg BD, which is higher than the current WHO-recommended dose, even in higher weight bands (17). Using the PODrTB cohort, Chirehwa et al. from our group, described a population PK model of cycloserine, which included the patients from our earlier NCA analysis (11). Chirehwa et al. showing that with current dosing guidelines of cycloserine dosed as terizidone (15-20mg/kg up to a maximum of 1000 mg daily), exposure time above MIC for ≥ 30% of the dosing interval was obtainable for 90% of participants, only if the participant's baseline sputum had an MIC value of ≤16 mg/litre (6). However, the proportion of patients achieving 100% exposure time above MIC, which is associated with the prevention of resistance, was more than 90% only at MICs of ≤ 8 mg/litre (6). At a similar time, Mulubwa et al. described an interesting population pharmacokinetics model of terizidone and cycloserine, quantifying both analogues in the plasma of patients treated for MDR-TB (18), which contradicts our observation that terizidone was not detectable in plasma. The method used by Mulubwa et al. to measure the terizidone and cycloserine concentrations was high-performance liquid chromatography–UV method. Using liquid chromatography mass spectrometry, which is the gold standard for drug quantification, we did not observe terizidone in the plasma of

PODrTB patients, which corresponds with the understanding that terizidone hydrolyses pre-systemically to cycloserine (10). Terizidone consists of two molecules of cycloserine, joined by a terephthalaldehyde moiety (9). There are several possibilities, which we considered to explain why Mulubwa et al. observed terizidone in plasma. First, considering that terizidone is understood to hydrolyse to cycloserine, it is possible that Muluwba et al. measured terizidone in plasma prior to the drug's separation to form cycloserine – however, if this was the case, we would have expected to detect some terizidone in plasma using the mass spectrometer, which we were unable to do. The second (more plausible) explanation for the contradicting measurement of terizidone, is a difference in the assay method (liquid chromatography mass spectrometry vs liquid chromatography UV method), but this requires further study.

The recommended cycloserine target therapeutic concentration is 20–35 μg/mL (19). An early study of cycloserine in the management of TB described psychotic symptoms in several patients with cycloserine concentrations >40 mg/mL (20). A more recent report also described psychosis in an MDR-TB patient treated with cycloserine with a plasma cycloserine concentrations of >35 mg/mL (21). The WHO has acknowledged that severe central nervous system toxicity including seizures, depression, psychosis, and suicidal ideation are more likely to occur with peak cycloserine concentrations >35 mg/mL, but also highlights that these events can occur with therapeutic cycloserine concentrations (17).

Peripheral neuropathy, which has been uncommonly reported as an adverse effect in MDR-TB patients treated with cycloserine/terizidone, was the largest contributor to the incidence of neuropsychiatric adverse events in the PODrTB cohort - 34.7% of participants developed either new or worsening neuropathy during the 12-week intensive phase of MDR treatment. We found participants with a cycloserine C_{max} of >35 μg/mL to be approximately twice as likely to develop incident or worsening peripheral neuropathy, and found cycloserine $AUC_{0\text{-}24,}$ cycloserine clearance, and high dose pyridoxine to be significantly associated with the incidence of peripheral neuropathy on univariate analyses. Cycloserine clearance maintained its association with neuropathy after adjusting for the effect of high-dose pyridoxine – see Chapter 3. During the time of study recruitment, local guidelines suggested the inclusion of high-dose pyridoxine (150-200mg daily) in MDR-TB treatment regimens

including terizidone, to prevent peripheral neuropathy (1). Cycloserine and pyridoxine are understood to be antagonistic; cycloserine also increases the renal elimination of pyridoxine (22). There is, however, accumulating evidence that high-dose pyridoxine is toxic to peripheral nerves (23–26), and was likely a significant contributor to the high rate of neuropathy we observed. In the mid 1980's, Dalton and Parry in separate studies described symptoms of peripheral neuropathy in patients with an increased intake of pyridoxine. The toxicity appeared to be related to the dose and duration of pyridoxine ingestion – symptoms also improved when the drug was withdrawn (23-24). In a more recent review in 2014, Ghavanini and Kimpinski completed a systematic review of pyridoxine toxicity and suggested that 6mg of pyridoxine per day was likely sufficient to prevent the neuropathic effects related to pyridoxine deficiency, but that daily doses of > 50 mg is likely to be harmful (26). As discussed in chapter 3, we also cannot exclude the contribution of other comorbidities, including HIV and diabetes, which may have caused or predisposed PODrTB patients to develop neuropathy.

The data generated from PODrTB has contributed significantly to the current understanding of cycloserine pharmacokinetics, including justification of current recommendations on dosing frequency, and highlights the limitation of current WHO dosing guidelines, particularly in patients with higher MICs. Although PK/PD relationships of cycloserine/terizidone related to toxicity requires further study, there is a signal that the higher dose of cycloserine required to achieve target exposures may increase the risk of neuropsychiatric adverse effects, including peripheral neuropathy.

Until an important meta-analysis published in 2018 showed the efficacy of kanamycin to be poor when compared with some TB drugs (23), kanamycin was considered a key second line TB drug. Consequently, kanamycin is no longer recommended by the WHO for inclusion in MDR-TB treatment regimens, a decision which has come as a relief to patients and practitioners alike, considering the drug's burdensome toxicity profile, which includes irreversible hearing loss (24). The incidence of audio-toxicity related to the long-term use of aminoglycosides in MDR-TB cohorts has repeatedly been shown to be unacceptably high, with rates of hearing loss reported to be as high as 90% in some cohorts (27,28). In the PODrTB cohort, Ghafari et al. detected hearing loss in 84/102 (82.4%) of patients with

analysable hearing data, with severe hearing loss observed in 20/84 (23.8%) (29); similar rates of hearing loss have been reported by others (27,30,31). In the same paper, Ghafari et al also found kanamycin $AUC_{0\text{-}10}$ to be significantly associated with the incidence of hearing loss (29). The activity of kanamycin is concentration dependent (32); the relationship between AUC and audio-toxicity has been well described (29), and appears to be cumulative (32). Despite the widely reported ototoxicity, kanamycin and other aminoglycosides are still used in some settings where newer TB drugs, including bedaquiline are not yet available (33).

The pain caused by the intramuscular administration of kanamycin, which may with repeat injections cause a painful lump at the injection site (34), has not always been included in reports of adverse events related to MDR-TB treatment. Completion rates of MDR-TB treatment have been poor with only approximately 50% of patients completing the previously long treatment regimen. Although not always reported in MDR-TB treatment adherence studies, there is an understanding that the pain caused by the injection of aminoglycosides was one of the factors, which contributed to poor MDR-TB treatment completion rates (35). Lidocaine, a local anaesthetic, has been used successfully to relieve pain caused by the intramuscular administration of drugs in other clinical settings, including ceftriaxone and penicillin G (36,37). At the time of study, it was unknown whether lidocaine could reduce the pain experienced by participants treated with kanamycin for MDR-TB, and second, if the addition of lidocaine had any effect on kanamycin pharmacokinetics. We therefore aimed to explore whether lidocaine reduces the pain experienced by adults treated with kanamycin for MDR-TB. Using a single-blinded crossover study design, we showed that lidocaine reduces the pain experienced by patients immediately, and at 15 minutes post intramuscular administration of kanamycin - this is important considering the highest pain scores were reported in the 0–15-minute time period post-dose. Further to this, we demonstrated that lidocaine had no effect on kanamycin pharmacokinetics. Unlike similar studies where both participant and carer were blinded to the constituents of the drug formulation administered (36–39), for logistical reasons, we were unable to blind the nurse/doctor administering the kanamycin injection. We therefore cannot exclude the possibility of bias in the way the injection was administered, which may have affected the participant pains scores. The reading of the pain scales, however, was performed, as far as

possible, without interference from the investigator. Shortly following the completion of our study, a paediatric research group in Cape Town similarly showed, using a randomised double-blind crossover design, that lidocaine is effective in reducing the pain experienced by children > 5 years of age treated with intramuscular amikacin, a similar aminoglycoside used in the treatment of MDR-TB (38). The same group also found lidocaine to have no effect on the pharmacokinetics of amikacin (38). Currently, WHO guidelines do allow the inclusion of amikacin (or streptomycin) in treatment regimens for adult MDR-TB patients ≥ 18 years if an appropriate oral regimen is not available (17) - drug-sensitivity testing demonstrating sensitivity to aminoglycosides, and appropriate facilities to monitor ototoxicity are mandatory (17). Amikacin has a favourable toxicity profile compared with streptomycin, and is therefore a preferred choice if an aminoglycoside is to be included in an MDR-TB treatment regimen; amikacin may be used in children only as salvage therapy under the same conditions as adults (17).

There is limited data exploring the utility of a local anaesthetic with intramuscularly administered drugs. In 2019, following the completion of our study, Estrada et al. published a study exploring the effect on injection-related pain when a local anaesthetic (mepivacaine) was added to Penicillin G for the treatment of patients with primary syphilis (39). Using a double-blinded randomised crossover study design, the investigators also explored whether using a longer needle with a larger lumen (19 Gauge) rather than a shorter needle (21 Gauge) for administration of Penicillin G would reduce the pain experienced by participants. Participants were randomized 1:1:1:1 to 4 arms with/without mepivacaine administered with a longer/shorter needle. The investigators found that the addition of mepivacaine significantly reduced pain experienced by participants immediately post injection with no difference observed between the groups at six- and 24 hours post dose. The size of the needle used for dosing had no significant effect on participant pain scores (39).

Considering the toxicity profile of aminoglycosides, the recent transition of MDR-TB treatment to an injectable-free regimen has been welcomed by the MDR-TB community. Although kanamycin is now seldom used, amikacin or alternatively streptomycin are still recommended as substitute drugs (group C) where aminoglycoside sensitivity can be

demonstrated (2). If included in treatment regimens, aminoglycosides should be administered together with a local anaesthetic to reduce injection-related pain. A less painful injection is likely to improve adherence to injectable-containing MDR-TB treatment regimens, which has historically been poor.

Tablet crushing is a method commonly used in an attempt to improve the tolerability of many orally dosed medications, including TB drugs. Tablet crushing, which is a common practice at some TB treatment centres (40), is particularly useful for patients with a depressed level of consciousness where crushed tablets may be mixed with water and administered via a nasogastric tube, or for children who may be unable to swallow whole tablet formulations - this is especially relevant in the setting of MDR-TB where the pill burden is heavy (34). During the data collection period in PODrTB, I noticed that some MDR-TB patients preferred ingesting crushed medications (usually mixed with water) rather than swallowing whole tablet formulations, often reporting less nausea with the crushed preparation. The process of crushing tablets has been shown to affect the bioavailability of some important drugs, including rifapentine and lopinavir (41,42) but has no effect on others e.g., bedaquiline (43–46). A case report of a patient treated in France demonstrated therapeutic exposures of bedaquiline, where the drug was administered as a crushed formulation via a nasogastric tube (47). Knowing that low TB drug exposure may affect clinical outcomes, including the development of drug resistance (48–50), we therefore aimed to investigate whether tablet crushing affected the exposure of commonly-used drugs for the treatment of MDR-TB.

The design of the PODrTB crushed study was similar in design to a study by Best et al. who explored the effect of crushing on the exposure of ritonavir-boosted lopinavir in HIV-infected children (42), showing that children dosed with crushed lopinavir achieved lower exposures compared with children dosed with whole tablet formulations. A similar study investigating the effect of crushing on bedaquiline, where there is currently no commercially available paediatric formulation, showed no significant difference in exposure compared to whole tablets, when bedaquiline was dispersed in water (46). Unlike the lidocaine-kanamycin substudy (chapter 4), for logistical reasons we were unable to randomise

participants in our crushed study with a crossover design – we therefore cannot include the possibility of a sequence effect on our findings.

By comparing individual drug exposures using geometric mean ratios, we found the bioavailability of crushed isoniazid to be approximately 42% less than the whole tablet equivalent. The exposure of other drugs in the treatment regimen were also reduced with crushing, although we found the differences not to be significant – see figure 6. Compared with the other drugs in the treatment regimen, we also observed greater variability in isoniazid exposures, likely reflecting variability in dosing: 5mg/kg was prescribed for patients with rifampicin-monoresistant TB; (10-15mg/kg) for patients with MDR-TB. Further to this. we also could not exclude the effect of the N-acetyltransferase 2 (Nat2) genotype on isoniazid variability as these were not sequenced (51,52). Another group in Cape Town also described low isoniazid exposures in children treated for MDR-TB, many of whom received crushed medications (53), although the study design did not randomise participants to receive crushed/whole tablet formulations. Terizidone has previously been reported to reduce the absorption of isoniazid via a potential drug-drug interaction – it is a possibility that this interaction is enhanced when terizidone and isoniazid tablets are crushed and mixed together with water prior to administration. A recent study in mice showed that ethionamide exposure, which is structurally similar to isoniazid, was reduced when ethionamide was co-administered with cycloserine although this effect was not seen when the drugs were dose separately, suggesting that the exposure effect may be a drug-drug interaction related to absorption (54). We also considered than an interaction of isoniazid with an excipient used in in the production of any one of the drugs in the treatment regimen may have caused or accentuated the low isoniazid concentrations we observed, when isoniazid was crushed together with other drugs in the treatment regimen.

We therefore recommend that the crushing of isoniazid be avoided if possible, and that paediatric isoniazid syrup formulations be used instead if tablet crushing be clinically indicated. Further studies are required to explain the reduced exposure we observed with crushed isoniazid. I presented this manuscript at the 11th International Conference on TB pharmacology, October 2018, The Hague, Holland. Abstract no: 25.

This thesis has, in parallel, answered practical questions related to the dosing of MDR-TB treatment, and made significant contributions to the understanding of PK/PD associations, particularly for cycloserine dose as terizidone. Answering one research question satisfactorily will most often lead to new questions – similarly, our research has highlighted knowledge gaps in in PK/PD relationships of key second line drugs, where further data requires rapid accumulation. With enhanced understanding of PK/PD associations of second line drugs, new and repurposed treatment options have improved outcomes, bringing new hope in the treatment of MDR-TB - the work in this thesis has made a tangible contribution to the global effort in accomplishing this goal.

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ANNEX: PODRTB STUDY DESIGN AND OUTCOMES

The general aim of PODrTB (Pharmacometric Optimisation of second line Drugs in the treatment of multidrug-resistant TB) was to identify pharmacokinetic exposure thresholds associated with treatment response using serial MGIT sputum cultures, considering the effect of minimum inhibitory concentrations. Exposure thresholds with specific-treatment related toxicities were also explored by prospectively collecting adverse event data during the first 12 weeks of standard therapy for pulmonary MDR-TB. See Table 1 for the standard MDR-TB treatment regimen during the time of study.

Table 17. Standard regimen used for multidrug-resistant tuberculosis in South Africa during the study period, per weight band

6 MONTH INTENSIVE PHASE - Daily doses by weight				
	<33kg mg/kg)	**33–50 kg**	**51–70 kg**	**> 70 kg**
Kanamycin	15–20	500–750 mg	1000 mg	1000 mg
Pyrazinamide	30–40 mg/kg	1000–1750 mg	1750–2000 mg	2000–2500 mg
Ethionamide	15–20 mg/kg	500 mg	750 mg	750–1000 mg
Moxifloxacin	400 mg	400 mg	400 mg	400 mg
Terizidone	15–20 mg/kg	500–750 mg	750 mg	750–1000 mg
18 MONTH CONTINUATION PHASE - Daily doses by weight				
	< 33 kg	**33–50 kg**	**51–70 kg**	**> 70 kg**
Ethionamide	15–20 mg/kg	500 mg	750 mg	750–1000 mg
Moxifloxacin	400 mg	400 mg	400 mg	400 mg
Pyrazinamide	30–40 mg/kg	1000–1750 mg	1750–2000 mg	2000–2500 mg
Terizidone	15–20 mg/kg	500–750 mg	750 mg	750–1000 mg
Ethionamide	15–20 mg/kg	500 mg	750 mg	750–1000 mg

The inclusion and exclusion criteria for PODrTB (per protocol) were as follows:

Eligibility:

- Adults ≥ 18 years of age
- Current diagnosis of pulmonary MDR-TB or rifampicin-monoresistant TB with either baseline sputum sample with positive GeneXpert® MTB test showing rifampicin resistance, or confirmed positive mycobacterium tuberculosis culture displaying resistance to rifampicin with or without isoniazid resistance on standard drug-sensitivity testing.
- Eligibility for standard MDR-TB treatment regimen or started on standard MDR-TB regimen within the previous month.
- Pregnant women fulfilling other eligibility criteria were eligible for recruitment
- Written informed consent.

Exclusion:

- Critically ill or medically unstable e.g., organ failure – requiring ventilatory support, receiving dialysis for acute renal failure, fulminant hepatitis or severe haemoptysis.
- Unwilling to participate, or unable to understand the participant information and provide full informed consent.

Participants were recruited at Brooklyn Chest and DP Marais TB Hospitals in Cape Town between July 2015 and September 2017. Participants were followed up two-weekly from treatment initiation to 12 weeks with adverse event surveillance including scheduled biochemistry for to screen for liver and renal impairment; sputums were sampled weekly for measurement of time to sputum culture conversion. Intensive pharmacokinetic sampling was scheduled at steady state on one occasion at six serial timepoints: predose and at 2,4,6,8 and 10 hours post-dose, approximately one to six weeks post treatment initiation. All drug concentration assays were performed at the University of Cape Town, Division of

Clinical Pharmacology using liquid chromatography tandem mass spectrometry. The PODrTB study schema, per protocol, is shown in figure 7.

Figure 7. PODrTB study schema during the first 12 weeks of MDR-TB therapy

		WEEK on MDR-TB treatment											
	BL	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Enrolment period													
Sputum culture	x[1]	x	x	x	x	x	x	x	x	x	x	x	x
MIC	x[1]								x				
CXR analysis	x												
Clinical evaluation	x		x		x		x		x		x		x
Serum biochem.[2]	x				x				x				x
TSH	x				x				x				x
PN	x				x				x				x
CNS	x		x		x				x				x
PTA	x				x				x				x
PK period[3]													

[1]Pre-treatment sputum culture to be used for MIC (minimum inhibitory concentration)
[2]Serum potassium, creatinine and alanine transaminase
[3]The target time for PK sampling: 4 weeks (with a Day 15-42 window) after starting MDR-TB treatment.
TSH: Thyroid stimulating hormone
PN: Peripheral neuropathy assessment
CNS: Psychiatric disturbance scales
BL: Baseline i.e., at enrolment
PTA: Pure tone audiometry

Seven hundred and thirty-one participants were screened for recruitment for PODrTB, of whom 144 were enrolled. One hundred and thirty-one participants completed the intensive pharmacokinetic sampling schedule of whom 98 patients completed the 12-week in-patient observation period. Breakdown of the 46 participants who did not complete the study follow-up period is as follows: six patients died as a result of MDR-TB related complications; nine were determined to have pre-XDR or XDR-TB (extensively drug-resistant TB) and therefore transferred for further care at another facility, and the remainder (n=31) were

either discharged by the primary carers prior to the end of the follow-up period; absconded from hospital or withdrew their consent.