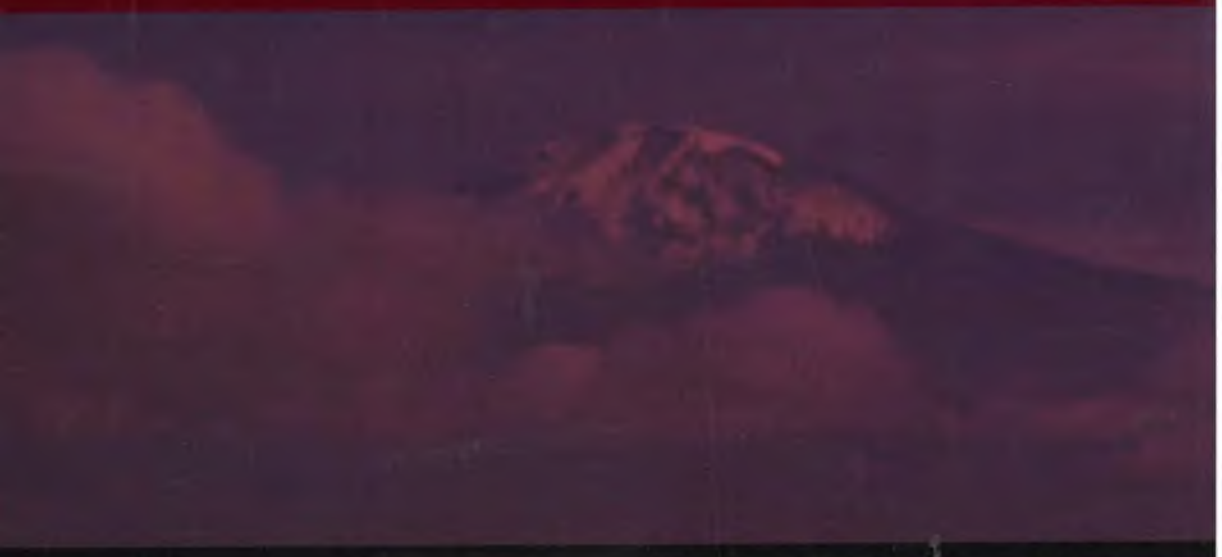
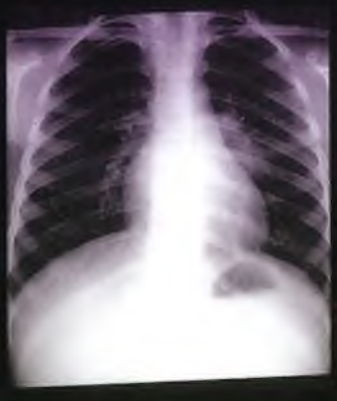
IMPROVING THE MANAGEMENT OF TUBERCULOSIS IN TANZANIA:

CLINICAL AND EPIDEMIOLOGICAL STUDIES




Charles Michael Mtabho






STOP
TB!
WORKING TOGETHER TO
STOP TB!

IMPROVING THE MANAGEMENT OF TUBERCULOSIS IN TANZANIA: CLINICAL AND EPIDEMIOLOGICAL STUDIES

Charles Mtabho

Improving the management of tuberculosis in Tanzania:
clinical and epidemiological studies.
Thesis, Radboud University Medical Center, The Netherlands

ISBN
978-94-6259-459-3

Design and lay-out
Promotie In Zicht, Arnhem

Print
Ipskamp Drukkers, Enschede

Printing and dissemination of this thesis was financially supported by
Radboud University Medical Center.

IMPROVING THE MANAGEMENT OF TUBERCULOSIS IN TANZANIA: CLINICAL AND EPIDEMIOLOGICAL STUDIES

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen,
op gezag van de Rector Magnificus prof. dr. Th.L.M. Engelen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op woensdag 10 december 2014
om 14.30 uur precies

door

Charles Michael Mtabho
geboren op 17 juni 1976
te Magu, Tanzania

Promotoren

Prof. dr. A.J.A.M. van der Ven

Prof. dr. G.S. Kibiki, Kilimanjaro Christian Medical College, Moshi, Tanzania

Copromotoren

Dr. R.E. Aarnoutse

Dr. M.J. Boeree

Manuscriptcommissie

Prof. dr. F.G.M. Russel *(voorzitter)*

Prof. dr. D. van Soolingen

Prof. dr. T.S. van der Werf *(Rijksuniversiteit Groningen)*

IMPROVING THE MANAGEMENT OF TUBERCULOSIS IN TANZANIA: CLINICAL AND EPIDEMIOLOGICAL STUDIES

Doctoral Thesis

to obtain the degree of doctor
from Radboud University Nijmegen
on the authority of the Rector Magnificus prof. dr. Th.L.M. Engelen,
according to the decision of the Council of Deans
to be defended in public on Wednesday, December 10th 2014
at 14.30 hours

by

Charles Michael Mtabho
Born on 17th June 1976
in Magu, Tanzania

Supervisors

Prof. dr. A.J.A.M. van der Ven

Prof. dr. G.S. Kibiki, Kilimanjaro Christian Medical College, Moshi, Tanzania

Co-supervisors

Dr. R.E. Aarnoutse

Dr. M.J. Boeree

Doctoral Thesis Committee

Prof. dr. F.G.M. Russel *(chair)*

Prof. dr. D. van Soolingen

Prof. dr. T.S. van der Werf *(University of Groningen, the Netherlands)*

Dedication

To my lovely father Christopher Nyakibhatare Mtabho
To my lovely mother Specioza Ndabacha Makungu Mtabho
To my loving wife Penina Mbuke Masanja and children Dorinda Nyambura Mtabho
and Specioza Ndabacha Mtabho

Contents

CHAPTER 1

General Introduction

Burden of tuberculosis

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) that mostly affects the lungs (pulmonary TB, PTB). However, other organs may be affected as well, including the meninges, bone, pericardium, kidney, lymphnodes: these cases are called extra-pulmonary TB (EPTB)[1, 2].

TB continues to be a worldwide problem despite the availability of potent anti-mycobacterial pharmacological agents for many years[2]. Globally, the disease affects approximately 9 million people every year. In 2012, 8.6 million new cases were documented and 1.3 people died from the disease[3]. Apart from its direct health related effects, TB causes also a significant loss of productive work force which results into economic losses and expenditures for health facilities which would otherwise be used for other development activities. For instance in 2011, the 104 low- and middle-income countries spent over US$ 4.4 billion for the treatment of TB cases, including multidrug-resistant TB (MDR-TB)[3].

The last decades, HIV has been fuelling TB because of its negative effects on the immune system, whereby alarming figures were reached around the year 2000 [4-6]. Not only the number of TB cases sharply increased as a result of HIV infection, but also the diagnosis, the clinical manifestations and treatment of TB became much more complex [7, 8]. The highest number of HIV infected patients live in Sub-Saharan Africa and in 2012 the African region also accounted for an estimated 23% of all TB cases and 25% of TB-related mortality. In addition, African countries account for about 75% of TB cases among people living with HIV [3]. TB is generally considered a Poverty Related Disease as 88% of the incident TB cases occur in 22 high-burden countries that are mostly low- or middle-income countries in Africa (n=9) or South-East Asia.

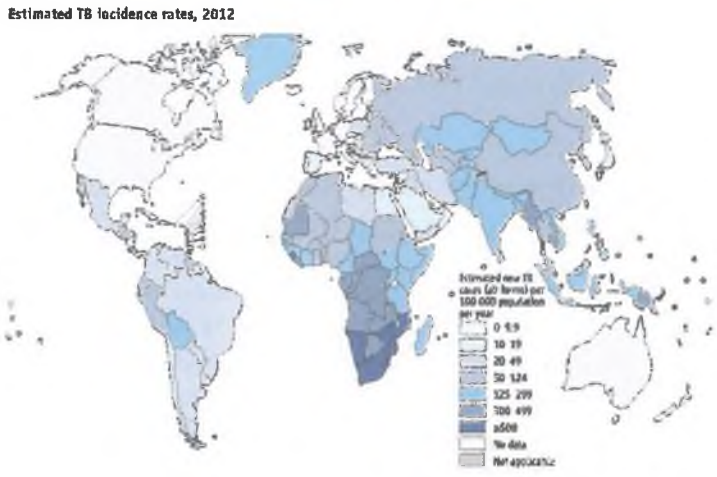
TB treatment is not only challenged by the increases in TB burden and the dual infection with HIV, but also by increasing resistance formation [9]. Multidrug-resistant TB, defined as resistance to isoniazid and rifampicin, has emerged as a major and growing challenge to global TB control efforts [9-11]. In 2012 there were an estimated 450,000 new cases of MDR-TB worldwide and approximately 170,000 MDR-TB cases died. More recently, extensively drug-resistant tuberculosis (XDR-TB) is being recognized which is defined as TB that has developed resistance to at least rifampicin and isoniazid as well as to any member of the fluoroquinolone family and at least one of the following second-line anti-TB injectable drugs: kanamycin, capreomycin, or amikacin [12-15].

The current treatment of drug susceptible TB comprises a six-month regimen whereby four first line drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) are given for 2 months and dual therapy with isoniazid and rifampicin is given for another 4 months thereafter. The World Health Organization (WHO) estimates that standard TB treatment was successfully administered in 87% in 2011, while 2% treatment failure, 4% default, and 4% mortality were reported, and 4% of the cases were not evaluated [3]. Treatment of MDR-TB is however more complex and lengthy, moreover, the second line drugs required to treat MDR-TB are expensive and more toxic than the standard TB drugs. Additional recommended drugs for MDR-TB include amikacin/ kanamycin/capreomycin, ofloxacin/levofloxacin/moxifloxacin, ethionamide/ prothionamide, cycloserine/terizidone and para-aminosalicylic acid (PAS), amongst others. Treatment for XDR-TB is becoming more complex as the number of remaining drugs with proven efficacy against *M. tuberculosis* is more limited for these patients[14, 15].

TB management in Tanzania

Tanzania is among the 22 high TB burden countries. In 2012, it had an estimated incidence (all forms) of 169 per 100,000 inhabitants[16]. Because of HIV, the incidence has increased since the 1980s which is illustrated by the number of new TB cases that increased nearly 3 fold from 45,405 in 1990 to approximately 120,000 in 2007[17]. Fortunately the incidence has started declining after 2000[18]. On the other hand, drug resistance has been notified in Tanzania as well: a study from 2010 reported a 8.3% adjusted prevalence of *Mycobacterium tuberculosis* strains resistant to any of the four first-line drugs in new patients while the prevalence of multidrug-resistant TB (MDR-TB) was 1.1%[19]. In addition, approximately 1,000 new cases of MDR-TB were estimated to occur in Tanzania in 2012 [3].

Tanzania adapted the WHO and IUATLD recommendations for TB treatment. in Tanzania, TB management is overseen by the Tanzanian National Tuberculosis and Leprosy Program (NTLP). Prior to the inception of the short-course chemotherapy regimen, Tanzania was using the long course (12 months) regimens with isoniazid, thiacetazone and streptomycin. The first short-course regimen of 8 months was introduced in Tanzania in 1987 for sputum smear positive PTB cases, consisting of two months intensive phase with rifampicin, isoniazid, pyrazinamide, and ethambutol followed by a six months continuation phase with isoniazid and ethambutol. In the year 2002, the national program introduced a new regimen to replace the standard regimen for smear negative and EPTB patients for all regions in the country[20]. In 2006 the six months short-course regimen was introduced for all new smear positive, smear negative and extra-pulmonary TB[21]. This six months regimen contains



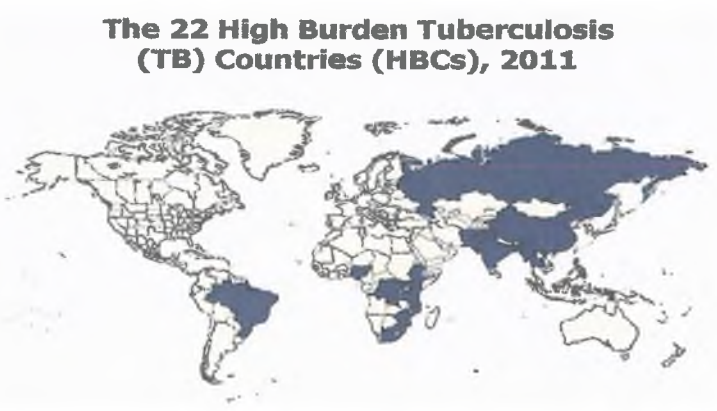


Figure 1 Estimated TB incidence rates, 2012, and the 22 High burden TB countries, 2011 (Source: WHO TB report 2013).

rifampicin throughout, and consists of rifampicin, isoniazid, pyrazinamide, and ethambutol administered daily in the first two months (intensive phase) and rifampicin and isoniazid daily in the next four months (continuation phase).
To ensure compliance and combat abandonment of therapy, directly observed therapy (DOT) has been widely endorsed since 1986. However due to constraints in human resource for health, an overloaded health system, and comparable efficacy, Tanzania switched from the facility based DOT to community based DOT (the so called Patient Centered TB treatment - PCT) in 2007[21, 22].

Analyses of TB treatment cohorts in Tanzania shows that treatment success has been ranging between 77% and 88.8% from 1985 to 2010. Mortality rates among new smear positive patients ranged from 8% in 2006 to 4.9% in 2009[22-24].

The need for improving management of tuberculosis in the general population and in risk groups

The burden of TB in Tanzania is significant, despite the decline in TB incidence as is recently seen together with the efforts to fight the TB and HIV epidemic. Also in Tanzania, HIV has complicated both the presentation and management of TB and from the 1980s through the 2000s the TB incidence has been increasing and the number of reported cases peaked around 2005. The increases in TB incidence has been linked to the spread of HIV[18].

In Tanzania, the TB burden is especially high among certain groups such as children and in patients with co-pathology such as TB/HIV and TB/Diabetes Mellitus.
Based on case notifications reported to the National TB program, children below 15 years contribute to the TB burden significantly by more than 6%[24]. Diagnosis is difficult due to the nature of the disease in the pediatric population, but also because appropriate specimens for microbiological analysis are difficult to obtain in children[25-27]. Apart from that, children have more often an unfavorable treatment outcome compared to adults[4, 28, 29]. Despite the higher TB burden and worse response to treatment, children are generally less often included in clinical research as policies and programs have been essentially adult focused[4]. Management of TB in children remains therefore highly challenging.

Patients with diabetes mellitus (DM) are also known to have a higher risk of developing TB[30-32]. DM is generally considered a disease of industrialized countries however recent evidence indicates that DM is now also increasing in the developing world[33]. According to the International Diabetes Foundation (IDF), the prevalence of diabetes in Tanzanian adults between 20–79 years of age was estimated 3.2% in 2010[34] but 7.8% in 2013[33]. A Tanzanian study reported that diabetes was strongly associated with incident TB, and a high prevalence of DM was found among controls in a case-control study looking at diabetes as a risk factor for TB[35]. Studies have also shown that TB patients with DM have a less favorable treatment outcome[36-38]. So far it is unclear whether this is caused by the inherent nature of the disease of impairing host immunity or the effect of the disease on the pharmacokinetics of drugs[39]. Some studies have been performed outside the African setting, however with varying and conflicting results[40-42].

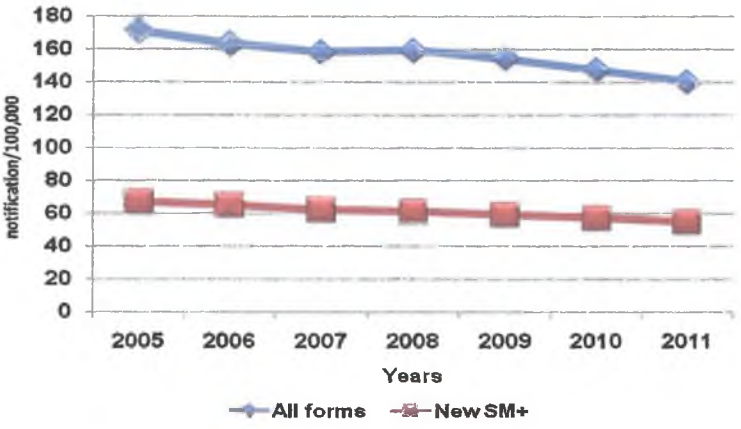


Figure 2 Trends of TB notification rates from 2005 – 2011 for all-forms and new smear positives. (Source: Tanzania NTLP Annual report 2011).

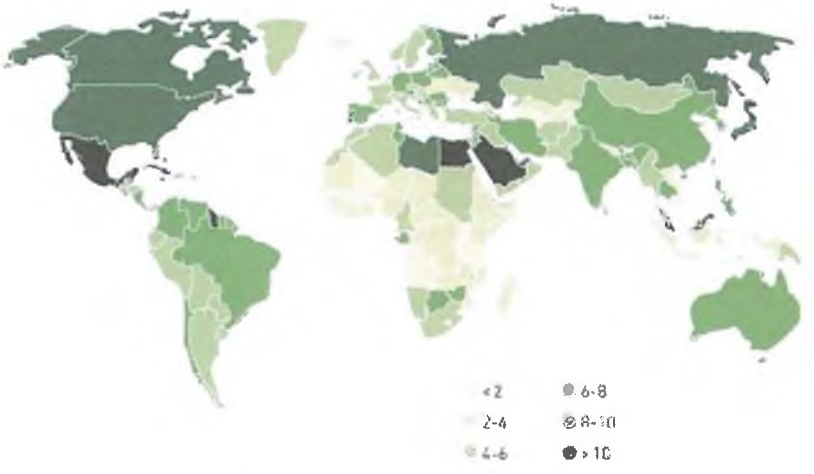


Figure 3 Percentage of TB cases attributable to diabetes mellitus. Source:http/www.idf.org/site map 4.3

The interpretation of poor treatment outcome for TB in Tanzania in vulnerable groups like children, patients with DM or a concurrent HIV infection is complicated by the fact that no data are available about exposure to TB drugs in Tanzanian pulmonary TB patients, or in relevant subgroups such as HIV-co-infected patients or patients with TB and DM. This is relevant considering that low plasma concentrations of TB drugs

and interpersonal variability in these concentrations are recognized to contribute to suboptimal treatment response[43-49], apart from poor adherence to treatment (no drug concentrations) which is a well-established reason for suboptimal response. A recent study is illustrative, showing that the total exposures to pyrazinamide, isoniazid and rifampicin in serum are the top 3 predictors of long-term outcome in South-African pulmonary TB patients[50]. Such pharmacokinetic-pharmacodynamic (PK-PD) evaluations with existing TB drugs and similar studies in each of the phases of development of new TB drug regimens[51] will hopefully allow for PK-PD optimized dosing of TB drugs in the future. Still most future patients will probably receive the same 'fixed' dose of TB drugs, albeit optimized for the population or subpopulation (eg DM patients) as a whole, considering the complex logistics involved in the programmatic treatment of the large number of TB patients worldwide. Some selected centers around the world, however, do apply the concept of Therapeutic Drug Monitoring (TDM) of TB drugs[52-57]. In contrast to administering the same dose to all patients, TDM is seeking to individualize drug doses, guided by measurement of serum (or plasma) drug concentrations.

Whereas serum drug level measurement with High-Performance Liquid Chromatography (HPLC) methods is not readily feasible in low income countries like Tanzania, we need to look into other tools that can be used to optimize treatment response and identify treatment failure. Plasma and whole-blood bactericidal/activity assays are possible alternative tools, as they are cheap and easy to perform in resource limited setting[58, 59]. In addition these assays are useful tools in optimization of TB regimens and therefore in studies of development of new TB drugs. With these methods, organisms from patients undergoing TB treatment are cultured in blood or plasma of the same patients and the amount of killing of the organism is determined, which will reflect the influence of both the drug taken and the body's immune system and in this way predict the treatment outcome beforehand.

As outlined above, important knowledge gaps are still hampering TB treatment in Tanzania which formed the basis for the epidemiological and clinical studies in different groups of Tanzanian TB patients in this thesis. These studies generally aimed to contribute to the fight against TB in Tanzania, and to improve its management.

Outline of the thesis

All epidemiological and clinical studies presented in this thesis were conducted in the Kilimanjaro region in Tanzania. The patients' enrollment sites involved in these studies were the Tanzanian National Tuberculosis Hospital (KNTH) located in Siha district, and the Mawenzi regional hospital and its satellite district TB treating units in Kilimanjaro region. All studies were coordinated from the Kilimanjaro Clinical Research Institute (KCRI) located at the Kilimanjaro Christian Medical Center (KCMC) in Moshi, Kilimanjaro region.

Chapter 1 describes the general introduction to the thesis. This chapter gives a brief general situational analysis (burden of disease) of TB and its management in Tanzania.

In chapter 2, the burden of TB is described for the pediatric population in the Kilimanjaro region. The epidemiology of TB in this vulnerable and forgotten population is described, in contrast to the burden in adults.

Besides poor adherence to treatment, low blood levels of TB drugs is another possible cause for unfavorable treatment outcome. Whereas the former has been well studied, the latter has not been investigated thoroughly in Tanzanian patients. In Chapter 3 the pharmacokinetics of first line TB drugs are therefore described in Tanzanian patients.

Treatment response monitoring and diagnosis of unfavorable response are recognized problems in both the pediatric and adult populations. Chapter 4 investigates whether saliva can be used as an alternative matrix in pharmacokinetic studies and for therapeutic drug monitoring. This may be particularly advantageous for children where venous blood sampling is challenging.

Furthermore, routine therapeutic drug levels monitoring with High-Performance Liquid Chromatography (HPLC) is not feasible in resource limited settings like Tanzania. In Chapter 5 we investigate and set up a blood and plasma culture assay for *Mycobacterium tuberculosis* to assess whether it can be an alternative to plasma drug measurement in assessing treatment response and early diagnosis of treatment failure.

Next, we focus on TB management in patients with co-morbidity. Chapter 6 analyses the pharmacokinetics of first line TB drugs in TB patients with diabetes mellitus. The study compares this vulnerable group to those with TB only, in an attempt to look into ways of improving drug dosing in TB/diabetes patients.

Chapter 7 describes the management of TB/HIV co-infected patients as concomitant administration of drugs for TB and HIV is challenged by high pill loads, drug-drug interactions and increased toxicity. The study investigates the efficacy, tolerability, and pharmacokinetics of a fixed dose combination of emtricitabine/tenofovir/efavirenz (Atripla) when co-administered with first line TB drugs.

Chapter 8 provides a summary of all findings of the studies, a general discussion and identification of future steps that need to be taken as well as recommendations to improve the management of TB in Tanzania.

References

1. World Health Organisation. Tuberculosis. WHO Fact Sheet No 104. Revised March 2006.
2. S. D. Lawn and A. I. Zumla, "Tuberculosis," *Lancet* 378, no. 9785 (2011): 57-72.
3. World Health Organization. Global tuberculosis report 2013, WHO, available at http://www.who.int/tb/publications/global_report/en/. 2013.
4. B. Rekha and S. Swaminathan, "Childhood Tuberculosis - Global Epidemiology and the Impact of HIV," *Paediatr.Respir.Rev.* 8, no. 2 (2007): 99-106.
5. World Health Organization. *Global tuberculosis control: surveillance, planning, financing. WHO report 2006*. Geneva. WHO/HTM/TB/2006.362. 2006.
6. T. R. Frieden et al., "Tuberculosis," *Lancet* 362, no. 9387 (2003): 887-899.
7. A. Zumla et al., "Impact of HIV Infection on Tuberculosis," *Postgrad.Med. J.* 76, no. 895 (2000): 259-268.
8. S. K. Sharma, A. Mohan, and T. Kadhiravan, "HIV-TB Co-Infection: Epidemiology, Diagnosis & Management," *Indian J.Med.Res.* 121, no. 4 (2005): 550-567.
9. N. R. Gandhi et al., "Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis: a Threat to Global Control of Tuberculosis," *Lancet* 375, no. 9728 (2010): 1830-1843.
10. D. Falzon et al., "Resistance to Fluoroquinolones and Second-Line Injectable Drugs: Impact on Multidrug-Resistant TB Outcomes," *Eur.Respir.J.* 42, no. 1 (2013): 156-168.
11. G. B. Migliori et al., "Drug Resistance Beyond Extensively Drug-Resistant Tuberculosis: Individual Patient Data Meta-Analysis," *Eur.Respir.J.* 42, no. 1 (2013): 169-179.
12. World Health Organization. WHO MDR-TB Factsheet. October 2013 Update. Available at www.who.int/tb/. 2014.
13. World Health Organization. Towards universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis by 2015: WHO PROGRESS REPORT 2011. 2011.
14. Ministry of Health Tanzania. Operational Guideline for the Management of Drug Resistant TB in Tanzania. 2010.
15. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis, 2011 update. Available at http://www.who.int/tb/challenges/mdr/programmatic_guidelines_for_mdrtb/en/. 2011.
16. World Health Organisation. Global tuberculosis report 2012. 1-89. 2012.
17. World Health Organization. *Global tuberculosis control: surveillance, planning, financing. WHO report 2008*. Geneva. 2008.
18. E. M. Netto, C. Dye, and M. C. Raviglione, "Progress In Global Tuberculosis Control 1995-1996, With Emphasis on 22 High-Incidence Countries. Global Monitoring and Surveillance Project," *Int.J.Tuberc.Lung Dis.* 3, no. 4 (1999): 310-320.
19. T. M. Chonde et al., "National Anti-Tuberculosis Drug Resistance Study in Tanzania," *Int.J.Tuberc.Lung Dis.* 14, no. 8 (2010): 967-972.
20. Ministry of Health. National tuberculosis and Leprosy control Programme-Annual report-2002. 2002.
21. The United Republic of Tanzania, MoHSW. Manual of the National Tuberculosis and Leprosy Programme in Tanzania. Fifth edition. 2006.
22. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2007. 2008.
23. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2006. 2007.
24. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2011. 2012.
25. B. J. Marais et al., "Diagnostic and Management Challenges for Childhood Tuberculosis in the Era of HIV 2," *J.Infect.Dis.* 196 Suppl 1 (2007): S76-S85.
26. S. M. Graham et al., "Childhood Tuberculosis: Clinical Research Needs 1," *Int.J.Tuberc.Lung Dis.* 8, no. 5 (2004): 648-657.
27. A. C. Hesseling et al., "A Critical Review of Diagnostic Approaches Used in the Diagnosis of Childhood Tuberculosis," *Int J Tuberc Lung Dis* 6, no. 12 (2002): 1038-1045.

28. L. J. Nelson et al., "Epidemiology of Childhood Tuberculosis in the United States, 1993-2001: the Need for Continued Vigilance," *Pediatrics* 114, no. 2 (2004): 333-341.
29. Rie A. van et al., "Childhood Tuberculosis in an Urban Population in South Africa: Burden and Risk Factor," *Arch.Dis Child* 80, no. 5 (1999): 433-437.
30. C. Y. Jeon and M. B. Murray, "Diabetes Mellitus Increases the Risk of Active Tuberculosis: a Systematic Review of 13 Observational Studies," *PLoS.Med.* 5, no. 7 (2008): e152.
31. F. Mugusi et al., "Increased Prevalence of Diabetes Mellitus in Patients With Pulmonary Tuberculosis in Tanzania," *Tubercle.* 71, no. 4 (1990): 271-276.
32. P. Baghaei et al., "Diabetes Mellitus and Tuberculosis Facts and Controversies," *J.Diabetes Metab Disord.* 12, no. 1 (2013): 58.
33. International Diabetes Federation. The IDF Diabetes Atlas, 6th Edition. 2013.
34. International Diabetes Federation. The IDF Diabetes Atlas, 4th Edition. 2010.
35. D. Faurholt-Jepsen et al., "Diabetes Is a Risk Factor for Pulmonary Tuberculosis: a Case-Control Study From Mwanza, Tanzania," *PLoS.ONE.* 6, no. 8 (2011): e24215.
36. S. A. Syed Suleiman et al., "Role of Diabetes in the Prognosis and Therapeutic Outcome of Tuberculosis," *Int.J.Endocrinol.* 2012 (2012): 645362.
37. M. A. Baker et al., "The Impact of Diabetes on Tuberculosis Treatment Outcomes: a Systematic Review," *BMC.Med.* 9 (2011): 81.
38. A. D. Harries et al., "The Looming Epidemic of Diabetes-Associated Tuberculosis: Learning Lessons From HIV-Associated Tuberculosis," *Int.J.Tuberc.Lung Dis.* 15, no. 11 (2011): 1436-44, i.
39. G. R. Wilkinson, "Drug Metabolism and Variability Among Patients in Drug Response," *N.Engl.J.Med.* 352, no. 21 (2005): 2211-2221.
40. H. M. Nijland et al., "Exposure to Rifampicin Is Strongly Reduced in Patients With Tuberculosis and Type 2 Diabetes," *Clin.Infect.Dis* 43, no. 7 (2006): 848-854.
41. R. Ruslami et al., "Pharmacokinetics of Antituberculosis Drugs in Pulmonary Tuberculosis Patients With Type 2 Diabetes," *Antimicrob.Agents Chemother.* 54, no. 3 (2010): 1068-1074.
42. A. Requena-Mendez et al., "Pharmacokinetics of Rifampin in Peruvian Tuberculosis Patients With and Without Comorbid Diabetes or HIV," *Antimicrob.Agents Chemother.* 56, no. 5 (2012): 2357-2363.
43. J. G. Pasipanodya, S. Srivastava, and T. Gumbo, "Meta-Analysis of Clinical Studies Supports the Pharmacokinetic Variability Hypothesis for Acquired Drug Resistance and Failure of Antituberculosis Therapy," *Clin.Infect.Dis.* 55, no. 2 (2012): 169-177.
44. S. Srivastava et al., "Multidrug-Resistant Tuberculosis Not Due to Noncompliance but to Between-Patient Pharmacokinetic Variability," *J.Infect.Dis.* 204, no. 12 (2011): 1951-1959.
45. M. Weiner et al., "Low Isoniazid Concentrations and Outcome of Tuberculosis Treatment With Once-Weekly Isoniazid and Rifapentine," *Am.J.Respir.Crit Care Med.* 167, no. 10 (2003): 1341-1347.
46. J. B. Mehta et al., "Utility of Rifampin Blood Levels in the Treatment and Follow-Up of Active Pulmonary Tuberculosis in Patients Who Were Slow to Respond to Routine Directly Observed Therapy," *Chest* 120, no. 5 (2001): 1520-1524.
47. M. E. Kimerling et al., "Low Serum Antimycobacterial Drug Levels in Non-HIV-Infected Tuberculosis Patients," *Chest* 113, no. 5 (1998): 1178-1183.
48. M. Weiner et al., "Association Between Acquired Rifamycin Resistance and the Pharmacokinetics of Rifabutin and Isoniazid Among Patients With HIV and Tuberculosis," *Clin.Infect.Dis.* 40, no. 10 (2005): 1481-1491.
49. S. Chideya et al., "Isoniazid, Rifampin, Ethambutol, and Pyrazinamide Pharmacokinetics and Treatment Outcomes Among a Predominantly HIV-Infected Cohort of Adults With Tuberculosis From Botswana," *Clin.Infect.Dis.* 48, no. 12 (2009): 1685-1694.
50. J. G. Pasipanodya et al., "Serum Drug Concentrations Predictive of Pulmonary Tuberculosis Outcomes," *J.Infect.Dis.* 208, no. 9 (2013): 1464-1473.
51. C. Lienhardt et al., "New Drugs for the Treatment of Tuberculosis: Needs, Challenges, Promise, and Prospects for the Future," *J.Infect.Dis.* 205 Suppl 2 (2012): S241-S249.
52. C. Magis-Escurra et al., "Therapeutic Drug Monitoring in the Treatment of Tuberculosis Patients," *Pulm. Pharmacol.Ther.* 25, no. 1 (2012): 83-86.

53. C. A. Peloquin, "Therapeutic Drug Monitoring in the Treatment of Tuberculosis," *Drugs* 62, no. 15 (2002): 2169-2183.
54. S. K. Heysell et al., "Therapeutic Drug Monitoring for Slow Response to Tuberculosis Treatment in a State Control Program, Virginia, USA," *Emerg.Infect.Dis.* 16, no. 10 (2010): 1546-1553.
55. D. P. Holland et al., "Therapeutic Drug Monitoring of Antimycobacterial Drugs in Patients With Both Tuberculosis and Advanced Human Immunodeficiency Virus Infection," *Pharmacotherapy* 29, no. 5 (2009): 503-510.
56. A. Babalik et al., "Therapeutic Drug Monitoring in the Treatment of Active Tuberculosis," *Can.Respir.J.* 18, no. 4 (2011): 225-229.
57. Tongeren L. Van et al., "Therapeutic Drug Monitoring in the Treatment of Tuberculosis: a Retrospective Analysis," *Int.J.Tuberc.Lung Dis.* 17, no. 2 (2013): 221-224.
58. R. S. Wallis et al., "A Whole Blood Bactericidal Assay for Tuberculosis," *J.Infect.Dis.* 183, no. 8 (2001): 1300-1303.
59. R. S. Wallis et al., "Whole Blood Bactericidal Activity During Treatment of Pulmonary Tuberculosis," *J.Infect.Dis.* 187, no. 2 (2003): 270-278.

CHAPTER 2

Childhood Tuberculosis in the Kilimanjaro region: lessons from and for the TB Programme

Charles M. Mtabho, Constantine F. Irongo, Martin J. Boeree, Rob E. Aarnoutse and Gibson S. Kibiki

Tropical Medicine and International Health 2010, vol. 15, no. 5, pp. 496-501

Summary

Objective. To determine the magnitude of childhood TB and treatment outcome in Kilimanjaro region.

Methods. Retrospective review of registration-based data on TB notifications in Kilimanjaro region were retrieved for the period 2002-2006.

Results. Between 2002 and 2006, there were 1615 patients of childhood TB in Kilimanjaro region constituting 13% of total TB burden and the average case detection rate was 147/100 000 for urban and 41.8/100 000 for rural. Of them, 54.2% were men and 75.2% had pulmonary TB; 24.9% were tested for acid-fast bacilli (AFB) by Ziehl-Neelsen staining showing that 5.8% of all patients with TB were AFB smear positive. The remaining 94.2% were presumptively treated for TB. Treatment success rate was 79.9%, mortality 10.9%, and default rate was 7%. Unfavourable outcome was more common among unconfirmed TB patients. HIV testing was very rare but increasing after 2004 (< 2% before 2005, 11-16% afterwards).

Conclusion. The rate of childhood TB in Kilimanjaro region is among the highest in the world. Microbiological diagnosis for TB and AFB smear positivity are very low. Treatment outcome in this region is poor. These findings argue for specific TB control strategies to be designed for children such as more AFB testing using new tools such as induced sputum and laryngeal swabs, active case finding, HIV testing of all suspected TB children, promoting and monitoring adherence. Regular epidemiological studies are also needed.

Introduction

Tuberculosis (TB) remains the leading single infectious agent associated with high morbidity and mortality worldwide, with about two million deaths per year (WHO 2006, 2008) and about nine million cases per year worldwide. The burden of TB is increasing especially in Sub Saharan Africa and the increase has been accelerated by the HIV pandemic (WHO 2006; Rekha & Swaminathan 2007).

Children (under 15 years of age) constitute about 11% of all patients with TB. The reported percentage in children varies from 2% to 40%, the rates being highest in low and middle income countries. The TB burden in children has not been sufficiently addressed. Policies and programmes have been essentially adult focused, leading to less attention to the childhood burden (Rekha & Swaminathan 2007). Recent, the contribution of childhood TB to the general burden of the disease has been recognitized and childhood TB is becoming a public health interest (Donald 2004; Donald *et al.* 2007).

In most high TB burden countries including Tanzania, studies presenting data on various aspects of childhood TB are rare, such as the contribution of childhood TB to the burden of the disease or the influence of HIV on the burden. Treatment outcome based on HIV status has not been sufficiently described (Ministry of Health 2004; Ministry of Health and Social Welfare 2007). Because of the importance of childhood TB, we need to establish and document baseline data, which will then lay ground for planning and conducting TB studies in children.
In this study, we retrospectively analyzed 5-year data from Kilimanjaro region TB registry to determine the magnitude of the disease in children compared to adults. Kilimanjaro region TB registry is part of the Tanzanian National Tuberculosis and Leprosy Programme (NTLP).

Methods

We conducted a retrospective registration-based study and analyzed the data on TB notifications in Kilimanjaro region from the period 2002 to 2006. The region consists of six districts: Moshi Urban, Moshi rural, Hai, Mwanga, Same, and Rombo. Only Moshi urban district is urban, the rest have essentially rural populations. A child was defined as a person younger than 15 years. Children in Kilimanjaro region constitute 43% of the total population of 1 381 149 and the population has an annual growth rate of 1.6% (The United Republic of Tanzania 2003). In Kilimanjaro region, the children treated for TB are diagnosed at the regional hospital, district hospitals, health centers

or dispensaries either by laboratory confirmation or by clinical judgment. After diagnosis they are registered in TB registers at these health facilities and treatment is initiated there. The records and treatment are supervised by the respective District Tuberculosis and Leprosy Co-ordinator (DTLC) and Regional Tuberculosis and Leprosy Co-ordinator (RTLC).

The study was approved by the KCMC Research Ethics Committee. Data were retrieved from the archive at the RTLC's office, and from the DTLC's offices for all six districts.

All paediatric patients with TB registered and reported to the RTLC during the period 2002-2006 were included in the study. The records were retrieved, recorded in a specially designed standard record form for this study and double entered in the computer program. To calculate the prevalence (or case detection rates), extrapolations of successive annual population for individual districts were made from the 1988 and 2002 population census data (The United Republic of Tanzania 2003). The number of adult TB cases and total cases were obtained from district TB registers and quarterly reports. Prevalence, proportion of pediatric TB cases, demographic distribution, mode of diagnosis, type of TB, treatment outcome and proportion of reported HIV infection were determined and related to different other variables e.g. age, gender, location. Immunization (with BCG vaccine) records, which are recorded on patient and facility cards were not found on TB registers. Analysis was performed using SPSS version 14 for Windows (SPSS Inc., Chicago, USA).

Results

Proportion, prevalence and geographical distribution of childhood TB

In the period 2002-2006, childhood TB constituted 13% (1615/12614) of all TB burden in Kilimanjaro region. The contribution of childhood TB to the overall TB burden in the region was above 11% throughout these years; ranging from 13.9% in 2002 to 11% in 2005. In 2003, 2004, and 2006, the proportion of childhood TB was 12.4%, 13.1%, and 13.6% respectively.

Given the number of inhabitants in the Kilimanjaro region, the prevalence of childhood TB in those 5 years ranged from 41 to 59 per 100,000 population per year (average: 52.8 per year). It was highest in 2002 and lowest in 2005. There were declines in both proportion and prevalence of childhood TB in 2005, which were statistically significant (χ^2= 9.35 and 19.8, respectively, p value < 0.001 in both cases) (figure 1).

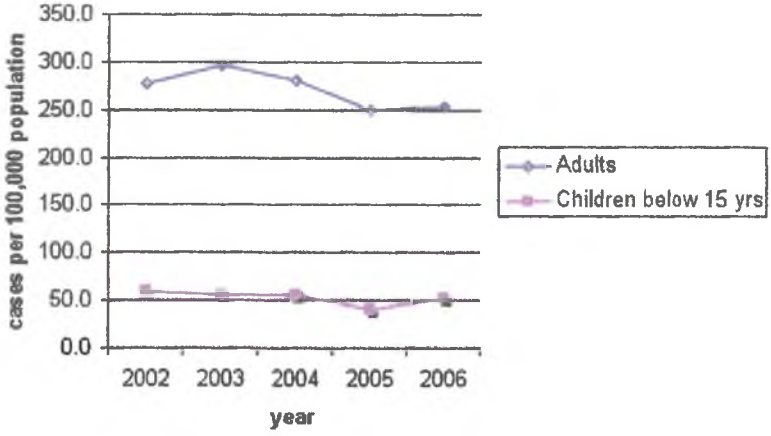


Figure 1 Trends in TB prevalence in Kilimanjaro region from 2002 to 2006.

Table 1 shows the distribution of childhood TB burden by districts. There were differences in the proportion of Childhood TB between the districts, with the highest being in Moshi Urban district and the lowest in the essentially rural, Same district. The difference between these two districts was statistically significant (χ^2=22.27, p<0.001).

Table 1 Proportion of childhood TB burden in Kilimanjaro Region by districts; 2002 – 2006

	No. of patients		
District	Children	All patients	% of children
Moshi Urban	472	3175	14.9%
Moshi Rural	376	2742	13.7%
Hai	490	3976	12.3%
Rombo	123	1130	10.9%
Mwanga	49	464	10.6%
Same	105	1127	9.3%
Total	1615	12614	12.8%

Most of the districts showed a decreasing trend in prevalence of childhood TB towards 2005 with a slight rise in 2006, except for Same district and neighbouring Mwanga district which showed fairly low and constant prevalences (Figure 2).

The average annual prevalence was 147 in urban and 41.8 (per 100,000 population) in rural. The difference was statistically significant (χ^2=118, p<0.001). Similarly the proportion of childhood TB was higher in urban than in rural populations (14.9% against 12.1%, respectively; χ^2=16, p<0.001).

Gender and age distribution of TB in Children

Of 1615 childhood TB cases, 875 (54.2 %) were men. Age distribution was positively skewed, with under-fives accounting for 49.3% (797/1615) of all childhood Tb cases. The median age was 5.0 years, with interquartile range 1.75 – 9 years.

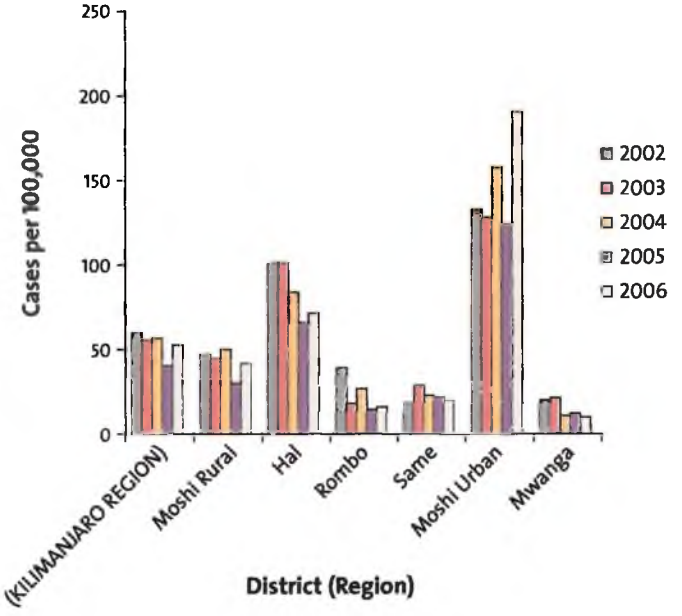


Figure 2 Childhood TB prevalence by district.

Type of TB

Pulmonary TB (PTB) was the most common type in this paediatric population with 75.2% (1215/1615) of patients; the remaining 24.8% were extrapulmonary TB (EPTB). Diagnosis of both PTB and EPTB was in fact presumptive as the vast majority was not microbiologically confirmed. The specific types for EPTB were (n=1615); 16.3% unclassified, 4.3% TB adenitis, 1.2% Miliary, 0.9% pleural effusion, 0.7% TB meningitis, and the remaining types (TB spine, peritonitis, arthritis, pericarditis, brain tuberculoma, and pericardial effusion) each comprised <0.5%. The proportion of EPTB for under-fives

was 19.3% (154/797); in children aged 5 to 10, it was 30.5% (150/491) and in children aged 11-15, it was 29.4% (96/327). EPTB was more common in older children than in under-fives (χ^2=25.19, p<0.001).

Acid fast Smear results

Of the 1615 children who were diagnosed to have TB and treated with anti tuberculosis drugs, only 24.9% (402/1615) had samples tested for acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) staining. All samples reported were from sputum. No other microbiological tests such as cultures for TB were carried out. 47.8% (772/1615) had no smear performed as the children did not produce sputum and/or diagnosis was based on history and clinical features. The remaining 27.3% (441/1615) had no record on AFB results.

Of those who were tested for AFB, 23.4% (94/402) were AFB smear positive and the remaining 76.6% were smear negative. The AFB smear positive patients were 5.8% (94/1615) of all patients treated for TB. Sputum smear negative cases constituted 19.1% (308/1615) of the all paediatric patients with TB.

Treatment outcome

Information on TB treatment outcome was obtained in 99.2% (1602/1615) of the children with TB. Among these, 72.9% (1168/1602) had treatment success i.e. were cured or completed treatment (3.6% or 69.4% respectively). Transfer out accounted for 9.2% and the default rate was 7.0% (112/1602). The overall case fatality rate during the course of TB treatment was 10.9% (table 2).

The highest case fatality rate was 13.3% in 2003; the lowest was 9.4% in 2004. Mortality generally showed a declining trend throughout the years although the trend was not perfectly linear (χ^2 for trend = 3.03, p=0.0817).

Table 2 Treatment outcome among childhood TB cases by TB type in Kilimanjaro region

Treatment outcome	Number (%)							
	All types		PTB Smear +		*PTB Smear -		EPTB	
Died	175	(10.9%)	6	(6.5%)	133	(11.9%)	36	(9.1%)
Completed/cured	1168	(72.9%)	67	(72.0%)	801	(72.0%)	300	(75.8%)
Transfer out	147	(9.2%)	13	(14.0%)	97	(8.7%)	37	(9.3%)
Default	112	(7.0%)	7	(7.5%)	82	(7.4%)	23	(5.8%)
Total	1602	(100%)	93	(100%)	1113	(100%)	396	(100%)

* by case definition, these included sputum smear negative and no smear cases

Outcome by type of TB, Smear results, age, and gender

Considering the outcomes of either treatment success or death, of the patients with PTB 13.8% (139/1007) died, and among EPTB, 10.7% (36/336) died. Of those with AFB smear positive TB, 8.2% (6/73) died, while 13.3% (169/1270) of microbiologically unconfirmed TB (i.e. smear negative or not done) died. Mortality for under-fives was 14.4 % (94/654) and 11.8% (81/689) in older children. Regarding gender, mortality was 11.7% (102/869) and 10.0% (73/733) for men and women, respectively. In all these categories, the differences were not statistically significant.

Comparing the mortality of patients with PTB who were AFB smear negative and those not tested for AFB, we found that the mortality in AFB smear negatives was 9.6% (21/219), while in those who were not tested, it was 15.8%(86/544). The death rate was statistically significantly higher in those not tested for AFB (χ^2=5.01, p=0.025). However, when adjusted for age this relationship became statistically not significant (p= 0.308 logistic regression analysis).

Childhood TB and HIV infection

Only 91 children out of all 1615 (6%) were tested for HIV between 2002 and 2006 and 82% (75/91) were positive. In 2002, 2003 and 2004, 4 out of 351, 1 out of 335, and 6 out of 343, respectively, were tested and all were positive. In 2005 10.7% (27/253) were tested, of which all except one (96.3%) were HIV positive. In 2006, 15.9% (53/333) of the children were tested for HIV and 38 (71.7%) were positive.

Regarding children of whom both HIV serostatus and AFB smear results were known: of the total 27 HIV seropositive children with TB, 7.4%(2/27) were smear positive. Among the HIV seronegative children 33.3% (2/6) were smear positive. The difference was not statistically significant (χ^2=3.1, p=0.142).

HIV seropositivity rate was 78.9 %(30/38) for under-fives and 85.5%(47/55) in children aged 5-14 (OR= 0.67, 95% CI 0.23-1.97).

HIV status and Mortality

TB Treatment outcome was recorded in 89% (81/91) of children whose HIV status was known. In the HIV seropositive group, 24.2% (16/66) died whereas 6.7% (1/15) died in the HIV seronegative group. However, the difference in mortality between these groups was not statistically significant (OR=4.48, 95%CI 0.55-36.78).
Report on anti-retroviral drugs (ARV) use was available for only 4% (3/75) of the HIV positive children.

Discussion

This study has shown that the burden of Childhood TB in the Kilimanjaro region is quite significant, contributing 13% of all patients with TB and with annual prevalence of 147 in urban and 42 (per 100,000 population) in rural areas. Young children <5 years were affected more, and there were low rates of smear testing for AFB (only about a quarter of all children were tested) and low rates of smear positive patients (6% of all cases). Treatment outcome was not very favourable; treatment success was fairly low (72.9%), and mortality significant (10.9%). Few children were tested for HIV (6% on average).

In general, TB in Kilimanjaro region (in both children and adults) is a major public health problem. In the year 2006 for instance, the overall prevalence of 166.6 per 100,000 population in the region, was comparable to the estimated national case detection rate of 164 per 100,000 (Ministry of Health and Social Welfare 2007). The proportion of childhood TB in the same year was 13.6% (17% for urban and 12.1% for rural), which is higher than the WHO estimate for the average proportion of childhood TB of 11% (WHO 2006; Rekha & Swaminathan 2007). This study describes the age-specific case notification rates in children and in adults thereby estimating the prevalence in children. The prevalence (average 52.8 per 100,000) is substantially higher than other reports with rates per 100,000 of 51 in Malawi (Harries *et al.* 2002), 13-15 in Thailand (Lolekha et al. 2008) and 1.5 in the United States (Nelson *et al.* 2004). In this region the prevalence was much higher in urban compared to rural populations. This could possibly be due to lifestyle related to housing and congestion in urban areas, but also better access to care and better education and awareness of the parents in urban areas. Given this high prevalence in the region it is recommended that childhood TB should receive special consideration by the TB control programme, and regular such and prospective studies need to be conducted.

Both the prevalence and proportion of children with TB have shown a slight decline in 2005. This happened just after ARVs started to be available free of charge to the general public in Tanzania in 2004. As the use of ARVs is known to reduce HIV transmission (Nunn & McAdam 1988), it is likely that this, coupled with improvement of the TB control programme, has led to the observed reduction in TB.

The prevalence of TB as well as smear positivity rate in children is lower than that of adults. The nature of the disease in children and the difficulty in making microbiological diagnosis (Hesseling *et al.* 2002) may have contributed to the observed lower case detection rate and smear positivity rate. In this study, only 23.2% of all children treated for TB produced sputum for microbiological TB diagnosis. Yet the observed

rate of smear positivity of 6% in Kilimanjaro region is higher than the WHO regional estimate for Africa which was 3.1% in 2004 (WHO 2006). Improving diagnosis by improving the existing diagnostic tools, introducing and evaluating new tools such as induced sputum, laryngeal swabs and nasopharyngeal aspiration and active case finding may improve case detection rates (Lighter & Rigaud 2009). This may also reduce the rate of presumptive treatment, overtreatment and overdiagnosis as studies have shown that many presumed smear negative TB are not TB cases at all (Dowdy et al. 2008).

Our analysis has shown that children share a disproportionate burden of TB. The age group 0-5 years had the highest proportion of patients with TB. These findings are consistent with other reports (van *et al.* 1999; Nelson *et al.* 2004; Rekha & Swaminathan 2007). Children with this age can rarely produce sputum for examination compared to the older ones. This study did not obtain information on BCG vaccination status of the children as such information is not recorded in the TB registers but rather on individual patient treatment cards. We recommend BCG status to be recorded in TB registers so that the information is readily accessible for evaluation. Nevertheless records show that BCG immunization coverage is quite good in Tanzania. In 2002, the coverage was 88% and increased to 99% in 2006 (WHO-UNICEF 2009). BCG is known to be protective against severe forms of tuberculosis in children e.g. miliary tuberculosis and tuberculous meningitis (Walker *et al.* 2006). Assuming the good immunization coverage in Kilimanjaro region, this may reflect the low occurrence of miliary tuberculosis (1.2%) and TB meningitis (0.7% of all cases) in this study.

The observed overall treatment success in children of 72.9% was below the figure for all ages for Tanzania in 2005 (82.9%) and globally (82%) for smear positive patients (WHO 2006). Although the figure for children was higher than that reported in Botswana and Malawi (Harries *et al.* 1998; Oeltmann *et al.* 2008), it is below the WHO's global target of 85% (Veen et al. 1998). Apart from the relatively low success rate, the default rate (7%) was relatively high compared to the global estimate of 6% (WHO 2007) and that of the general population for Kilimanjaro region of 4.1% (Ministry of Health and Social Welfare 2007). As TB treatment is provided in Tanzania freely, these findings suggest that adherence may be a serious problem in the pediatrics population. There is therefore a need to assess adherence to identify the determinants and ways to improve it.

There has been a gradual increase in HIV testing in children in this region. This reflects increased awareness by the community on the importance of testing, improved services to HIV patients like the availability of voluntary counseling and testing (VCT),

ARVs, and prevention of mother to child transmission (PMTCT). Moreover, since 2004, the Tanzanian Ministry of Health and Social welfare had put in place a policy of testing all patients with TB for HIV. In the study period, HIV testing was carried out to apparently highly HIV suspected patients; therefore, the proportion of children with HIV infection in this study cannot reflect the prevalence of HIV in children with TB. HIV testing needs to be further promoted by increasing awareness to parents and through sensitization and education. HIV treatment in both adults and children should also be promoted so as to reduce the rate of unfavorable outcome associated with HIV-TB co-infection (Jeena *et al.* 2002; Kiwanuka 2002; Lolekha *et al.* 2008).

Conclusion

This study has documented important baseline data for childhood TB in the Kilimanjaro region. It has demonstrated a high burden of childhood TB, low level of definitive diagnosis and HIV testing, and poor treatment outcome. These findings argue for regular epidemiological studies like this one to be conducted, and also specific TB control strategies to be designed for children. These include evaluation of new diagnostic tools and active case finding. Special attention is needed for promoting, supervising and monitoring adherence in children.

References

Donald PR (2004) Childhood tuberculosis: the hidden epidemic. *The International Journal of Tuberculosis and Lung Disease* 8, p 627-629.

Donald PR, Maher D, & Qazi S (2007) A research agenda to promote the management of childhood tuberculosis within national tuberculosis programmes. *The International Journal of Tuberculosis and Lung Disease* 11, p 370-380.

Dowdy DW, Lourenco MC, Cavalcante SC *et al.* (2008) Impact and cost-effectiveness of culture for diagnosis of tuberculosis in HIV-infected Brazilian adults. *PLoS.ONE* 3, p e4057.

Harries AD, Nyangulu DS, Kang'ombe C *et al.* (1998) Treatment outcome of an unselected cohort of tuberculosis patients in relation to human immunodeficiency virus serostatus in Zomba Hospital, Malawi. *Transactions of the Royal Society of Tropical Medicine Hygiene* 92, p 343-347.

Harries AD, Hargreaves NJ, Graham SM *et al.* (2002) Childhood tuberculosis in Malawi: nationwide case-finding and treatment outcomes. *The International Journal of Tuberculosis and Lung Disease* 6, p 424-431.

Hesseling AC, Schaaf HS, Gie RP, Starke JR, & Beyers N (2002) A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 6, p 1038-1045.

Jeena PM, Pillay P, Pillay T, & Coovadia HM (2002) Impact of HIV-1 co-infection on presentation and hospital-related mortality in children with culture proven pulmonary tuberculosis in Durban, South Africa. *The International Journal of Tuberculosis and Lung Disease* 6, p 672-678.

Kiwanuka JP (2002) Tuberculosis in children at Mbarara University Teaching Hospital, Uganda: diagnosis and outcome of treatment. *African Health Sciences* 2, p 82-88.

Lighter J & Rigaud M (2009) Diagnosing childhood tuberculosis: traditional and innovative modalities. *Current Problem Pediatric Adolescent Health Care* 39, p 61-88.

Lolekha R, Anuwatnonthakate A, Nateniyom S *et al.* (2008) Childhood TB epidemiology and treatment outcomes in Thailand: a TB active surveillance network, 2004 to 2006. *BMC Infectious Diseases* 8, p 94.

Ministry of Health (2004). *National tuberculosis and Leprosy control Programme-Annual report-2004*. MoH, Dar es salaam.

Ministry of Health and Social Welfare (2007). *National tuberculosis and Leprosy control Programme-Annual report-2006*. MoH, Dar es salaam.

Nelson LJ, Schneider E, Wells CD, & Moore M (2004) Epidemiology of childhood tuberculosis in the United States, 1993-2001: the need for continued vigilance. *Pediatrics* 114, p 333-341.

Nunn PP & McAdam KP (1988) Mycobacterial infections and AIDS. *British Medical Bulletin* 44, p 801-813.

Oeltmann JE, Chengeta B, Mboya JJ *et al.* (2008) Reported childhood tuberculosis treatment outcomes, Gaborone and Francistown, Botswana, 1998-2002. *The International Journal of Tuberculosis and Lung Disease* 12, p 186-192.

Rekha B & Swaminathan S (2007) Childhood tuberculosis - global epidemiology and the impact of HIV. *Paediatric Respiratory Reviews* 8, p 99-106.

The United Republic of Tanzania (2003). *The United Republic of Tanzania 2002 Population and Housing census*. Bureau of Statistics, Dare s salaam.

van RA, Beyers N, Gie RP *et al.* (1999) Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Archives of Disease in Childhood* 80, p 433-437.

Veen J, Raviglione M, Rieder HL *et al.* (1998) Standardized tuberculosis treatment outcome monitoring in Europe. Recommendations of a Working Group of the World Health Organization (WHO) and the European Region of the International Union Against Tuberculosis and Lung Disease (IUATLD) for uniform reporting by cohort analysis of treatment outcome in tuberculosis patients. *The European Respiratory Journal* 12, p 505-510.

Walker V, Selby G, & Wacogne I (2006) Does neonatal BCG vaccination protect against tuberculous meningitis? *Archives of Disease In Childhood* 91, p 789-791.

WHO (2006) *Global tuberculosis control: surveillance, planning, financing. WHO report 2006*. WHO, Geneva.

WHO (2007) *Global tuberculosis control: surveillance, planning, financing. WHO report 2007*. WHO, Geneva.

WHO (2008) *Global tuberculosis control: surveillance, planning, financing. WHO report 2008*. WHO, Geneva.

WHO-UNICEF (2009) *WHO-UNICEF estimates of immunization coverage: United Republic of Tanzania.* URL Available from: http://www.who.int/vaccines/globalsummary/immunization/timeseries/TSWUcoverageByCountry.cfm?country=TZA (accessed on 3 May 2009).

CHAPTER 3

Pharmacokinetics of first-line tuberculosis drugs in Tanzanian patients

Alma Tostmann, Charles M. Mtabho, Hadija H. Semvua, Jossy van den Boogaard, Gibson S. Kibiki, Martin J. Boeree, Rob E. Aarnoutse

Antimicrobial Agents and Chemotherapy 2013;57(7):3208

Abstract

Introduction. East Africa has a high tuberculosis (TB) incidence and mortality, yet there is very limited data on exposure to TB drugs in patients from this region. We therefore determined the pharmacokinetic characteristics of first-line TB drugs in Tanzanian patients using intensive pharmacokinetic sampling.

Methods. In twenty adult TB patients, plasma concentrations were determined just before and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after observed drug intake with food to estimate the area under the curve (AUC_{0-24h}) and peak plasma concentrations (C_{max}) of isoniazid, rifampicin, pyrazinamide and ethambutol. Acetylator status for isoniazid was assessed phenotypically using the isoniazid elimination half-life and the acetylisoniazid/isoniazid metabolic ratio at 3 hours post dose.

Results. The geometric mean AUC_{0-24h}s were as follows: isoniazid, 11.0 h*mg/liter; rifampicin 39.9 h*mg/liter; pyrazinamide 344 h*mg/liter; and ethambutol 20.2 h*mg/liter. The C_{max} was below the reference range for isoniazid in 10/19 patients and for rifampicin in 7/20 patients. In none of the patients were the C_{max}s for pyrazinamide and ethambutol below the reference range. Elimination half-life and metabolic ratio of isoniazid gave discordant phenotyping results in only 2/19 patients.

Discussion. A substantial proportion of patients had an isoniazid and/or rifampicin C_{max} below the reference range. Intake of drugs with food may partly explain these low drug levels, but such drug intake reflects common practice. The finding of low TB drug concentrations is concerning because low concentrations have been associated with worse treatment outcome in several other studies.

Introduction

The pharmacokinetic properties of tuberculosis (TB) drugs are well described, especially in Caucasian populations [1,2]. Pharmacokinetic data from East Africa are limited, especially data that are based on intensive pharmacokinetic sampling during the full dosing interval. Such intensive pharmacokinetic sampling enables an accurate assessment of the total exposure to TB drugs (area under the time versus plasma concentration curve from 0 to 24 h [AUC_{0-24h}]) and the peak plasma concentration (C_{max}) in individual patients. Large inter-patient variability in these key pharmacokinetic parameters of TB drugs generally exists [1]. As a result, a proportion of patients achieve drug concentrations that are below the reference range for TB drugs. Several studies have shown that such lower exposures to TB drugs are associated with a suboptimal response [3-8]. A recent study showed that pharmacokinetic variability to just a single drug in the multidrug TB drug regimen is associated with treatment failure and acquired drug resistance [9,10].

On the other hand, unduly high exposures may cause toxicity and interruption of TB treatment. It is therefore important to have more knowledge on pharmacokinetic properties of TB treatment in populations from East Africa, as a high TB incidence and TB mortality are found in this region [11]. HIV infection [12-14] and malnutrition [15] have been associated with decreased plasma concentrations of TB drugs. Both are highly prevalent amongst East African TB patients, and therefore low TB drug levels are to be expected in this population [11].

The objective of this study was to describe the pharmacokinetic parameters of isoniazid, rifampicin, pyrazinamide and ethambutol using intensive sampling during the full dosing interval in Tanzanian TB patients.

Materials and methods

Study design

We conducted an observational pharmacokinetic study at the Kilimanjaro Clinical Research institute at the Kilimanjaro Christian Medical Centre (KCMC) in Moshi, Tanzania, using the standard two-stage approach. With this approach individual pharmacokinetic parameters are estimated in the first stage. In the second stage, population characteristics of each parameter are derived by obtaining measures of central tendency and spread of all the subjects' individual parameters.

Study participants

Study participants were recruited from an outpatient tuberculosis treatment clinic at Mawenzi Hospital in Moshi. Adult TB patients who were in the intensive phase of treatment and who were not using medication that could interfere with TB drug plasma concentrations were eligible for participation. All participants gave written informed consent. The study was approved by the local Institutional Research Board at KCMC and by the Tanzanian National Institute of Medical Research.

Tuberculosis was treated with fixed-dose combination tablets (FDC tablets) that were manufactured by Sandoz, Mumbai India and are on the WHO List of Prequalified Medicinal Products. The drugs were donated by Novartis through the WHO Global Drug Facility (GDF), which only provides high quality drugs that meet stringent WHO standards. Patients with a body weight less than 50 kg use three FDC tablets per day (i.e., 225 mg isoniazid, 450 mg rifampicin, 1200 mg pyrazinamide and 875 mg ethambutol) and patients with a body weight>50 kg use four FDC tablets per day (i.e., 300 mg isoniazid, 600 mg rifampicin, 1600 mg pyrazinamide and 1100 mg ethambutol) [16].

Data collection

Basic demographic and clinical information was collected from all participants, including age, sex, body weight and height (to calculate body mass index [BMI]), co-morbidities (including HIV infection and hepatitis B and C) and concomitant drug use. Malnutrition was defined as BMI < 18.5 kg/m^2 [17].

Sample collection

Pharmacokinetic sampling took place at KCMC hospital. Patients had to be on TB treatment for at least ten days because of the expected steady state in the pharmacokinetics of the TB drugs at that point. Patients had refrained from food at least eight hours before drug intake. On the sampling day, patients took their drugs under our supervision at 8 a.m. They then had a standardized breakfast within 30 minutes after drug intake, which reflected the usual drug intake procedures in this population. The standardized breakfast consisted of a cup (125 ml) of tea with whole milk and sugar and either a small bowl of porridge or *maandazi*, a typical East African fried pasty (analogous to doughnut) that is rather fat.

Serial venous blood samples were collected just before, and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after observed TB drug intake. Plasma was separated and stored at -80°C immediately until transport on dry ice to The Netherlands for bio-analysis.

Bio-analysis

The plasma concentrations of isoniazid, rifampicin, pyrazinamide and ethambutol were assessed by validated high-performance liquid chromatography (HPLC) as described before [18]. Isoniazid and acetylisoniazid were measured with liquid-liquid extraction followed by Ultra Performance Liquid Chromatography (UPLC) with ultraviolet (UV) detection. Accuracy was between 97.8% and 106.7% for isoniazid and between 98.0% and 108.9% for acetylisoniazid, dependent on the concentration level. The intra- and inter-assay coefficients of variation were less than 13.4 % and less than 3.2% (dependent on the concentration), respectively, over the range of 0.05 to 15.1 mg/liter for isoniazid and less than 4.2% and less than 5.7%, respectively, over the range of 0.16 to 16.2mg/liter for acetylisoniazid. Lower limits of quantification were 0.05 mg/liter for isoniazid and 0.16 mg/liter for acetylisoniazid. Isoniazid and acetylisoniazid-containing samples were stable (<5% loss) for at least 12 months at -80°C.

Pharmacokinetic analysis

Pharmacokinetic evaluations were performed using non-compartmental methods in WinNonLin Version 5.3 (Pharsight Corp., Mountain View, California US). The highest observed plasma concentration was defined as C_{max} with the corresponding sampling time as T_{max}. The log-linear period (log C versus t) was based on the last data points (at least three). The absolute value of the slope (/2.303, in which is the first order elimination rate constant) was calculated using linear regression analysis. The elimination half-life (T) was calculated as 0.693/ . If the concentration at 24 h (C_{24h}) was below the limit of quantification of the assay (which was often the case for isoniazid and rifampicin), it was estimated based on the last measurable concentration (C_{last}) and using the formula $C_{24h} = C_{last}*e^{-(*(24-Tlast)}$. The area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24h}) was calculated using the linear/ log-trapezoidal rule from zero up to the last concentration at 24h. The apparent clearance of the drug (Cl/F; where F is bioavailability) was calculated as dose/ AUC_{0-24h} and the apparent volume of distribution (V_d/F) was calculated as (Cl/F)/ . The reference ranges for C_{max} were 3-5 mg/liter for isoniazid, 8-24 mg/ liter for rifampicin, 20-50 mg/ liter for pyrazinamide and 2-6 mg/ liter for ethambutol [19].

These reference ranges represent the normal C_{max}s concentrations that can be expected in adults after the standard doses of anti-TB drugs. They are based on data that were compiled from all available sources (both healthy volunteers and TB patients) by, amongst others, Holdiness [1] and Peloquin [2]. Subsequently, the ranges were validated in a range of phase I studies in healthy volunteers (C. A. Peloquin, presented at the 51st Intersci. Conf. Antimicrob. Agents Chemother., Chicago, IL, 17 to 20 September 2011) [20].

Determination of acetylator status

We determined the acetylator status phenotypically by assessing the t½ of isoniazid. Participants with at½ greater than 130 minutes were classified as slow metabolizers and those with a t½ of less than 130 minutes were classified as fast/intermediate metabolizers [21-23]. As another means to assess acetylator phenotype, the metabolic ratio of acetylisoniazid concentration to isoniazid concentration at 3 hours post dose was calculated. Patients with a ratio above 1.5 were considered fast/intermediate metabolizers, and patients with a ratio below 1.5 were considered slow metabolizers [21]. In addition, we also explored a metabolic ratio of 0.55 at 3 hours post dose as a cut-off to distinguish fast/intermediate from slow metabolizers, as this evolved from a study in African patients [23].

Statistical analysis

Most pharmacokinetic parameters were presented as geometric means. The sample size was considered too small to test for associations between patient characteristics and pharmacokinetic parameters. We tested the effect of acetylator status only on the pharmacokinetics of isoniazid. The correlations between C_{max} and AUC_{0-24h} of each of the drugs were explored using Spearman's Rho correlation.

To explore the potential of limited sampling for estimating the AUC_{0-24h} based on sampling early in the pharmacokinetic curve, univariate linear regression was used to determine the association between the plasma concentration at 2, 3, 4 and 6 hours post dose and the AUC_{0-24h} for each of the drugs. The r^2 value was presented as a measure of variance in the AUC_{0-24h} that is explained by the variance in the concentration at that time point. Statistical analyses were performed in STATA version 10.1 (Stata Corp LP, College Station, Texas, USA).

Results

Twenty tuberculosis patients were enrolled for this study. All were under community-based directly observed treatment. Their median age was 38 years (inter-quartile range [IQR] 30-42), 15 patients (75%) were male, seven (35%) were HIV positive and seven (35%) were considered malnourished based on their BMI (Table 1).

Descriptive pharmacokinetics

Intensive pharmacokinetic sampling took place after a median of 19 days after start of TB treatment (range 11-49 days).The pharmacokinetic parameters for isoniazid, rifampicin, pyrazinamide and ethambutol are presented in Table 2. In 10 patients (53%), the isoniazid peak plasma concentration was below the reference range (3-5

Table 1 Characteristics of 20 tuberculosis patients in northern Tanzania

Characteristic	
Male sex, n (%)	15 (75%)
Age, median years (IQR)	38 (30-42)
Body weight, mean kg (SD)	55.7 (6.4)
BMI, mean (SD)	19.5 (2.4)
Malnutrition, n (%)[a]	7 (35%)
HIV positive, n (%)	7 (35%)
Type of tuberculosis, n (%)	
Pulmonary	19 (95%)
Extra pulmonary	1 (5%)
Concomitant drugs, n (%)	
Amoxycillin	1 (5%)
ARV[b]	2 (10%)
Co-trimoxazole	3 (15%)
Diclofenac	3 (15%)
Vitamin B complex	2 (10%)
Dose (mg) per kg body weight, mean (SD)	
Isoniazid	5.2 (0.46)
Rifampicin	10.4 (0.91)
Pyrazinamide	27.8 (2.4)
Ethambutol	19.1 (1.7)
Acetylator status based on INH $t_{1/2}$, n (%)	
Slow	12 (63%)
Fast/ intermediate	7 (37%)
Acetylator status based on ac-INH/INH ratio (cut-off 1.5), n (%)	
Slow	10 (53%)
Fast/ intermediate	9 (47%)
Acetylator status based on ac-INH/INH ratio (cut-off 0.55), n (%)	
Slow	8 (42%)
Fast/intermediate	11 (58%)

IQR = interquartile range; BMI = body mass index; [a] Malnutrition defined as BMI < 18.5 kg/m²;[b] ARV = antiretroviral treatment.

mg/liter). In seven patients (35%), the rifampicin C_{max} was below the reference range (8-24 mg/ liter); in five of them isoniazid C_{max} was also below the reference range. In none of the patients the C_{max} of pyrazinamide or ethambutol was below the reference range.

Table 2 Pharmacokinetic parameters of tuberculosis drugs in 20 Tanzanian tuberculosis patients

	Isoniazid[a]	Rifampicin	Pyrazinamide	Ethambutol
PK parameters, geometric mean (min-max)				
AUC_{0-24}, h*mg/liter	11 (3.7-22.7)	39.9 (27.4-68.3)	344 (209-610)	20.2 (13.4-32.0)
C_{max}, mg/liter	2.8 (1.0-4.6)	8.9 (5.9-14.8)	38.2 (29.0-50.8)	3.3 (2.2-5.8)
t_{max}, h	1.2 (0.7-2.9)	1.3 (0.9-3.0)	1.2 (0.7-3.0)	1.5 (0.9-2.2)
CL/F, liters/h	25.8 (13.0-60.5)	14.4 (8.8-21.9)	4.5 (2.6-6.5)	52 (34.3-71.3)
Vd/F, liters	99 (69.6-173.4)	37.3 (22.7-56.5)	40.3 (29.5-98.3)	719 (491-965)
$t_{1/2}$, h	2.9 (1.3-4.2)	1.8 (1.1-3.8)	5.5 (4.1-15.7)	9.5 (6.9-13.5)
Reference range C_{max}[2], mg/liter	3-5	8-24	20-50	2-6
Proportion with C_{max} below reference range	10 (52%)	7 (35%)	0 (0%)	0 (0%)

[a]Isoniazid plasma concentrations were only determined in 19 patients.

There was a positive correlation between the AUC_{0-24h} and C_{max} of isoniazid (correlation coefficient 0.68; P=0.001), pyrazinamide (correlation coefficient 0.69; P=0.001) and ethambutol (correlation coefficient 0.72; P=0.003), but not between those of rifampicin (correlation coefficient 0.35 (P=0.14).

Assessment of acetylator status

Based on the elimination half-life of isoniazid, 7 (37%) patients were fast or intermediate metabolizers and 12 (63%) were slow metabolizers. Based on the 3-hour acetyl-INH/INH ratio with a cut-off of 1.5, nine (47%) patients were fast/intermediate metabolizers and 10 patients (53%) were slow metabolizers (Fig. 1). Two patients had

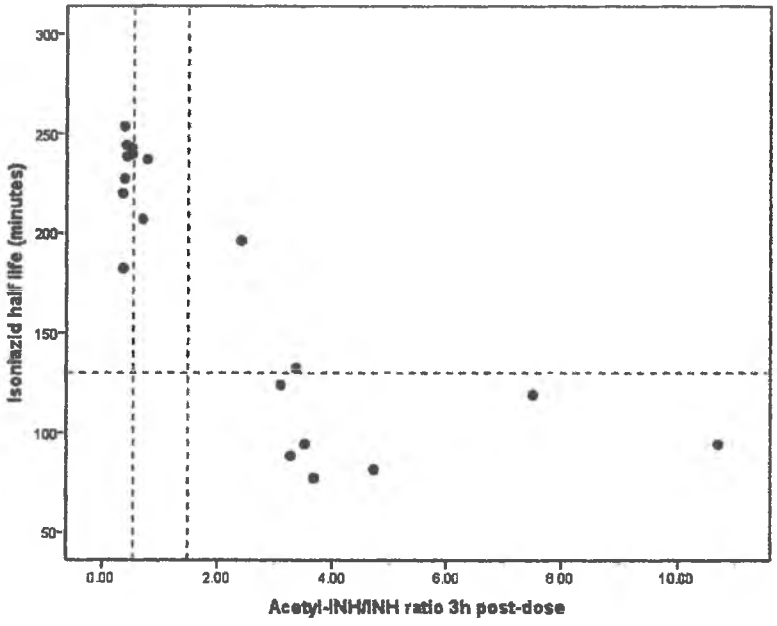


Figure 1 The relation between the acetylator status (slow versus fast/intermediate) based on two methods: the t½ of isoniazid and the acetyl-INH/INH metabolic ratio in the 3h post-dose plasma sample. Patients with a t½ below 130 minutes (=below the horizontal dashed line) were considered fast/intermediate metabolizers[17-19]. Patients with anacetyl-INH/INH ratio greater than 0.55 [19] or greater than 1.5 [17](=right of the first or second vertical dashed line, respectively) were considered fast/intermediate metabolizers based on metabolic ratio. Note that phenotyping by t½ and metabolic ratio resulted in discordant results (in the upper right quadrant) in 4 patients when a metabolic ratio of 0.55 was used as cut-off compared to 2 patients when a metabolic ratio of 1.5 was used as cut-off.

a discrepancy in acetylator phenotype determined by the two methods. With a cut-off of 0.55, 11 patients (58%) were fast/intermediate metabolizers and eight (42%) were slow metabolizers.

Acetylator status based on the half-life method was associated with the AUC_{0-24h} and C_{max} of isoniazid. In slow metabolizers, the geometric mean AUC_{0-24h} was 17.2 h*mg/liter (range 9.5-22.7) and C_{max} 3.4 mg/liter (range 2.0-4.6), and in intermediate/fast metabolizers, the geometric mean AUC_{0-24h} was 5.2 h*mg/liter (range 3.7-8.1) and C_{max}2.0 mg/liter (range 1.0-3.3); ($P<0.001$ and $P=0.01$, respectively).

Association between the 2, 3, 4 and 6h concentration and AUC_{0-24h}

The isoniazid concentrations at 2, 3, 4 and 6 hours post-dose were associated with AUC_{0-24h} and the r^2 value ranged from 0.91 to 0.96, meaning that 91-96% of the variance in the AUC_{0-24h} is explained by the variance in the concentration at a particular sampling time point. For rifampicin the r^2 value was highest for the concentration 4 hours post-dose (0.82). For pyrazinamide the r^2 value was highest for the concentrations 4 and 6 hours post dose (0.84 and 0.86, respectively). For the different ethambutol concentrations the r^2 value was low, varying from 0.40 to 0.71. This indicates that limited sampling to estimate the AUC_{0-24h} may be feasible for isoniazid, rifampicin and pyrazinamide but less for ethambutol.

Discussion

This report provides important data on pharmacokinetic characteristics of isoniazid, rifampicin, pyrazinamide and ethambutol based on intensive 24-hour pharmacokinetic sampling in East African TB patients.

Half of the patients from our study had an isoniazid peak plasma concentration (C_{max}) below the reference range, and a third of patients had a rifampicin C_{max} below the reference range. This may partly be explained by the breakfast that patients took within half an hour after the intake of TB drugs. This approach was chosen to mimic the real-life situation for TB patients, as most TB patients take their pills just before or with their breakfast in order to prevent or alleviate gastro-intestinal adverse effects. Previous studies have shown that intake of a meal with a high fat or carbohydrate content just before drug intake decreases the rate of absorption and significantly reduces the isoniazid C_{max} by 20% to 51% [24-27]. The rifampicin C_{max} can be reduced up to 36% when it is taken directly after a meal [24,28]. There is no significant effect of food on the C_{max}s of pyrazinamide and ethambutol [24,29,30]. Of note, in the current study the average T_{max} values for the TB drugs were short (about 1 h for

isoniazid, rifampicin and pyrazinamide) (table 2), indicating that the delay in absorption and a possible related decrease in C_{max} were probably limited. Furthermore, it should be noted that pharmacokinetic studies from other African countries found even higher proportions of patients with C_{max}s below the reference range for isoniazid (30% in Botswana [8,31], 89% in Kenya [32]) and rifampicin (69% in South Africa [12], 78-84% in Botswana [27,28] and 90% in Kenya [32]), despite administration of drugs on an empty stomach in the studies from Botswana and Kenya. The relatively high C_{max}s of the TB drugs in our study among African patients may also relate to the average body weight of 55.7 kg (table 1), which is just above 50 kg above which 4 FDC tablets (rather than 3) are administered.

Values for total exposure (AUC_{0-24h}) may be even more relevant to the efficacy of first-line TB drugs than the C_{max} [33]. AUC_{0-24h} values can best be compared to those recorded in Indonesian TB patients [18] and in a racially mixed population of patients in The Netherlands who used similar doses on a mg/kg basis (Magis-Escurra, H. M. J. Later-Nijland, J. W. C. Alffenaar, J. Broeders, D. M. Burger, R. van Crevel, M. J. Boeree, A. R. T. Donders, R. van Altena, T. S. van der Werf, and R. E. Aarnoutse, submitted for publication), as the pharmacokinetics in these studies were assessed with the same analytical methodology. Average exposures to rifampicin were 39.9, 48.5 and 41.1 h*mg/liter in the Tanzanian patients, the Indonesian patients and a mixed population, pyrazinamide AUC_{0-24h} values were 344, 473 and 380 h*mg/liter, respectively, and exposures to ethambutol were 20.2, 14.4 and 23.5 h*mg/liter. Isoniazid was not measured in Indonesian TB patients, and we used another analytical method with the mixed population (average AUC_{0-24h} was 15.2 h*mg/liter compared to 11.0 in the current study). These comparisons show no drastically lower total exposure to TB drugs between Tanzanian patients and the Indonesian patients and the mixed population from the Netherlands.

Even though the exposure to TB drugs in Tanzanian patients may be high compared to exposure from other African studies and similar to those in other populations, a large number of patients had low plasma concentrations of isoniazid and rifampicin. Several clinical studies have pointed towards a possible association between low TB drug concentrations and poor treatment outcome [3-5]. A preclinical model showed that interindividual variability in pharmacokinetics is relevant to the emergence of resistance [7], and a recent meta-analysis revealed that variability in exposure to isoniazid is associated with failure of therapy and acquired drug resistance [10]. On the other hand, other studies have found no association between plasma concentrations and effect to first-line TB drugs [34, 35].
We could not readily explain the observed low isoniazid and rifampicin AUC_{0-24h}s and C_{max}s by patient characteristics, as the sample size was considered too small for

analysis of association between patient characteristics and pharmacokinetic parameters. However, we evaluated the effect of genetic polymorphisms in N-acetyl transferase 2 (NAT2), a phase II metabolic enzyme, on the pharmacokinetics of isoniazid. Slow acetylation of isoniazid into acetylisoniazid is a homozygous recessive trait. Genotypically, homozygous fast, heterozygous fast (or intermediate) and slow acetylators are distinguished [22,36]. In our study acetylator status was assessed phenotypically using the isoniazid elimination half-life and the acetylisoniazid/isoniazid ratio 3 hours post-dose with a cut-off of 1.5.Based on these two methods, 63% or 53% of patients in our study, respectively, were slow metabolizers. This is consistent with available data showing that 50-60% percent of European (Caucasian), African, and Indian populations are slow metabolizers [37]. Clearly, assessment of the acetylator status by the metabolic ratio of acetylisoniazid/isoniazid at a single time point post dose is more convenient. There was good concordance between the two methods when 1.5 was used as the cut-off for the metabolic ratio (2 discordant patients). In the future, limited sampling may allow for an assessment of acetylator status as well as estimation of AUC values of TB drugs, considering the high correlation between concentrations measured in the early part of the pharmacokinetic curve and exposures to isoniazid, rifampicin and pyrazinamide. AUC_{0-24}s and C_{max}s isoniazid, pyrazinamide and ethambutol were also correlated, but for rifampicin no such association was found. We have considered whether this may be explained by the nonlinear, saturable pharmacokinetics of rifampicin, but disproportional changes in exposure above certain concentrations are expected to affect both C_{max} and AUC_{0-24}.

In summary, this study provides pharmacokinetic data of first-line TB drugs based on intensive 24h sampling in East African TB patients. Half of the patients had isoniazid peak plasma levels and a third had rifampicin peak plasma concentrations below the reference ranges. Although the pharmacodynamics of the currently used first line TB drugs is not entirely clear, low isoniazid and rifampicin concentrations have been associated with suboptimal treatment response. Exploring the effect, tolerability and pharmacokinetics of higher dose of isoniazid and rifampicin in African tuberculosis patients is therefore recommended.

Acknowledgements

We wish to thank the following people: all patients for participating in this study; Dr C. Irongo (Regional TB and Leprosy coordinator in the National TB and Leprosy Programme Tanzania), staff of the TB clinic at Mawenzi Hospital in Moshi and the research nurses at KCMC for their cooperation and effort; the laboratory technicians at KCMC and at the Department of Pharmacy of the RUNMC, The Netherlands for their technical support.

Financial support

A. Tostmann received the*UNESCO/L'Oreal For Young Women in Science Fellowship 2008* to coordinate this study. C. Mtabho, H. Semvua and J. van den Boogaard and bio-analysis of drugs were sponsored by the African Poverty Related Infection Oriented Research Initiative (APRIORI), a research network granted by the Netherlands-African partnership for Capacity development and Clinical interventions Against Poverty-related diseases (NACCAP).

References

1. Holdiness, M. R. 1984. Clinical pharmacokinetics of the antituberculosis drugs. Clin.Pharmacokinet. 9:511-544.
2. Peloquin, C. A. 1991. Antituberculosis drugs: pharmacokinetics., p. 59-88. *In* L. Heifets (ed.), Drug susceptibility in the chemotherapy of mycobacterial infections. CRC Press, Boca Raton.
3. Kimerling, M. E., P. Phillips, P. Patterson, M. Hall, C. A. Robinson, and N. E. Dunlap. 1998. Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. Chest 113:1178-1183.
4. Mehta, J. B., H. Shantaveerapa, R. P. Byrd, Jr., S. E. Morton, F. Fountain, and T. M. Roy. 2001. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest 120:1520-1524.
5. Weiner, M., W. Burman, A. Vernon, D. Benator, C. A. Peloquin, A. Khan, S. Weis, B. King, N. Shah, and T. Hodge. 2003. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am.J.Respir.Crit Care Med. 167:1341-1347.
6. Weiner, M., D. Benator, W. Burman, C. A. Peloquin, A. Khan, A. Vernon, B. Jones, C. Silva-Trigo, Z. Zhao, and T. Hodge. 2005. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. Clin.Infect.Dis. 40:1481-1491.
7. Srivastava, S., J. G. Pasipanodya, C. Meek, R. Leff, and T. Gumbo. 2011. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J.Infect.Dis. 204:1951-1959.
8. Chideya, S., C. A. Winston, C. A. Peloquin, W. Z. Bradford, P. C. Hopewell, C. D. Wells, A. L. Reingold, T. A. Kenyon, T. L. Moeti, and J. W. Tappero. 2009. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin.Infect.Dis. 48:1685-1694.
9. Egelund, E. F., and C. A. Peloquin. 2012. Editorial commentary: pharmacokinetic variability and tuberculosis treatment outcomes, including acquired drug resistance. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55:178-179.
10. Pasipanodya, J. G., S. Srivastava, and T. Gumbo. 2012. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55:169-177.
11. WHO. 2012. Global tuberculosis control: WHO report 2012. World Health Organization.
12. Mclleron, H., P. Wash, A. Burger, J. Norman, P. I. Folb, and P. Smith. 2006. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob.Agents Chemother. 50:1170-1177.
13. Gurumurthy, P., G. Ramachandran, A. K. Hemanth Kumar, S. Rajasekaran, C. Padmapriyadarsini, S. Swaminathan, P. Venkatesan, L. Sekar, S. Kumar, O. R. Krishnarajasekhar, and P. Paramesh. 2004. Malabsorption of rifampin and isoniazid in HIV-infected patients with and without tuberculosis. Clin. Infect.Dis. 38:280-283.
14. Sahai, J., K. Gallicano, L. Swick, S. Tailor, G. Garber, I. Seguin, L. Oliveras, S. Walker, A. Rachlis, and D. W. Cameron. 1997. Reduced plasma concentrations of antituberculosis drugs in patients with HIV infection. Ann.Intern.Med. 127:289-293.
15. Polasa, K., K. J. Murthy, and K. Krishnaswamy. 1984. Rifampicin kinetics in undernutrition. Br.J.Clin. Pharmacol. 17:481-484.
16. The United Republic of Tanzania, M. o. H. a. S. W. 2006. Manual of the National Tuberculosis and Leprosy Programme in Tanzania. Fifth edition.
17. Organization, W. H. 2012. BMI classification. In: WHO nutrition.
18. Ruslami, R., H. M. Nijland, B. Alisjahbana, I. Parwati, R. van Crevel, and R. E. Aarnoutse. 2007. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. Antimicrob Agents Chemother 51:2546-2551.
19. Peloquin, C. A. 2002. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 62:2169-2183.
20. Peloquin, C. A. 2011. 9/17/2011.

21. Hutchings, A., and P. A. Routledge. 1986. A simple method for determining acetylator phenotype using isoniazid. Br.J.Clin.Pharmacol. 22:343-345.
22. Parkin, D. P., S. Vandenplas, F. J. Botha, M. L. Vandenplas, H. I. Seifart, P. D. van Helden, B. J. van der Walt, P. R. Donald, and P. P. van Jaarsveld.1997. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. Am.J.Respir.Crit Care Med. 155:1717-1722.
23. Seifart, H. I., D. P. Parkin, F. J. Botha, P. R. Donald, and B. J. van der Walt. 2001. Population screening for isoniazid acetylator phenotype. Pharmacoepidemiol.Drug Saf 10:127-134.
24. Lin, M. Y., S. J. Lin, L. C. Chan, and Y. C. Lu. 2010. Impact of food and antacids on the pharmacokinetics of anti-tuberculosis drugs: systematic review and meta-analysis. Int.J.Tuberc.Lung Dis. 14:806-818.
25. Peloquin, C. A., R. Namdar, A. A. Dodge, and D. E. Nix. 1999. Pharmacokinetics of isoniazid under fasting conditions, with food, and with antacids. Int.J.Tuberc.Lung Dis. 3:703-710.
26. Zent, C., and P. Smith. 1995. Study of the effect of concomitant food on the bioavailability of rifampicin, isoniazid and pyrazinamide. Tuber.Lung Dis. 76:109-113.
27. Joshi, M. V., Y. S. Saraf, N. A. Kshirsagar, and V. N. Acharya. 1991. Food reduces isoniazid bioavailability in normal volunteers. J.Assoc.Physicians India 39:470-471.
28. Peloquin, C. A., R. Namdar, M. D. Singleton, and D. E. Nix. 1999. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest 115:12-18.
29. Peloquin, C. A., A. E. Bulpitt, G. S. Jaresko, R. W. Jelliffe, J. M. Childs, and D. E. Nix. 1999. Pharmacokinetics of ethambutol under fasting conditions, with food, and with antacids. Antimicrob.Agents Chemother. 43:568-572.
30. Peloquin, C. A., A. E. Bulpitt, G. S. Jaresko, R. W. Jelliffe, G. T. James, and D. E. Nix. 1998. Pharmacokinetics of pyrazinamide under fasting conditions, with food, and with antacids. Pharmacotherapy 18:1205-1211.
31. Tappero, J. W., W. Z. Bradford, T. B. Agerton, P. Hopewell, A. L. Reingold, S. Lockman, A. Oyewo, E. A. Talbot, T. A. Kenyon, T. L. Moeti, H. J. Moffat, and C. A. Peloquin. 2005. Serum concentrations of antimycobacterial drugs in patients with pulmonary tuberculosis in Botswana. Clin.Infect.Dis. 41:461-469.
32. Choudhri, S. H., M. Hawken, S. Gathua, G. O. Minyiri, W. Watkins, J. Sahai, D. S. Sitar, F. Y. Aoki, and R. Long. 1997. Pharmacokinetics of antimycobacterial drugs in patients with tuberculosis, AIDS, and diarrhea. Clin. Infect.Dis. 25:104-111.
33. Hall, R. G., R. D. Leff, and T. Gumbo. 2009. Treatment of active pulmonary tuberculosis in adults: current standards and recent advances. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy 29:1468-1481.
34. Narita, M., M. Hisada, B. Thimmappa, J. Stambaugh, E. Ibrahim, E. Hollender, and D. Ashkin. 2001. Tuberculosis recurrence: multivariate analysis of serum levels of tuberculosis drugs, human immunodeficiency virus status, and other risk factors. Clin.Infect.Dis. 32:515-517.
35. Chang, K. C., C. C. Leung, W. W. Yew, K. M. Kam, C. W. Yip, C. H. Ma, C. M. Tam, E. C. Leung, W. S. Law, and W. M. Leung. 2008. Peak plasma rifampicin level in tuberculosis patients with slow culture conversion. Eur.J.Clin.Microbiol.Infect.Dis. 27:467-472.
36. Aarnoutse, R. E. 2011. Pharmacogenetics of antituberculosis drugs, p. 176-190. *In* P. R. Donald and P. D. van Helden (ed.), Antituberculosis Chemotherapy. Progress in Resporatory Research, Basel, Switzerland.
37. Ellard, G. A. 1984. The potential clinical significance of the isoniazid acetylator phenotype in the treatment of pulmonary tuberculosis. Tubercle. 65:211-227.

CHAPTER 4

Associations between salivary, protein-unbound and total plasma concentrations of rifampicin

Charles M. Mtabho‡, Hadija H. Semvua‡, Marga Teulen, Angela Colbers, Rogate Machange, Arnold Ndaro, André van der Ven, David Burger, Gibson S. Kibiki, Rob E. Aarnoutse

‡ These authors contributed equally to this work

Manuscript submitted

Abstract

Introduction. Plasma is the traditional biological sample for PK studies and Therapeutic Drug Monitoring (TDM) of the pivotal anti-tuberculosis (TB) drug rifampicin. Saliva may be an attractive alternative matrix, also considering that it may reflect protein-unbound, active plasma concentrations. The objectives of this study were (1) to compare the PK of rifampicin in saliva and plasma and (2) to assess whether saliva could be an alternative matrix for PK studies and TDM with this drug.

Methods. A descriptive PK study was performed among 15 adult Tanzanian TB patients who were in the intensive phase of TB treatment. Time-matched samples of stimulated saliva (obtained with a Salivette® device containing citric acid) and plasma were collected at predose and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after intake of rifampicin. Salivary, total (protein-unbound plus bound) and unbound plasma concentrations of rifampicin were measured with validated HPLC methods. Salivary and plasma PK parameters were assessed, ratios of salivary to (total and unbound) plasma concentrations were calculated and the performance of salivary concentrations to predict plasma concentrations was evaluated using the jackknife method.

Results. The geometric mean AUC_{0-24} of rifampicin in saliva (3.1 h*mg/L) was slightly but significantly lower than the protein-unbound AUC_{0-24} in plasma (5.3 h*mg/L) and these were much lower than the plasma AUC_{0-24} based on total concentrations (32.7 h*mg/L). T_{max} and elimination half-lives of rifampicin based on salivary, protein-unbound and total plasma concentrations were similar. Geometric mean concentration ratios for salivary versus total and unbound plasma concentrations were 0.099 and 0.614 and these ratios were not dependent on time post dose or associated rifampicin plasma concentrations (repeated measures ANOVA). The prediction of total and unbound plasma concentrations based on salivary concentrations of rifampicin resulted in median percentage prediction errors (MPPE) of 13.4% and 6.0% and median absolute percentage prediction errors (MAPE) of 35.7% and 23.0%, respectively, which means that these predictions were sufficiently accurate (<15%) yet imprecise (>15%).

Conclusions. AUC_{0-24} and C_{max} of rifampicin in saliva were much lower than those in plasma based on total plasma concentrations. The AUC_{0-24} of rifampicin in saliva was of the same order of magnitude as the protein-unbound plasma AUC_{0-24}, but was significantly lower. It is not possible to predict total or protein-unbound plasma concentrations from salivary concentrations, due to inadequate precision associated with this prediction.

Introduction

The global burden of tuberculosis (TB) remains immense, with an estimated 8.7 million new cases of TB and 1.4 million people dying from this infectious disease in 2011 [1]. Still too many patients do not respond adequately to treatment, experience a relapse of TB after treatment completion, or are confronted with the emergence of drug resistant strains of *M. tuberculosis*.
Inadequate exposure to TB drugs constitutes one of the factors underlying suboptimal treatment response and development of resistance, as evidenced by results from the *in vitro* hollow fibre model [2], clinical studies on relationships between drug concentrations and response [3-7], and findings of a recent meta-analysis [8]. In view of the relevance of exposure to TB drugs, drug dosing may be *individualized* based on measurement of drug concentrations (Therapeutic Drug Monitoring, TDM), a practice applied in a few centres around the world [9-13]. Yet in the long term it seems preferable to develop new TB regimens that are pharmacokinetically optimized, enabling *fixed* dosing for all patients. This requires multiple pharmacokinetic studies during development of such regimens.

Plasma is the traditional biological sample for pharmacokinetic studies and TDM, but saliva may be an attractive alternative matrix. Advantages of saliva include the easy sample collection which can be standardized with specific saliva collection devices; and the painless collection of saliva which seems particularly advantageous for children [14,15]. Furthermore, salivary concentrations may reflect the protein-unbound (free) fraction of drug in plasma, which is active and available to be transported to the sites of action, rather than the total (protein-unbound plus bound) plasma concentration [14]. A clear prerequisite for the use of saliva in pharmacokinetic studies and TDM is a strong relationship between the salivary concentrations and the (unbound or total) plasma concentrations of a drug.

The plasma pharmacokinetics of the TB drug rifampicin are well-characterized [16-18]. This pivotal TB drug is now being repurposed as a component of future TB drug regimens for adults, but at higher doses [19-20]. In addition, higher doses of rifampicin are currently advised for treatment of TB in children, requiring pharmacokinetic evaluations as well [21]. Therefore the objectives of this study were (1) to describe the pharmacokinetics of rifampicin in saliva and plasma (total and protein-unbound concentrations) and (2) to assess whether saliva could be an alternative matrix for pharmacokinetic studies and TDM with this essential TB drug.

METHODS

Study design and population

We performed a descriptive pharmacokinetic study in Moshi, Tanzania. Adult TB patients who were in the intensive phase of TB treatment were eligible for participation. They had to be on TB treatment for at least two weeks because of the expected steady state of rifampicin at that time. Patients with a body weight less than 50 kg used three Fixed Dose Combination (FDC) tablets per day (i.e. 225 mg isoniazid, 450 mg rifampicin, 1200 mg pyrazinamide and 825 mg ethambutol) and patients with a body weight fff50 kg used four FDC tablets per day (i.e. 300 mg isoniazid, 600 mg rifampicin, 1600 mg pyrazinamide and 1100 mg ethambutol). All participants gave written informed consent. Intensive pharmacokinetic sampling for both plasma and saliva took place during 24 h.

This was an explorative study and no sample size calculation was performed. The study aimed to recruit 15 patients, providing for an expected number of circa 80 time-matched detectable salivary, protein-unbound and total (protein-unbound plus bound) concentrations of rifampicin.

Study participants were recruited from the outpatient TB clinic at Mawenzi Hospital in Moshi and pharmacokinetic sampling was done at the patient unit of the Kilimanjaro Clinical Research Institute. The study was approved by the Research Ethics Committee at the Kilimanjaro Christian Medical Centre (KCMC) and by the Tanzanian National Institute for Medical Research.

Pharmacokinetic sampling

Patients were asked not to eat for at least six hours (i.e. overnight) before start of intensive pharmacokinetic sampling. On the sampling day, patients took their drugs under supervision of a nurse in the morning, and then had a standardized breakfast within 30 minutes after drug intake, which is the usual procedure in this population. The standardized breakfast consisted of a cup (250 ml) of tea with milk and sugar and two pancakes. Serial venous blood samples were collected just before, and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after TB drug intake. Plasma was centrifuged at 2800 rpm for 10 minutes and stored at -80°C immediately thereafter.

Stimulated saliva was collected immediately (i.e within 2 minutes) after every blood sample. The patients were asked to rinse their mouth with water. Then a Salivette® was provided, which is a plastic device containing a dental cotton roll impregnated with citric acid that stimulates the salivary flow (Sarstedt, Etten-Leur, The Netherlands). The patients were asked to chew on the cotton roll inside the Salivette®. After one minute, the patient was asked to put the cotton roll back in the insert of the Salivette® centrifuge vessel. The centrifuge vessel was centrifuged at 3000g for 5 minutes, which allows saliva to be collected in the extended tip of the Salivette®

tube. The saliva was put in polypropylene tubes and stored at -80 °C. The total process until storage in the freezer took a maximum of 45 minutes after sampling.
Both the plasma and saliva samples were transported on dry ice to The Netherlands, where bio-analysis took place.

Bio-analysis

Total (protein-bound plus unbound) plasma concentrations of rifampicin were measured with a validated HPLC method as described before [22].
For the analysis of rifampicin in saliva, liquid-liquid extraction followed by HPLC was used. Briefly, 250 µL of saliva was mixed with 25 µL ascorbic acid (20 g/L), 25 µL 0.2 M acetate buffer pH5, 25 µL of internal standard (sulindac) solution and 1 ml of iso-octane / dichloro-methane (3:2 %v/v) and the mixture was centrifuged at 1910 g for 5 minutes. The water layer was frozen in a cryobath (-40°C), the organic layer was decanted and evaporated at 37°C under a N_2 flow until the tube was dry. The residu was reconstituted in 200 µL H_2O / CH_3CN (70/30 %v/v) at 4°C by shaking it for 10 minutes. Immediately after centrifugation at 1910 g for 10 minutes at 4°C, 50 µL was injected into the HPLC system.
To measure protein-unbound rifampin, ultrafiltration followed by HPLC was used. Briefly, 0.5 ml of plasma was added into a Centrifree YM-30 tube (Millipore, Amsterdam, the Netherlands). The tube was centrifuged for 15 min at 1650 g at 25°C and 50 µL of the clear ultrafiltrate was injected into the HPLC system.
One HPLC system was developed for measurement of salivary and protein-unbound rifampicin concentrations. The analytical column was an OmniSpher 5 C18 column (250 4.6 mm ID; particle size 5 µm) protected by a Chromguard RP ss 10 x 3 mm column (Varian, Middelburg, The Netherlands). The mobile phase components were 30% acetonitrile and 70% 10 mM phosphate buffer with pH 5, run during a HPLC gradient with different flow rates. Total run time was 17 min. UV detection was set at 334 nm. The autosampler liquid system was set at 4°C.
For measurement of rifampicin in saliva, accuracy and intra-day precision ranged from 98 to 105% and from 3.7 to 7.3 %, respectively, dependent on the concentration which ranged from 0.1 (limit of quantitation) to 10.4 mg/L. For measurement of unbound plasma concentrations of rifampicin, the average accuracy of ultrafiltrate spiked with rifampin was 107% and intraday precision in measurement of rifampicin in ultrafiltrate varied from 1.5 to 1.9% dependent on the concentration measured. The range of the method for unbound rifampicin plasma concentrations was from 0.06 mg/L (limit of quantitation) to 13 mg/L.

Pharmacokinetic analysis

The area under the plasma concentration-time curve (AUC_{0-24h}), the highest observed concentration (C_{max}) with the corresponding sampling time T_{max}, apparent clearance (Cl/F; in which F is bioavailability), apparent volume of distribution (V_d/F) and

elimination half-life ($T_{}$) were assessed by noncompartmental pharmacokinetic methods using WinNonLin Version 5.3 (Pharsight Corp., Mountain View, California US), as described before [22]. C_{24h} was estimated based on the last measurable concentration (C_{last}) taken at time T_{last} and the elimination rate constant , using the formula $C_{24h} = C_{last} * e^{-*(24-Tlast)}$.

Statistical analysis

Differences in pharmacokinetic parameters of rifampicin based on salivary, protein-unbound and total plasma concentrations were assessed with repeated-measures (within-subject) analysis of variance (ANOVA) performed on log-transformed pharmacokinetic parameters, followed by three separate paired-samples T-tests performed at a Bonferroni-corrected significance level of 0.05/3. T_{max} values of rifampicin were tested with the related-samples (within-subject) Friedman's analysis of variance by ranks. Correlation between pharmacokinetic parameters was performed on untransformed data using Spearman's rho (rank correlation).

To assess whether saliva could be an alternative matrix for pharmacokinetic studies and TDM, the time-matched salivary concentrations, protein-unbound and total plasma concentrations of rifampicin were first correlated to eachother using Spearman's rho. Subsequently ratios of salivary concentrations versus total and protein-unbound plasma concentrations of rifampicin were calculated for all time-matched samples. The effect of sampling time and the effect of the associated rifampicin plasma concentrations on the concentration ratios was assessed with repeated-measures (within-subject) ANOVA on log-transformed concentration ratios. Geometric means were calculated for the saliva to plasma (total and unbound) concentration ratios; the reciprocal average concentration ratios were used as conversion factors to estimate total and unbound rifampicin plasma concentrations based on the measured salivary concentrations [23].

The predictive performance of these estimations was assessed by calculation of conversion factors based on datasets in which one out of the 15 patients was omitted, followed by use of these conversion factors to estimate the (total and unbound) plasma concentrations of the 15th patient (jackknife method [24]). Such predictions were performed for plasma concentrations of all 15 patients. Potential bias in the predictions was assessed using the median percentage prediction error (MPPE). This is the median of all percentage prediction errors, which were defined as: 100% * (predicted concentration – measured concentration) / measured concentration. Imprecision was assessed using the median absolute percentage prediction error (MAPE). This is the median of all absolute percentage prediction errors. The absolute percentage prediction error was defined as: 100% * absolute ((predicted concentration – measured concentration) / measured concentration). For an acceptable predictive performance, both MPPE and MAPE had to be <15% [24,25].

As an alternative means to estimate plasma concentrations and assess predictive performance, a linear regression analysis was performed on log-transformed salivary concentrations versus log-transformed plasma (total or protein-unbound) concentrations of rifampicin, yielding a least squares regression line with equation: log (plasma concentration) = a + b* log (salivary concentration), in which a is the intercept and b is the slope of the regression line [26]. This equation was derived in a randomly selected set of 8 patients (index set) and was used to estimate plasma concentrations in the remaining 7 patients (validation set) [24,26]. This procedure was repeated three times. Again MPPE and MAPE were used as measures of bias and imprecision.

All statistical analyses were performed with SPSS for Windows version 20.0 (SPSS Inc, Chicago, IL).P values of below 0.05 were considered significant.

Results

Patients

Fifteen TB patients were enrolled in the study. Twelve patients were male (80%), their median age was 37 years (range 19-50 years), their median body weight was 49.5 kg (range 41.5-73.6 kg) and they received a median rifampicin dose of 10.2 mg/kg (range 8.2 -11.3 mg/kg).

Pharmacokinetics of rifampicin based on salivary, protein-unbound and total plasma concentrations

The geometric mean AUC_{0-24} of rifampicin in saliva (3.1 h*mg/L) was slightly lower than the geometric mean protein-unbound AUC_{0-24} in plasma (5.3 h*mg/.L) and these were much lower than the geometric mean plasma AUC_{0-24} based on total concentrations (32.7 h*mg/L, table 1, figure 1). Repeated-measures ANOVA on log-transformed AUC_{0-24} values showed a significant main effect based on type of concentration measured (<0.001) and paired t-tests with Bonferroni correction revealed significant differences between all three AUC_{0-24} values ($p<0.001$). For C_{max} values of rifampicin, the difference in rifampicin C_{max} in saliva and C_{max} based on protein-unbound plasma concentrations was not significant, but both these C_{max} values were significantly lower than the C_{max} based on total plasma concentrations ($p<0.001$).

Rifampicin AUC_{0-24} values based on salivary concentrations, protein-unbound plasma concentrations and total plasma concentrations were all strongly (Spearman's rho at least 0.71) and significantly (pffi 0.003) correlated to eachother. Rifampicin C_{max} values based on protein-unbound and total concentrations were also correlated (rho = 0.9, $p<0.001$) but these were not correlated with rifampicin C_{max} values in saliva.

Table 1 Pharmacokinetics of rifampicin in saliva and plasma (n=15)[1]

PK parameter	saliva[2]	Plasma, protein-unbound conc.[2]	Plasma, total conc.[2,3]	Ratio saliva / plasma protein-unbound[4]	Ratio saliva / plasma total[3,4]	Ratio plasma protein-unbound / plasma total[3,4]
AUC_{0-24} (h*mg/L)	3.1 (0.70-8.6)	5.3 (1.8-10.3)	32.7 (11.7-73.6)	0.59 [0.50-0.71]	0.096 [0.077-0.119]	0.161 [0.146-0.178]
C_{max} (mg/L)	0.64 (0.12-1.7)	1.0 (0.50-2.2)	6.8 (3.2-17.4)	0.62[0.42-0.91]	0.094 [0.063-0.140]	0.151 [0.136-0.167]
T_{max} (h)	3.0 (1.0-6.1)	2.1 (1.0-6.0)	2.1 (1.0-6.0)	-	-	-
Cl/F (L/h)	164.1 (69.9-646.3)	97.6 (58.1-256.5)	15.7 (8.2-38.6)	1.68 [1.42-2.00]	10.4 [8.4-12.9]	6.21 [5.61-6.87]
Vd/F (L)	469.3 (223.3-1673.6)	318.8 (167.9-1022.4)	47.7 (24.4-139.5)	1.47 [1.26-1.72]	9.8 [8.1-11.9]	6.69 [5.69-7.85]
$T_{1/2}$(h)	2.0 (1.3-2.7)	2.3 (1.6-4.9)	2.1 (1.4-3.9)	0.88 [0.76-1.01]	0.94 [0.84-1.06]	1.08 [0.96-1.21]

1. Abbreviations: AUC_{0-24}: area under the concentration versus time curve, C_{max}: peak plasma concentration, T_{max}: time to peak plasma concentration, Cl/F: apparent clearance, Vd/F: apparent volume of distribution, $T_{1/2}$: elimination half-life, conc.: concentration
2. Geometric means and ranges, apart from Tmax (median and range)
3. Total plasma concentrations refer to protein-unbound plus bound concentrations
4. Geometric means and 95% confidence intervals

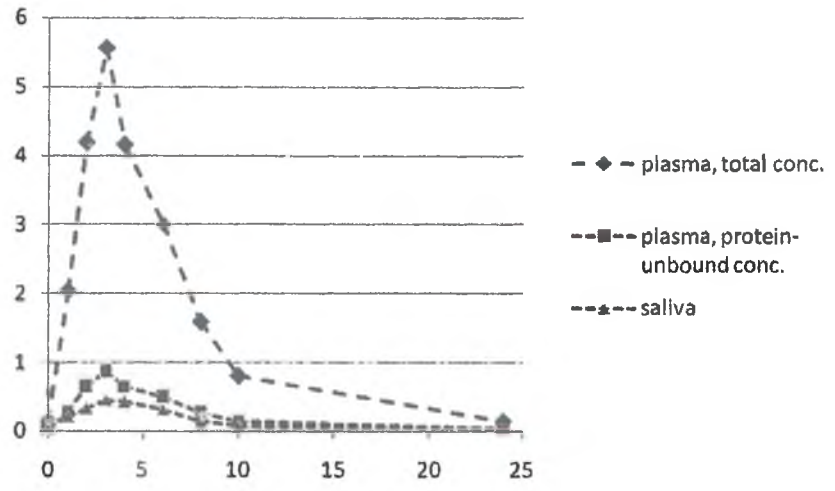


Figure 1 Geometric mean steady-state concentrations of rifampicin versus time (N=15)[1].

1 Concentrations of rifampicin (Y-axis) are displayed in mg/L. Time post dose (X-axis) is in hours.

T_{max} of rifampicin in saliva appeared to be larger in saliva compared to plasma (3.0 h versus 2.1 h and 2.1 h, table 1), but this difference did not reach statistical significance (p=0.08). Elimination half-lives of rifampicin based on salivary, protein-unbound and total plasma concentrations were similar (see table 1, p=0.10).

Evaluation of saliva as alternative sampling matrix: correlation analysis and time and concentration dependency of concentration ratios

As expected based on the short elimination half-life of rifampicin, loose concentrations of rifampicin were below the limits of quantitation at predose (T=0h) and at 24 h post dose for all salivary, protein-unbound and total plasma concentration measurements. Salivary and unbound plasma concentrations were still below the limit of quantitation at T=1 (6 patients) and T=2h (1 patient) and they returned below the limit of quantitation at T=8h (2 patients) and T =10h post dose (T=8 patients). A total of 88 time-matched salivary and protein-unbound and total plasma concentrations were available based on concentrations measured above the limits of quantitation of the assays. In 100 time-matched samples, protein-unbound and total plasma concentrations were measurable.

Salivary concentrations were significantly correlated with total plasma concentrations (rho = 0.777, p<0.001, n=88) and with protein-unbound plasma concentrations (rho = 0.787, p<0.001, n=88, figure 2). Protein-unbound and total plasma concentrations showed a stronger correlation (rho = 0.960, p<0.001, n=100).
The geometric mean concentration ratio for salivary versus total plasma concentrations was 0.099 (95% CI: 0.089-0.110, range 0.014-0.539) and that for salivary versus protein-unbound plasma concentrations was 0.614 (95% CI: 0.553-0.682, range 0.086-5.51). The concentration ratio of salivary versus total plasma concentrations of rifampicin did not seem to be dependent of time of sampling (table 2) and this was confirmed by repeated-measures ANOVA performed over the sampling times 1h to 10h (p=0.59, n=4), 2h to 10h (p=0.91, n=6) and 2h to 8h (p=0.43, n=12) post dose; no trends over the sampling times were shown either. Concentration ratios for salivary versus unbound plasma concentrations were not dependent of sampling time either (1h to 10h post dose: p=0.23, n=5; 1h to 8 h: p=0.31, n=9). However, concentrations of unbound versus total plasma concentrations showed an increase with sampling time according to a significant linear trend (sampling times 1h to 10h: p=0.01, n=12, table 2).

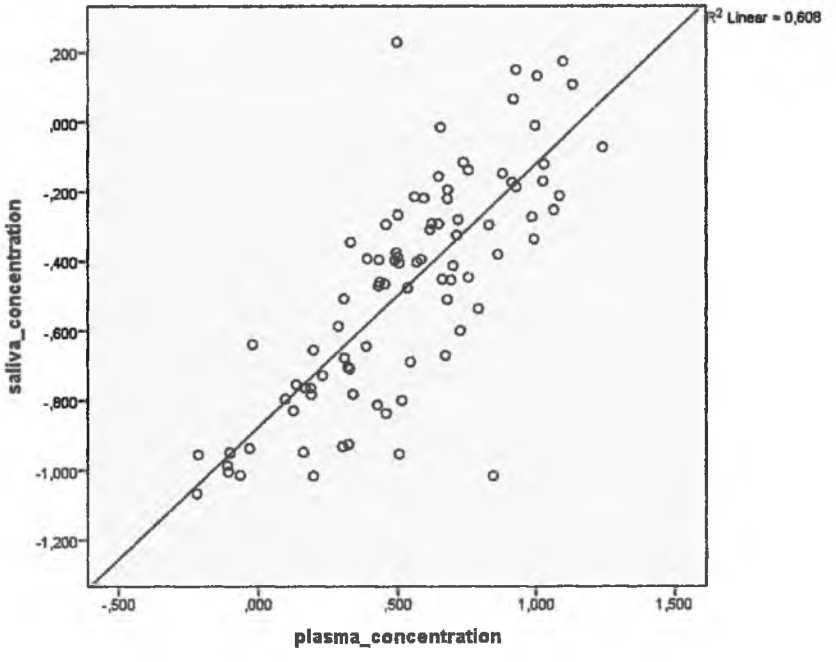


Figure 2 Scatter plot and regression line of log-transformed salivary and total (protein-bound plus unbound) plasma concentrations of rifampicin (R^2 = 0.608, P<0.001, n=88).

Table 2 Effect of sampling time and associated concentrations on concentration ratios

Time post dose (h)[1]	Total plasma concentration (mg/L, geometric mean)[2]	Concentration ratio of saliva to protein-unbound plasma concentrations [3]		Concentration ratio of saliva to total plasma concentrations [2,3]		Concentration ratio of protein-unbound to total plasma concentrations[2,3]	
1	2.0	0.773 (0.301-5.511)	N=9	0.111 (0.048-0.539)	N=9	0.145 (0.098-0.197)	N=12
2	4.2	0.575 (0.338-1.067)	N=14	0.091 (0.047-0.142)	N=14	0.154 (0.106-0.229)	N=11
3	5.6	0.528 (0.087-1.593)	N=15	0.082 (0.014-0.214)	N=15	0.155 (0.110-0.218)	N=15
4	4.2	0.658 (0.289-1.011)	N=15	0.104 (0.045-0.168)	N=15	0.157 (0.113-0.229)	N=15
6	3.0	0.656 (0.313-1.072)	N=15	0.107 (0.047-0.171)	N=15	0.163 (0.105-0.238)	N=15
8	1.6	0.581 (0.210-1.015)	N=13	0.098 (0.035-0.210)	N=13	0.169 (0.100-0.232)	N=14
10	0.79	0.593 (0.376-1.086)	N=7	0.114 (0.061-0.182)	N=7	0.184 (0.116-0.263)	N=14

1. Both salivary and protein-unbound plasma concentrations were below the limit of quantitation in all samples at T=0 h and T=24 h post dose.
2. Total plasma concentrations refer to protein-unbound plus bound concentrations
3. Geometric means and ranges

Evaluation of saliva as alternative sampling matrix: predictive performance of salivary concentrations

The conversion factor to predict total (protein-bound plus unbound) rifampicin plasma concentrations from salivary concentrations was 10.14 (n=88, all 15 patients) and this factor varied slightly when derived from different sets of 14 patients (range: 9.64 to 10.53). The jackknife method showed that the median percentage prediction error (MPPE) for estimation of total rifampicin plasma concentrations based on salivary concentrations was 13.4% and the median absolute percentage prediction error (MAPE) was 35.7%.

The conversion factor to predict protein-unbound rifampicin plasma concentrations from salivary concentrations was 1.63 (n=88, all 15 patients, varying from 1.56 to 1.70 in the sets of 14 patients). The MPPE and MAPE for estimation of protein-unbound rifampicin plasma concentrations based on salivary concentrations were 6.0 and 23.0%.

Use of a linear regression equation to predict total plasma concentrations in three index and validation sets resulted in MPPE values of 43.1, 3.8 and -22.4%, and MAPE values were 43.3, 37.5 and 34.6%. For prediction of protein-unbound plasma concentrations, MPPE and MAPE values were 23.5 and 28.1% for one index and validation set, data for the other two sets are pending.

Discussion

The first objective of this study was to describe the pharmacokinetics of rifampicin in saliva and compare these to plasma pharmacokinetics based on protein-unbound and total concentrations. It appeared that the salivary AUC_{0-24} and C_{max} of rifampicin were much lower than those in plasma as based on total (protein-bound plus unbound) concentrations (table 2). This was anticipated, as only the protein-unbound (free) fraction of a drug in plasma can pass into the salivary compartment [14,15,27,28]. In fact, saliva concentrations of most drugs are thought to reflect unbound drug concentrations in plasma [14,15,27,28]. In this study we actually measured the protein-unbound rifampicin plasma concentrations by using a validated analytical method based on ultrafiltration.

The AUC_{0-24} of rifampicin in saliva was of the same order of magnitude as the protein-unbound plasma AUC_{0-24}, yet it was significantly lower (table 1). This may be explained by one or several other factors that (apart from protein binding) determine the extent of diffusion of drugs into saliva, including lipid solubility, the degree of ionisation in blood and in saliva, and salivary flow [14,15]. The rifampicin molecule exhibits high lipid solubility [16]; its degree of ionisation is dependent on the pH of the solution in which it recides. In solution, rifampin behaves as a zwitterion. Its acidic

function (pKa = 1.7) is associated mainly to its hydroxyl groups, while its basic function (pKa = 7.9) is related to a piperazine-nitrogen functional group. In acidified saliva obtained with the Salivette® device (pH=3 [29]), the hydroxylgroup (pKa 1.7) will be largely unprotonated and charged negative, whereas the piperazine-nitrogen will be charged positive, resulting in a neutral molecule. The neutral molecule of rifampicin is not expected to be 'trapped' in saliva (causing higher salivary than plasma concentrations) as is the case for many basic drugs that are charged positive in acidified saliva.
The appearance of rifampicin into saliva was rapid as reflected in a T_{max} of 3.0h (table 1) and salivary to plasma concentration ratios were not dependent on sampling time or associated rifampicin concentrations. The rapid appearance of rifampicin in saliva and the absence of saturation of passage of rifampicin from blood into saliva suggest that this TB drug enters saliva by passive diffusion instead of active transport.
Relatively few comparable studies are available on diffusion of rifampicin into saliva. Kenny and Strates have summarized the literature on rifampicin up to 1981 and stated that highest rifampicin levels in saliva and sputum are obtained up to 4 hr after peak serum levels and that these are only 17-25% of peak serum levels [16]. Gurumuthy et al showed that peak concentrations of rifampicin in saliva were 10.6% of those in plasma [30], very similar to data from our study. Contrary to the current study, Gurumuthy et al found a clear increase in the saliva to serum concentration ratio with the sampling time post dose. Orisakwe et al found a much higher saliva to plasma ratio for AUC_{0-24} (i.e. a ratio of 0.67) in their study with chewing gum stimulated saliva flow and intensive pharmacokinetic sampling in healthy volunteers [31].

This is one of very few studies that assessed protein-unbound concentrations of rifampicin in all samples of the recorded pharmacokinetic curves. The average protein binding of 84% as assessed in this study based on unbound/total plasma AUC_{0-24} ratios (table 1) is very close to the 80% protein binding which is often stated for rifampicin [17,18]. Of note, the ratio of protein-unbound versus total concentrations of rifampicin steadily increased with higher (total) rifampicin concentrations, which is relevant to rifampicin being repurposed at higher doses.

The second objective of this study was to assess whether saliva could be used as an alternative matrix for pharmacokinetic studies and TDM with rifampicin. Based on experience in the past [29,32] we deliberately chose to sample 'stimulated' saliva using a standardized, reproducible and convenient sampling technique with the Salivette® device. It was considered that stimulated collection results in less variation of salivary pH and possibly less variation in saliva to plasma ratios compared to simple expectoration of saliva [14]. The patients were asked to rinse their mouth with water before sampling of saliva to avoid contamination of the oral cavity with drugs due to prior drug administration, which could lead to spurious findings [14]. As required for a

good prediction, saliva to plasma concentration ratios were not dependent of sampling time and rifampicin plasma concentrations.
Prediction of total or unbound rifampicin plasma concentration based on salivary concentrations resulted in an acceptable median percentage prediction error (MPPE), which means that systematic bias was limited when many individual predictions (each with positive or negative bias) were considered. Unfortunately, the average *absolute* error as measured by the median absolute percentage prediction error (MAPE) was above the predefined criterium, which means that the overall predictive performance of salivary concentrations was insufficient. Use of a regression equation rather than a straightforward conversion factor resulted in a similarly imprecise prediction of (total or unbound) plasma concentrations based on salivary concentrations. Of note, the current study also showed that monitoring of saliva concentrations of rifampicin has limited value in evaluating adherence to this drug, as salivary concentrations of rifampicin were below the limits of quantitation at predose (T=oh), at T=1 and starting from T=1oh post dose in many patients who took rifampicin under observation. This means that early or late sampling of saliva may incorrectly suggest nonadherence to rifampicin.

In summary, this study showed that total exposure (AUC_{0-24}) and C_{max} of rifampicin in saliva were much lower than those in plasma based on total (protein-unbound plus bound) plasma concentrations. The AUC_{0-24} of rifampicin in saliva was of the same order of magnitude as the protein-unbound plasma AUC_{0-24}, but was significantly lower. Rifampicin appeared rapidly in saliva and probably enters saliva through passive diffusion. It appeared not to be possible to predict total or protein-unbound plasma concentrations from salivary concentrations, due to inadequate precision associated with this prediction.

Acknowledgements
We wish to thank all people who in one way or another facilitated the conduct of this study, particularly all patients for participating in this study; dr Chelangwa (Kilimanjaro Regional TB and Leprosy coordinator in the National TB and Leprosy Programme Tanzania), the staff of the TB clinic at Mawenzi Hospital in Moshi and the research nurses Rose Malya, Mono Batuli and Taji Mnzava for their cooperation and effort during pharmacokinetic sampling; the laboratory technicians at KCMC-Biotechnology and at the Department of Pharmacy of Radboud University Medical Centre, Nijmegen, The Netherlands for analysing samples; and the Kilimanjaro Clinical Research Institute (KCRI) staff for their support.

Financial support

The African Poverty Related Infection Oriented Research Initiative (APRIORI), a research network granted by the Netherlands-African partnership for Capacity development and Clinical interventions Against Poverty-related diseases (NACCAP), sponsored the activities of PhD students involved in this project. The Kilimanjaro Clinical Research Institute (KCRI) sponsored the transport, participants incentives and food during pharmacokinetic sampling. Bio-analysis of drugs was sponsored by the Department of Pharmacy of Radboud University Medical Centre, Nijmegen, The Netherlands.

References

1. Global Tuberculosis Report 2012, WHO, 2012. Available at http://www.who.int/tb/publications/global_report/en/
2. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis 2011; 204:1951-9.
3. Kimerling ME, Philips P, Patterson P, Hall M, Robinson CA, Dunlap DA. Low serum antimycobacterial drug levels in non-HIV infected tuberculosis patients. Chest 1998;113:1178-83.
4. Mehta JB, Shantaveerapa H, Byrd RP Jr, Morton SE, Fountain F, Roy TM. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest 2001;120:1520-4.
5. Weiner M, Burman W, Vernon A, Benator D, Peloquin CA, et al. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am J Resp Crit Care Med 2003;167:1341-7.
6. Weiner M, Benator D, Burman W, Peloquin CA, Khan A, et al. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. Clin Infect Dis 2005;40:1481-91.
7. Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, et al. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin Infect Dis 2009;48:1685-94.
8. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis:2012;55:169-77.
9. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 2002;62:2169-83.
10. Ray J, Gardiner I, Marriott D. Managing antituberculosis drug therapy by therapeutic drug monitoring of rifampicin and isoniazid. Internal Medicine Journal 2003;33:229-34.
11. Holland DP, Hamilton CD, Weintrob AC, Engemann JJ, Fortenberry ER, et al. Therapeutic drug monitoring of antimycobacterial drugs in patients with both tuberculosis and advanced human immunodeficiency virus infection. Pharmacotherapy 2009;29:503-10.
12. Heysell SK, Moore JL, Keller SJ, Houpt ER. 2010. Therapeutic drug monitoring for slow response to tuberculosis treatment in a state control program, Virginia, USA. Emerging infectious diseases 2010;16:1546-53.
13. Magis-Escurra C, van den Boogaard J, Ijdema D, Boeree M, Aarnoutse R. Therapeutic drug monitoring in the treatment of tuberculosis patients. Pulmonary pharmacology & therapeutics 2012;25:83-6.
14. Liu H, Delgado MR. Therapeutic drug concentration monitoring using saliva samples. Focus on anti-convulsants. Clin Pharmacokinet. 1999;36:453-70.
15. Mullangi R, Agrawal S, Srinivas NR. Measurement of xenobiotics in saliva: is saliva an attractive alternative matrix? Case studies and analytical perspectives. Biomed Chromatogr. 2009;23:3-25.
16. Kenny MT, Strates B. Metabolism and pharmacokinetics of the antibiotic rifampin. Drug Metab Rev. 1981;12:159-218.
17. Acocella G. Clinical pharmacokinetics of rifampicin. Clin Pharmacokinet. 1978;3:108-27.
18. Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. Clin Pharmacokinet 1984;9:511-44
19. Ginsberg AM. Drugs in development for tuberculosis. Drugs 2010;70:2201-14.
20. van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, Gillespie SH, Boeree MJ. Why Do We Use 600 mg of Rifampicin in Tuberculosis Treatment? Clin Infect Dis 2011;52:e194-9.
21. Rapid Advice. Treatment of tuberculosis in children. WHO, 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241500449_eng.pdf
22. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. Antimicrob Agents Chemother 2007;51:2546-51
23. Van Heeswijk RPG, Veldkamp AI, Mulder JW, Meenhorst PL, Beijnen JH, Lange JMA, Hoetelmans RMW. Saliva as an alternative body fluid for therapeutic drug monitoring of the nonnucleoside reverse transcription inhibitor nevirapine. Ther Drug Monit. 2001;23:255-8.

24. Ting LS, Villeneuve E, Ensom MH. Beyond cyclosporine: A systematic review of limited sampling strategies for other immunosuppressants. Ther. Drug Monit. 2006; 28:419–30.
25. Barraclough KA, Isbel NM, Johnson DW, Hawley CM, Lee KJ, McWhinney BC, Ungerer JP, Campbell SB, Leary DR, Staatz CE. A limited sampling strategy for the simultaneous estimation of tacrolimus, mycophenolic acid and unbound prednisolone exposure in adult kidney transplant recipients. Nephrology. 2012;17:294-9.
26. Koks CHW, Crommentuyn KML, Hoetelmans RMW, Mathot RAA, Beijnen JH. Can fluconazole concentrations in saliva be used for therapeutic drug monitoring ? Ther Drug Monit 2001;23:449-53.
27. Jusko WJ, Milsap RL. Pharmacokinetic principles of drug distribution in saliva. Ann N Y Acad Sci. 1993;694:36-47.
28. Haeckel R. Factors influencing the saliva/plasma ratio of drugs. Ann N Y Acad Sci. 1993;694:128-42.
29. Hugen PW, Burger DM, de Graaff M, ter Hofstede HJ, Hoetelmans RM, Brinkman K, Meenhorst PL, Mulder JW, Koopmans PP, Hekster YA. Saliva as a specimen for monitoring compliance but not for predicting plasma concentrations in patients with HIV treated with indinavir. Ther Drug Monit. 2000;22:437-45.
30. Gurumurthy P, Rahman F, Narayana ASL, Sarma GR. Salivary levels of isoniazid and rifampicin in tuberculous patients. Tubercle 1990;71: 29-33.
31. Orisakwe OE, Akunyili DN, Agbasi PU, Ezejiofor NA. Some plasma and saliva pharmacokinetics parameters of rifampicin in the presence of pefloxacin. Am J Ther. 2004;11:283-7.
32. L'homme RF, Muro EP, Droste JA, Wolters LR, van Ewijk-Beneken Kolmer NW, Schimana W, Burger DM. Therapeutic drug monitoring of nevirapine in resource-limited settings. Clin Infect Dis. 2008;47:1339-44.

CHAPTER 5

Plasma drug activity assay for treatment optimization in tuberculosis patients

Scott K. Heysell, Charles Mtabho, Stellah Mpagama,
Solomon Mwaigwisya, Suporn Pholwat, Norah Ndusilo, Jean Gratz,
Rob E. Aarnoutse, Gibson S. Kibiki, Eric R. Houpt

Antimicrob.Agents Chemother. 55, no. 12 (2011): 5819-5825.

Abstract

Background. Low anti-tuberculosis (TB) drug levels are common, but their clinical significance remains unclear and methods of measurement are resource intensive.

Methods. Subjects initiating treatment for sputum smear positive pulmonary TB were enrolled from Kibong'oto National TB Hospital, Tanzania, and levels of isoniazid, rifampin, ethambutol and pyrazinamide were measured at time of typical peak plasma concentration (C_{2hr}). To evaluate the significance of the observed drug levels on *Mycobacterium tuberculosis* growth, a plasma TB drug activity (TDA) assay in the Bactec MGIT system was developed. Time-to-detection of plasma co-cultured *M. tuberculosis* versus time-to-detection of control growth was defined as a TDA ratio. TDA assays were later performed using the subject's own *M. tuberculosis* isolate and C_{2h} plasma from the Tanzanian cohort and compared to drug levels and clinical outcomes.

Results. Sixteen subjects were enrolled with a mean age of 37.8 years ± 10.7. Fourteen (88%) had C_{2h} rifampin levels and 11 (69%) had isoniazid levels below 90% of the lower limit of the expected range. Plasma spiked with varying concentrations of anti-tuberculous medications found TDA to be unaffected by ethambutol or pyrazinamide. Yet with a range of isoniazid and rifampin concentrations, TDA exhibited a statistically significant correlation with drug level and drug MIC, and a TDA of ~1.0 identified multidrug-resistant TB. In Tanzania, low (ffi2.0) TDA was significantly associated with both lower isoniazid and rifampin C_{2h} levels, and very low (ffi1.5) TDA corresponded to a trend toward lack of cure.

Conclusions. Study of TDA compared to additional clinical outcomes and as a therapeutic management tool is warranted.

Introduction

Tuberculosis (TB) is the leading cause of death from a curable infectious disease worldwide, and resource-limited settings bear a disproportionate burden of TB prevalence and poor treatment outcomes [30]. Even in TB patients receiving appropriate multidrug therapy, treatment outcomes can be poor due to immunosuppressive co-morbidities, delayed presentation to medical care, and impaired adherence to treatment requirements but can also be secondary to inadequate pharmacotherapy. Peak plasma levels (estimated C_{max}) of anti-tuberculosis drugs below the expected range occur commonly in patients, yet the exact role of low drug levels in treatment outcome is not fully understood [2,3,11,13-15]. Given that the majority of patients with TB reside in resource-limited settings, widespread application of drug level monitoring is impractical and too costly [2, 14]. Thus, alternative means of identification of patients possibly at risk of poor treatment outcome due to low drug levels, and strategies to optimize existing drug regimens, are of critical research importance [6, 21, 22].

We therefore developed an assay that could potentially determine the impact of low drug levels and serve as an accessible clinical tool. The assay uses a patient's plasma or serum collected during TB treatment and their own *Mycobacterium tuberculosis* isolate and measures time-to-detection in liquid culture. The principles of the assay are derived from prior study of serum bactericidal dilutions for the management of endocarditis [19]. Results are reported as a ratio of time-to-detection of plasma co-cultured *M. tuberculosis* to the time-to-detection of *M. tuberculosis* alone. We modeled the assay on the work by Wallis et al. in whole-blood culture studies [25-27] in which time-to-detection in the Bactec MGIT system (Becton Dickinson, Sparks, MD) is used in replacement of conventional colony counting. Others have found a 1-log decrement in bacilli to be approximately equivalent to a 1.2 to 1.3-fold increase in time-to-detection [4,18]. We additionally pursued the MGIT system for its ease of reproducibility and current WHO-endorsed suitability for scaling up for use in intermediate-volume laboratories in resource-limited areas. Furthermore, we utilized plasma or serum without leukocytes to constrain analysis to drug effects.

Materials and methods

Tanzania

We first sought to describe the extent of low drug levels in a population of patients starting treatment in a setting of high TB prevalence and at relatively low risk of malabsorption. Subjects initiating TB treatment at Kibong'oto National TB Hospital

(KNTH) in Kilimanjaro, Tanzania, were recruited for enrollment. Inclusion criteria specified subjects ≥18 years of age who had no prior TB treatment history, were HIV-negative, and had newly diagnosed sputum smear-positive pulmonary TB. Per KNTH protocol, all subjects received fixed dose combination tablets that included isoniazid (INH) (75 mg), rifampin (RMP) (150 mg), ethambutol (EMB) (275 mg), and pyrazinamide (PZA) (400 mg) based on weight: for patients who weighed <50 kg, 3 tablets were administered; and for patients who weighed ≥50 kg, 4 tablets were administered. Subjects who had any recent history of nausea, vomiting or diarrhea were excluded. Specimen processing and analysis was performed at Kilimanjaro Christian Medical College (KCMC) in Moshi, Tanzania. Written consent was obtained from all subjects and protocols were approved by the Institutional Review Boards of Tumaini University at KCMC and the University of Virginia.

Prior to the initiation of TB treatment, sputum was collected for culture in the automated Bactec MGIT 960 system. Standard analyses of drug-susceptibilities to INH, RMP, EMB and streptomycin were performed with the Bactec SIRE kit to detect critical concentrations of INH of (0.4 μg/ml), RMP (1.0), EMB (5.0), and streptomycin (1.0). Blood was collected at 14 days following TB treatment initiation and at 2 hours after observed administration of all anti-tuberculosis medications. Subjects were served a meal of porridge approximately 1 hour before medication administration per hospital routine. Blood was transported on ice to KCMC, where plasma was separated from the blood draw within 2 hours and stored at -80°C for shipment to Radboud University, Nijmegen Medical Centre in the Netherlands, where high-performance liquid chromatography (HPLC) measurement for INH, RMP, EMB and PZA were performed by validated methods. For INH, measurement of the acetyl-INH level was also performed to determine the acetylator phenotype. A plasma acetyl-INH/INH ratio >1.0 was categorized as representing a "fast" metabolizer of INH and a ratio ≤1.0 as representing a "slow" metabolizer [22]. An intermediate INH acetylator phenotype was not described, as, when the assay had been performed previously, it had required blood draws at additional time points following dose administration. Drug levels were compared against established C_{2h} reference ranges and categorized as "low" if below 90% of the expected lower limit [12,15]. Subjects were re-evaluated at 2 months and 6 months for sputum smear conversion, change in weight, subjective improvement based on assessment of a local TB clinician blinded to study results, and mortality. TB drug activity (TDA) testing was later performed at KCMC with the patient's own *M. tuberculosis* isolate and plasma.

TB Drug Activity (TDA) assay development

Concentrations within and below the expected C_{2h} range for INH and RMP (Sigma-Aldrich, St. Louis, MO, USA) were spiked into plasma of a healthy, tuberculin skin

test-negative, volunteer. Expected C_{2h} concentrations of EMB (MP Biomedicals, Solon, OH, USA) and PZA (BD Diagnostic System, Sparks, MD, USA) were also added for further comparison. INH, EMB and PZA were dissolved in sterile distilled water; RMP in dimethyl sulfoxide.

The *M. tuberculosis* isolates were H37Rv (ATCC 27294), two recent clinical isolates susceptible to INH, RMP, EMB and PZA, and two recent clinical isolates resistant to INH and RMP (referred to here as multidrug-resistant [MDR-TB] isolates). For susceptibility testing, a 1.0 McFarland suspension was made, and 10^{-2} and 10^{-4} were inoculated onto Middlebrook 7H10 agar with and without drug and incubated at 35°C. Conventional testing was carried out according to the 1% proportions method using established critical concentrations for INH, RMP and EMB [31]. Serial dilutions of INH, RMP and EMB were used to establish the MIC, defined as the lowest concentration of drug that inhibited more than 99% of the bacterial population after 21 days from inoculation. PZA susceptibility testing was performed in PZA-specific Bactec MGIT media.

TDA assays were performed by adding a 500 µl suspension of a 10^{-1} dilution of 0.5 McFarland for each *M. tuberculosis* isolate to a 2 ml screw-top tube; the suspension was centrifuged at 12,000 rpm for 10 min at room temperature, the supernatant removed, and 300 µl of PBS added and followed by 300 µl of plasma. Tubes were incubated for 72 hours at 37°C and then centrifuged at 12,000 rpm for 5 minutes, and the supernatant removed. A 1-ml volume of sterile distilled water was then added with repeat vortexing, prior to final centrifugation at 12,000 rpm for 10 minutes. The supernatant was discarded, 500 µl of Middlebrook 7H9+ 10%OADC was added, and the mixture was subjected to vortexing, transferred to a prefilled 7-ml MGIT tube, and incubated in the MGIT 320 machine until time to detection. For control tubes, identical 500 µl inocula were added for each of the isolates and incubated until the time to detection was determined. TDA was reported as the ratio of the time-to-detection of plasma co-cultured TB in hours to time-to-detection of control. All experiments were approved by the Institutional Biosafety Committee of the University of Virginia.

Statistics

For all analyses, means were compared using *t* test or medians by a Mann-Whitney test for nonparametric data. All *M. tuberculosis* TDA cultures were performed in duplicate or triplicate, except in Tanzania where they were performed once. The correlation between TDA ratios and C_{max} / MIC ratios for INH or RMP was calculated using the Pearson coefficient. TDA ratios were compared to clinical characteristics and outcomes in the Tanzanian cohort by -square or Fisher's exact testing when appropriate. All p-values were from two-tailed tests.

Results

Tanzania: plasma drug levels

A total of 16 subjects with newly diagnosed smear-positive pulmonary TB were evaluated. All were inpatients and received directly observed medication administration. The mean age was 37.8 years ± 10.7, and 13 (81%) were male. The mean weight at the time of diagnosis was 48.8 kg ± 7.9. Eight (50%) of subjects were below 50 kg and hence were prescribed 3 fixed-dose combination tablets. Drug-susceptibility testing revealed 15 of the subject's isolates to be susceptible to INH, RMP, EMB and streptomycin. One subject's isolate was resistant to EMB only.

HPLC testing demonstrated the majority of patient's plasma to have very low levels of INH, RMP and EMB [Table 1]. Fourteen (88%) had low C_{2h} levels of RMP (expected C_{2h} range 8-24 μg/ml), with a mean of 2.5 ± 2.9 μg/ml. Two patients had trace RMP levels below the limit of quantification. Eleven (69%) patients had low C_{2hr} levels of INH (expected C_{2hr} range 3-5 μg/ml) with a mean of 1.85 μg/ml ± 1.3. Acetyl-INH testing revealed a fast acetylator phenotype in 6 (38%) patients. Of patients with low C_{2hr} levels of INH, 3 (27%) were fast acetylators compared to 1 (20%) of those with levels in the expected range (p= not significant [NS]). An additional 12 (75%) patients had low C_{2h} levels of EMB (expected C_{2h} range 2-6 μg/ml) with a mean of 1.25 ± 0.8 μg/ml. Drug levels were not explained by the concentrations of medication administered. For instance, of the 8 patients with the highest RMP dose (ffl11.0 mg/kg), 5 (63%) had RMP C_{2h} levels <1 μg/ml.

Development of the TDA assay

We postulated that the TDA would predominantly reflect the activity of INH and RMP, since pyrazinamide (PZA) is inactive at the pH of standard MGIT media and EMB is largely bacteriostatic [23]. To examine this supposition, we compared the mean TDA for plasma without drug to the mean TDA for plasma spiked with EMB at 5 μg/ml (expected C_{2h} range 2-6 μg/ml) and plasma spiked with PZA at 30 μg/ml (expected C_{2h} range 20-50 μg/ml) (see Fig.S1 in the supplemental material). For the drug-susceptible clinical isolates (isolates 1 and 2) and H37Rv, the EMB MICs were 2.5 μg/ml for each. Mean TDA values were 0.99 ± 0.05 for plasma across all isolates, 1.16 ± 0.1 with addition of EMB and 0.9 ± 0.16 with the addition of PZA (p=NS).

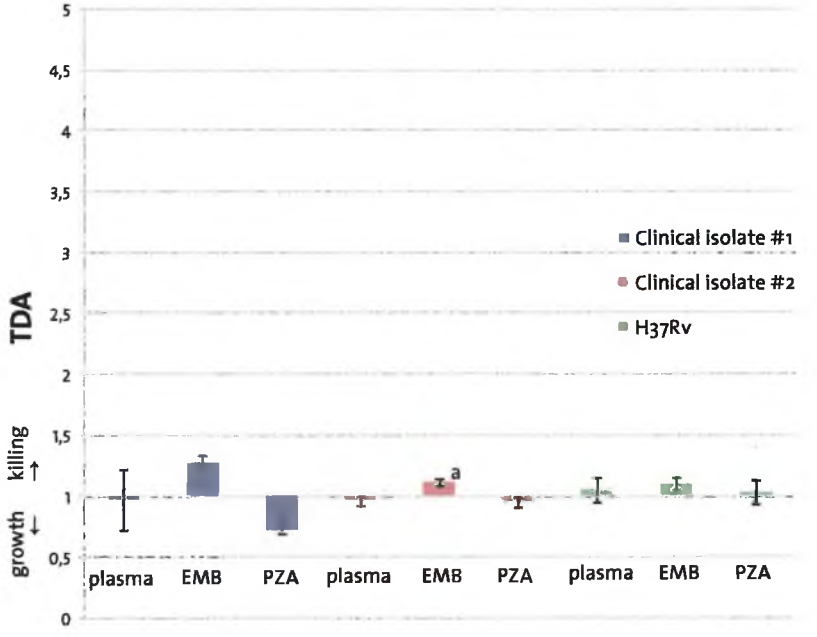
Given the finding of minimal to no activity of EMB and PZA, we sought to examine the effects of INH and RMP. Concentrations of INH (0-5 μg/ml) and RMP (0-16 μg/ml) starting from below the expected C_{2h} range to the highest in the expected range were studied in checkerboard format (Fig. 1). A dose-response relationship was observed in which higher plasma concentrations of drug resulted in a measurable

Table 1 C_{2hr} levels of anti-tuberculosis drugs and dose received from fixed pill combination in Tanzanian subjects at 14 days of treatment for pulmonary TB

Study number	Weight kg	INH mg/kg	C_{2hr} INH	INH acetylator	RMP mg/kg	C_{2hr} RMP	EMB mg/kg	C_{2hr} EMB	PZA mg/kg	C_{2hr} PZA
1	52	5.8	2.5	slow	11.5	7.9	21.1	2.9	30.7	33
2	41	5.5	1.3	slow	11.0	0.19	20.1	0.70	29.3	17
3	45	5.0	2.8	fast	10.0	7.8	18.3	2.5	26.7	38
4	66	4.5	0.45	fast	9.1	0.17	16.6	0.53	24.2	17
5	49	4.6	3.6	slow	9.2	6.1	16.8	2.1	24.5	32
6	41	5.5	1.2	slow	11.0	0.17	20.1	0.66	29.3	20
7	40	5.6	0.5	fast	11.3	0.48	20.6	0.76	30.0	20
8	51	5.8	0.25	slow	11.8	trace	21.6	0.36	31.3	7.7
9	60	5.0	1.6	slow	10.0	0.97	18.3	1.6	26.7	24
10	51	5.8	1.2	slow	11.8	0.79	21.6	0.99	31.3	26
11	52	5.7	3.6	slow	11.5	3.8	21.2	1.2	30.8	30
12	51	5.8	4.2	slow	11.8	4.0	21.6	1.0	31.3	34
13	58	5.2	0.23	fast	10.3	trace	19.0	0.55	27.6	8
14	42	5.4	1.9	slow	10.7	0.75	19.6	1.0	28.6	19
15	37	6.1	3.2	slow	12.1	6.2	22.3	2.5	32.4	36
16	45	5.0	1.1	slow	10.0	0.23	18.3	0.71	26.7	17

[a] The fixed-dose tablets administered included isoniazid (INH) (75 mg), rifampin (RMP) (150mg), ethambutol (EMB) (275 mg), and pyrazinamide (PZA) (400 mg); for patients weighing <50 kg, 3 tablets were given, and for those weighing ffl50 kg, 4 tablets were given. Values shown in bold are within 90% of the expected $C_{2\,h}$ range (reported in micrograms per milliliter). Expected $C_{2\,h}$ range for INH, 3 to 5 mg/ml; for RMP, 8 to 24 mg/ml; for EMB, 2 to 6 mg/ml; for PZA, 20 to 50 mg/ml. INH acetylator phenotype values represent ratios of plasma levels of acetyl-isoniazid to those of isoniazid. Values greater than 1.0 were categorized as representing fast acetylation; values less than or equal to 1.0 were categorized as representing slow acetylation.

increase in TDA. Keeping a single drug concentration constant at the lower range of normal but increasing the alternate drug produced a significantly increased TDA. However, the response attributable to INH or RMP was more pronounced in the isolates for which the MICs of those agents were lower (Fig. 1). For example, the mean change in TDA upon increasing INH from 0 to 5.0 μg/ml across fixed RMP concentrations was 1.48 ± 0.36 for isolate 1 (INH MIC <0.03 μg/ml; RMP MIC, 0.25 μg/ml), but was only 0.95 ± 0.1 for isolate 2, which had a higher INH MIC (INH MIC, 0.06 μg/ml, RMP MIC, 0.5 μg/ml) (p=0.03). The effect of altering the RMP concentration was more pronounced; the mean change in TDA resulting from increasing RMP from 0 to 16.0 μg/ml across fixed INH concentrations was 1.95 ± 0.35 for isolate 1, but was only 0.53 ± 0.15 for the isolate 2, for which the RMP MIC was higher (p<0.001). Indeed, combinations that included RMP at 8.0 μg/ml or greater were completely sterilizing for the laboratory isolate H37Rv tha had the lowest RMP MIC (Fig. 1c). As predicted, there was no significant change in TDA for isolates 1 and 2 when EMB at 5 μg/ml was added to combinations of INH of 3 μg/ml plus RMP of 8 μg/ml or INH of 5 μg/ml plus RMP 16 μg/ml (data not shown).



Supplemental figure 1 TB drug activity (TDA) for ethambutol and pyrazinamide for drug-susceptible *M. tuberculosis* isolates.

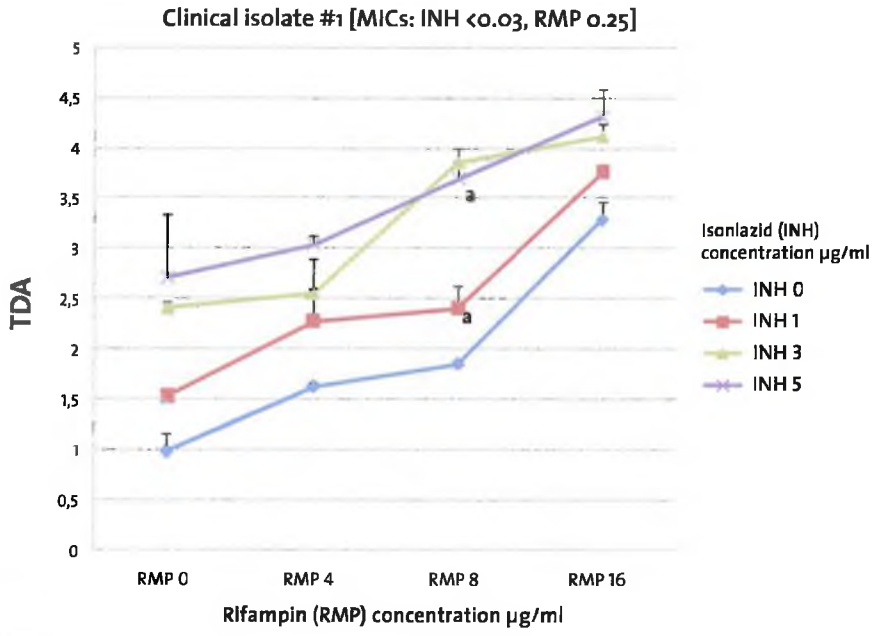


Figure 1a

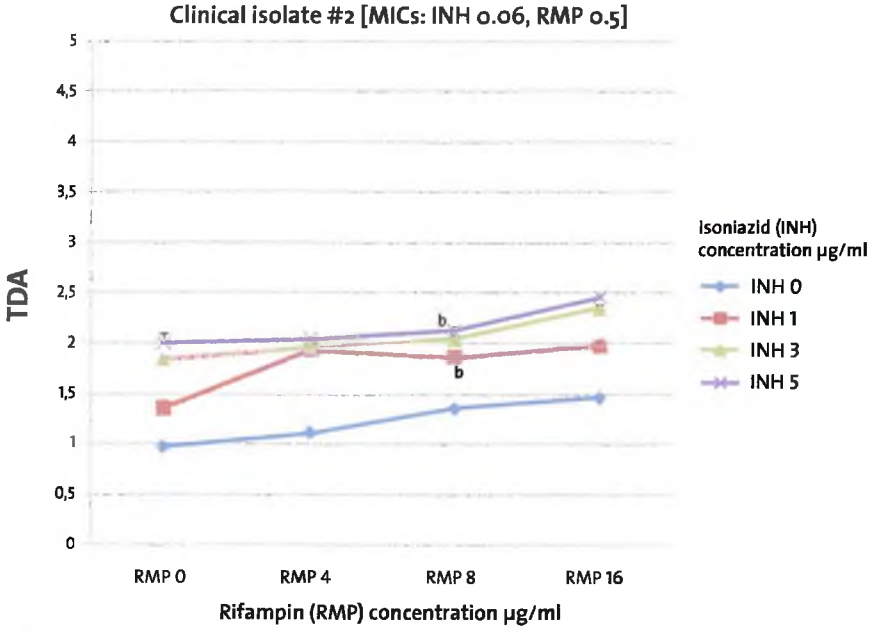


Figure 1b

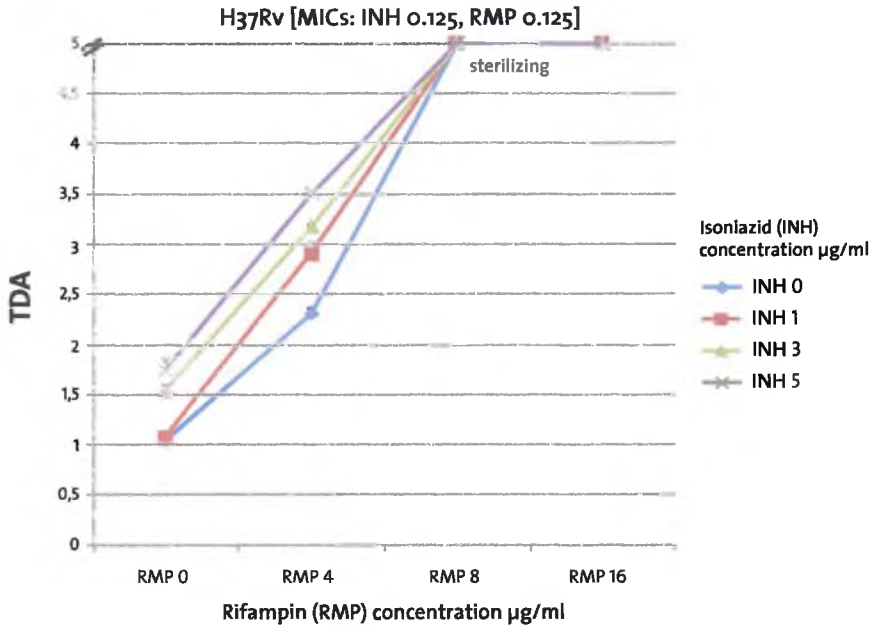


Figure 1c

FIG. 1. Comparison of anti-TB drug activities (TDA) of isoniazid and rifampin concentrations tested with the *M. tuberculosis* clinical isolates and laboratory strain H37Rv presented in checkerboard format. TDA assays were performed using *M. tuberculosis* isolates and volunteer plasma alone or plasma spiked with various concentrations of isoniazid (INH) or rifampin (RMP), including concentrations below the expected $C_{2\,h}$ range (INH, 1 mg/ml; RMP, 4 mg/ml), in the low-normal range (INH, 3 mg/ml; RMP, 8 mg/ml), and in the high-normal range (INH, 5 mg/ml; RMP, 16 mg/ml). The expected C_{max} range for INH was 3 to 5 mg/ml and for RMP was 8 to 24 mg/ml. TDA was reported as a ratio of time to positivity of plasma-cocultured *M. tuberculosis* versus time to detection of *M. tuberculosis* alone, where a TDA ratio of 1.0 indicates stasis, a TDA ratio of >1.0 indicates killing, and a TDA ratio of <1.0 indicates growth. All isolates were susceptible to INH and RMP according to results determined by the 1% proportions method. For the combination of INH at 1 µg/ml with RMP at 8 µg/ml and INH at 5 µg/ml with RMP at 8 µg/ml, the mean TDA levels were 2.55 6 0.06 and 4.11 6 0.18 for isolate 1 (*P* 5 0.008) (panel a) and 1.86 6 0.02 and 2.12 6 0.06 for isolate 2 (*P* 5 0.03) (panel b). For H37Rv, RMP concentrations of µ8.0 mg/ml were sterilizing.

We then examined the extent to which TDA correlated with INH or RMP drug levels. Since both INH and RMP affect TDA, for this analysis we examined these drugs in isolation. While a statistically significant correlation between TDA and drug level for both INH and RMP was observed (Fig. 2a and c], the correlation was improved when TDA was analyzed against INH level/MIC or RMP level/MIC, respectively, and was highest for the latter (Fig. 2b and d]. Thus, the TDA assay yielded a metric of both drug level and MIC, particularly for RMP level/MIC.

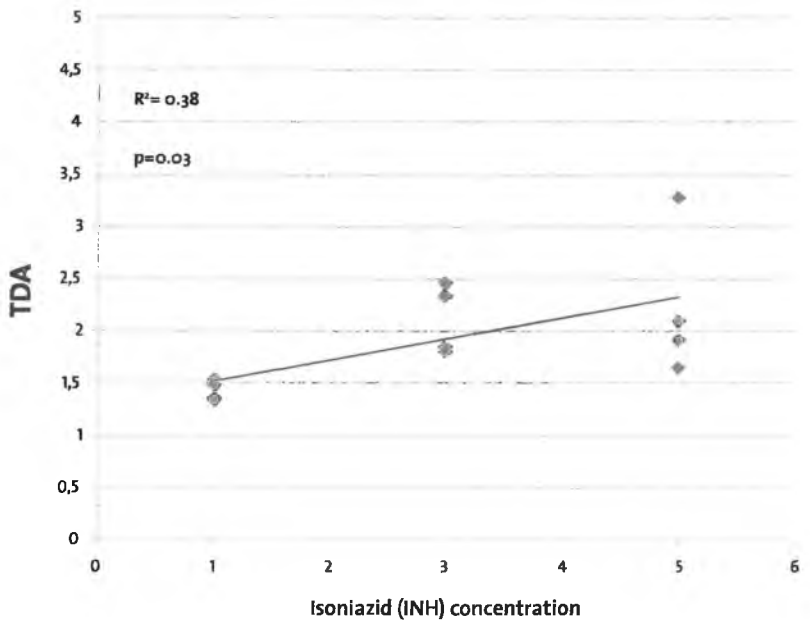


Figure 2a

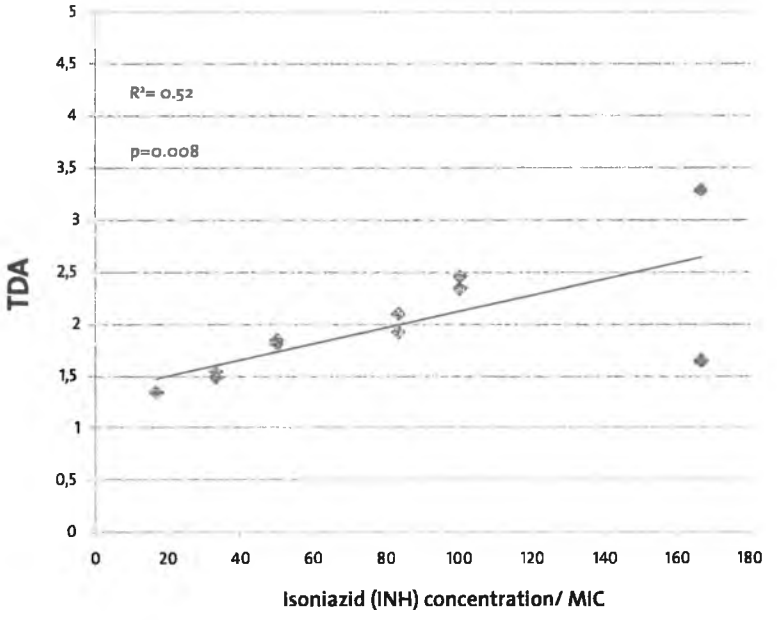


Figure 2b

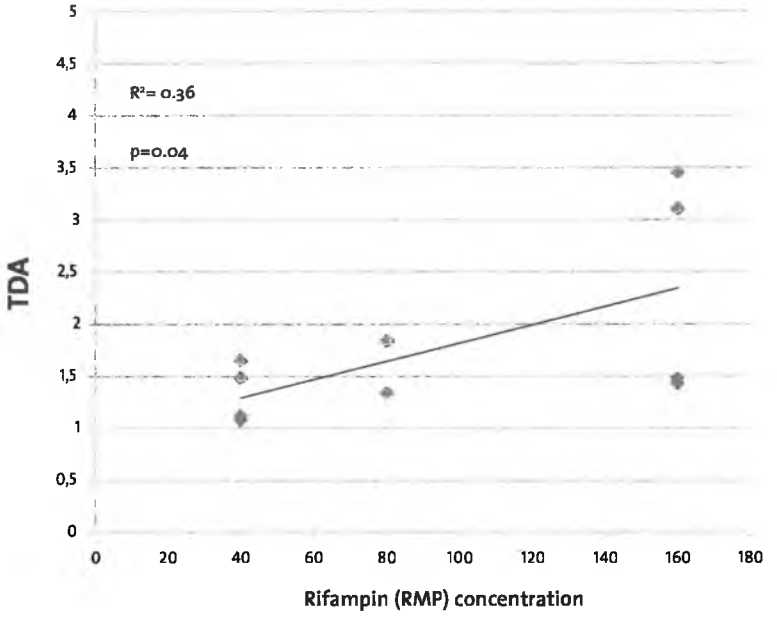


Figure 2c

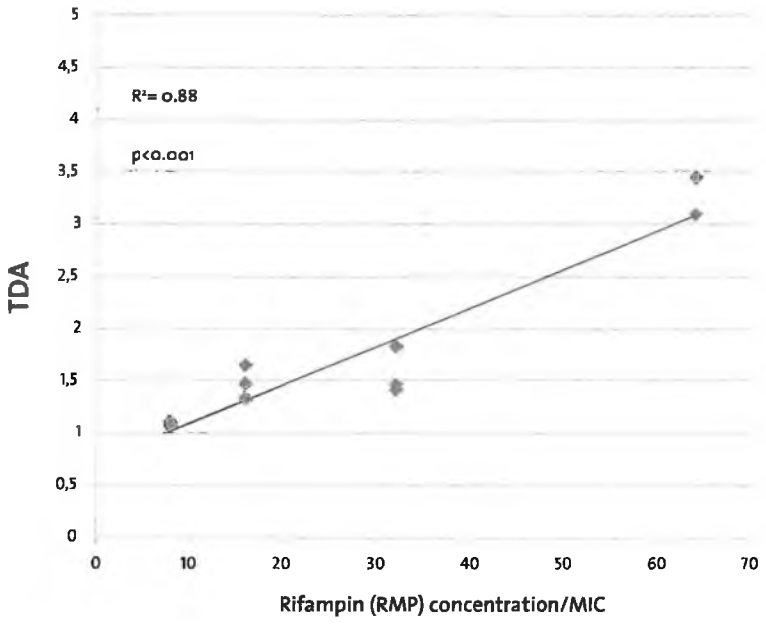


Figure 2d

FIG. 2. Comparison of TB drug activity (TDA) to isoniazid and rifampin drug concentrations and drug concentrations/MIC. TDA assays were performed using plasma spiked with isoniazid (INH) or rifampin (RMP) in isolation for clinical isolates. TDA was compared to INH concentration (a), INH concentration/MIC (b), RMP concentration (c), and RMP concentration/MIC (d). Correlation determinations were performed using the Pearson coefficient

Tanzania: TDA assay and clinical outcomes

We then sought to examine TDA for the 16 subjects from Tanzania on 4-drug therapy. TDA assays were performed onsite in Tanzania where MIC testing was not available. The mean time-to-detection for control tubes among all isolates was 146.4 hours ± 40.8, and the mean TDA ratio for all isolates was 1.9 ± 0.5 (range 1.1 to 3.2). No patient's plasma was completely sterilizing and low values were common. For instance 5 (31%) subjects had a TDA of ffi 1.5, which was clearly at the lowest end of our in vitro results.

In subjects with low TDA (ffi2.0), statistically lower mean concentrations of INH, RMP and EMB were found compared to those with TDA >2.0 (Table 2). Furthermore, in

analyzing subjects with the lowest TDA (ffi1.5), 3 (60%) converted to smear negative compared with 10 (91%) subjects with TDA>1.5 (p=0.17); and 2 (40%) were cured (sputum smear negative at 5 months and 6 months of medication completed), and 10 (91%) were cured (p=0.06). Mean weight gain at 6 months was 5.6 kgs ± 4.4 versus 7.4 ± 4.5 in the two groups, respectively (p=0.1), and mortality was 0% in the cohort.

Table 2 TB drug activity (TDA) values and C_{2h} drug levels at 14 days of TB treatment in Tanzanian patients[a]

C_{2hr} drug levels µg/ml	TDA ffi2.0 N=9	TDA >2.0 N= 7	p-value
Isoniazid mean level ± SD	1.31 ± 1.2	2.56 ±1.2	p=0.05
Rifampin mean level ± SD	0.77 ± 1.3	4.65 ± 3.2	p=0.005
Ethambutol mean level ± SD	0.83 ± 0.37	1.68 ± 0.93	p=0.03
Pyrazinamide mean level ± SD	20.3 ± 7.3	28.0 ± 10.7	p=0.11

[a]Plasma used for C_{2h} drug level and TDA measurement were from same blood draw. Comparison of C_{2h} levels for isoniazid and rifampin by *t* test.

TDA performance in MDR-TB

Given that TDA assay yielded information on drug concentration/MIC, we predicted it should indicate MDR-TB, for which MICs are extremely high. In testing a range concentrations of INH and RMP in plasma against two MDR-TB isolates, TDA values were near 1.0 and were never observed above 1.19, even when EMB was added to EMB-susceptible MDR TB isolate 1 (Fig. 3). In Tanzania, two locally identified strains of MDR-TB were used for testing plasma from four of the previously enrolled patients on a drug-susceptible regimen (Fig. 4). Each strain was susceptible to EMB. Plasma from two patients (patients 10 and 11) was tested against MDR-TB isolate A and plasma from two other patients (patients 1 and 2) was tested against MDR-TB isolate B. These plasma samples exhibited a mean TDA of 0.92 ± 0.03 against the MDR-TB isolates, versus 2.0 ± 0.38 with their own susceptible isolates (p<0.001). The individual who produced MDR-TB isolate B was able to be subsequently enrolled while receiving ethambutol, pyrazinamide, amikacin, levofloxacin and cycloserine and his TDA on this regimen was 2.2 (Fig. 4).

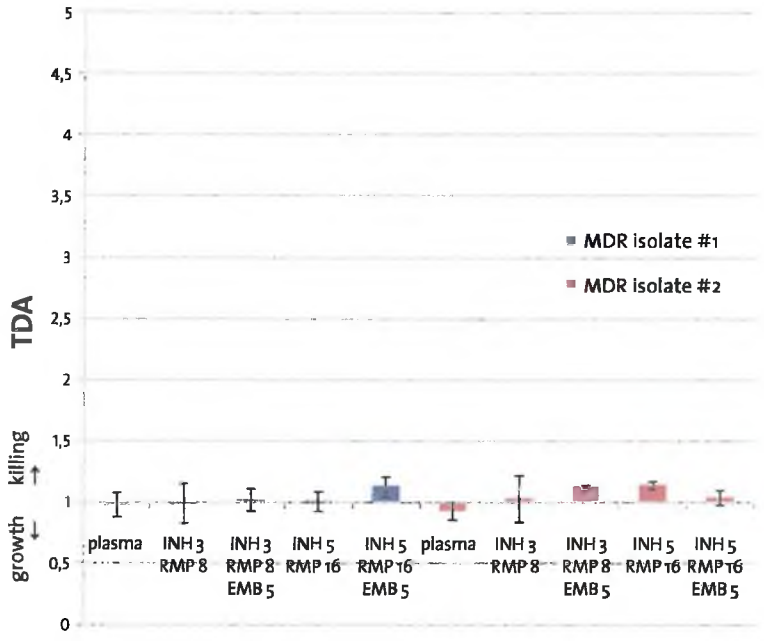


Figure 3 TB drug activity (TDA) with plasma alone compared to concentrations of isoniazid, rifampin, and ethambutol for multidrug-resistant (MDR) *M. tuberculosis* isolates. TB drug activity (TDA) ratios were determined for volunteer plasma without drug versus plasma spiked with a various concentrations of isoniazid (INH), rifampin (RMP), and ethambutol (EMB) within the expected $C_{2\,h}$ range. The drug MICs for MDR isolate 1 were as follows: INH, 32 µg/ml (100% resistant as determined by the proportions method); RMP, 32 µg/ml (100% resistant); EMB, 5 mg/ml (<1% susceptible). The drug MICs for MDR isolate 2 were as follows: INH, 16 µg/ml (63% resistant); RMP, 16 µg/ml (77% resistant); EMB, 10 µg/ml (27% resistant).

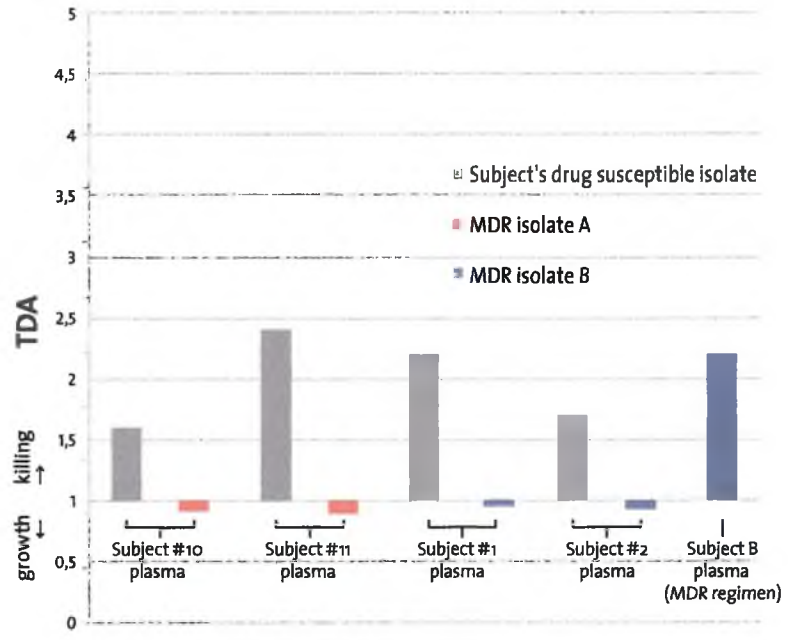


Figure 4 Use of TB drug activity (TDA) determinations in MDR-TB treatment. Plasma from subjects 1, 2, 10, and 11 under treatment for drug-susceptible TB with a regimen of isoniazid, rifampin, ethambutol, and pyrazinamide administration was tested using the TDA assay and recent MDR-TB isolates from Kibong'oto National TB Hospital in Tanzania. In addition to the initial 16 subjects with drug-susceptible TB, subject B produced MDR-TB isolate B and had a TDA assay performed while on an MDR-TB regimen of ethambutol, pyrazinamide, amikacin, levofloxacin, and cycloserine.

Discussion

The major result of this work is the development of an assay that can provide individualized measure of INH and RMP activity in TB patients on treatment. Importantly, low circulating drug levels of INH and RMP confer a cost of less killing that that is quantifiable *in vitro,* particularly for isolates with higher MICs still considered susceptible by conventional testing. Therefore, TDA may provide an adjunctive tool to optimize TB therapy in certain patients.

The fact that TDA represents a metric whose determinations are largely constrained to INH and RMP activities in patients on a typical 4-drug regimen is in our view

fortunate, since these are the medications most important in therapeutic outcome, most often continued throughout the entirety of the treatment course, and most likely to be dose adjusted. Indeed, in our experience, INH and RMP doses can be increased for low levels without toxicity and to within the expected range with a single adjustment, which may be of clinical benefit in patients with slow response to TB therapy [11]. A growing body of evidence suggests that RMP in particular may be at the lower end of the dose response curve, and reports are emerging that higher dose RMP may be able to inhibit strains with low-level resistance (MIC~1.0-2.0 μg/ml) [5,9,20,24]. Determinations of TDA provide some substantiation for dose adjustment through enhanced killing and increase in C_{max}/MIC, especially for strains that are highly INH or RMP susceptible.

Interestingly, TDA was integrally dependent upon the *M. tuberculosis* isolate to INH and RMP MICs for *M. tuberculosis* isolate. While not surprising for conventionally "resistant" isolates, this result is not altogether obvious for isolates deemed "susceptible" as low drug levels remain above the MIC. Thus, this work suggests a possible role for MIC testing among susceptible isolates, particularly when dose adjustment is being considered. As regimens based on higher dose RMP are being studied in clinical trials [24], MIC testing may aid interpretation of results.

Overall, the TDA values observed in this Tanzanian cohort were surprisingly low. Values reached as low as 1.1, which *in vitro* was akin to having MDR-TB. The low TDA was presumably due to subjects having very low C_{2h} INH and RMP levels and is particularly worrisome, considering that potential subjects who had HIV or gastrointestinal symptoms that would have predisposed to malabsorption were excluded [15,30,31]. Few studies have examined drug levels in similar African settings, but in a cohort from Botswana that included HIV infected patients, a similar 88% had low C_{max} levels of RMP [3]. Unfortunately, determining which subjects have low INH or RMP levels is not readily predictable: in our study the fast acetylator phenotype did not account for the majority of those with low INH levels, and there was no association with initial dosing concentration for other medications based on standard fixed-dose combination. These findings reinforce the need for individualized patient management tools such as TDA.

Additionally, we envision a role for TDA in the management of MDR-TB but that necessitates further study. As there are no randomized trials to guide the treatment of MDR-TB with currently available drugs [7] and as the optimal treatment duration is unknown [8], whether patients with favorable clinical improvement can be treated for a shorter course of drug administration has yet to be determined [1, 17]. For a patient with known MDR-TB and limited therapeutic options, TDA may offer an

indication of therapeutic activity. For instance, since MDR patient B had a respectable TDA of 2.2 on the 5-drug regimen and we understand EMB and PZA to have little effect, TDA may represent the activity of other second-line drugs with extracellular and concentration-dependent killing.

There are several limitations to this work. The Tanzanian cohort was not designed to discern a relationship between TDA and later clinical outcomes such as relapse and acquired drug resistance. While a trend toward lack of cure at 6 months was found in subjects with the lowest TDA values, we speculate that repeated measurements may be more informative than a single value. Despite recent *in vitro* models and animal studies that suggest the area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC) RATIO may be the best pharmacodynamic index to explain RMP and INH activity, C_{max} and AUC do correlate well [10]. We also acknowledge that the use of C_{2h} plasma may not identify patients with delayed absorption and therefore drug activity in some subjects may have been underestimated [15]. Food intake may have been further delayed C_{max} for RMP and may explain why some subjects with very low C_{2h} drug levels were still able to convert their sputum smear to negative and improve symptomatically.

Furthermore, since TDA is particularly affected by certain drugs (INH, RMP), TDA determinations may underestimate the overall activity resulting from a treatment regimen and accordingly may not reveal intracellular killing levels as effectively as whole-blood culture [26]. That said, we feel that erring on the side of underestimation is safer than the alternative. Additionally, TDA levels are dependent upon circulating drug levels, which may not represent concentrations achieved at the predominant site of infection [32]. In the absence of data on MIC values, it may be difficult to determine the predominant effect of either INH or RMP within the assay for any given patient. Yet among the members of the current cohort, no subject with a TDA value of <2.0 had an RMP level within the expected range, posing the clinical issue of whether all of the members of this at-risk subset should have their RMP doses increased.
Finally, as in any study of *M. tuberculosis* culture, the populations of bacilli isolated may not have been representative of the entirety of populations within the host, and thus TDA may select for the measurement of killing of subpopulations only in rapid-growth phase.

Despite these caveats, there are precious few therapeutic management tools to bring to bear on treatment issues for individual TB patients; this is particularly so for those with poor treatment response. In this context TDA assays are accessible to laboratories capable of liquid culture experiments and may offer a useful adjunct to standard testing.

Acknowledgements

This work was supported by a National Institutes of Health grant [R01 AI093358 to E.R.H.]; a National Institutes of Health/ Fogarty training grant [D43 TW008270 to E.R.H. and G.S.K.]; and the Virginia Department of Health.

We are grateful to Dr. Robert Wallis for initial guidance in assay development.

References

1. Caminero, J.A. 2006. Treatment of multidrug-resistant tuberculosis: evidence and controversies. Int J Tuberc Lung Dis. 10:829-37.
2. Chang, K.C., C.C. Leung, W.W. Yew, K.M. Kam, C.W. Yip, C.H. Ma, C.M. Tam, E.C. Leung, W.S. Law and W.M. Leung. 2008. Peak plasma rifampicin level in tuberculosis patients with slow culture conversion. Eur J Clin Microbiol Infect Dis. 27(6):467-72.
3. Chideya, S, C.A. Winston, C.A. Peloquin CA, W.Z. Bradford, P.C. Hopewell, C.D. Wells, A.L. Reingold, T.A. Kenyon, T.L. Moeti and J.W. Tappero. 2009. Isoniazid, rifampin, ethambutol and pyrazinamide pharmacokinetics and treatment outcomes among predominately HIV-infected cohort of adults with tuberculosis from Botswana. Clin Infect Dis. 48:1685-94.
4. Diacon, A.H., J.S. Maritz, A. Venter, P.D. van Helden, K. Andries, D.F. McNeeley and P.R. Donald. Time to detection of the growth of Mycobacterium tuberculosis in MGIT 960 for determining the early bactericidal activity of antituberculosis agents. 2010. Eur J Clin Micro Infect Dis. 29(12): 1561-65.
5. Diacon, A.H., R.F. Patientia, A. Venter, P.D. van Helden, P.J. Smith, H. McIlleron, J.S. Maritz, P.R. Donald. 2007. Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears. Antimicrob Agents Chemother. 51:2994–96
6. Dooley, K.E., and R.E. Chaisson. 2009. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect Dis. 9:737-46.
7. Espinal, M., and P. Farmer. 2009. The Cambridge Declaration: towards clinical trials for drug-resistant tuberculosis. Int J Tuberc Lung Dis. 13(1):1-2.
8. Gandhi, N.R., P. Nunn, K. Dheda, H.S. Schaaf, M. Zignol, D. van Soolingen, P. Jensen, and J. Bayona. 2010. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet. 375:1830-43.
9. Goutelle, S., L. Bourguignon, P.H. Maire, M. van Guilder, J.E. Conte, and R.W. Jelliffe. 2009. Population modeling and Monte Carlo simulation study of the pharmacokinetics and antituberculosis pharmacodynamics of rifampin in lungs. Antimicrob Agents Chemother. 53:2974–81.
10. Hall, R.G., R.D. Leff, and T. Gumbo. 2009. Pharmacotherapy. 29(12):1468–1481.
11. Heysell, S.K., J.L. Moore, S.J. Keiler, and E.R. Houpt. 2010. Therapeutic drug monitoring among slow responders to tuberculosis therapy in a state control program, Virginia, USA. Emerg Infect Dis. 16:1546-53.
12. Holland, D.P., C.D. Hamilton, A.C. Weintrob, J.J. Engemann, E.R. Fortenberry, C.A. Peloquin, and J.E. Stout. 2009. Therapeutic drug monitoring of antimycobacterial drugs in patients with both tuberculosis and advanced human immunodeficiency virus infection. Pharmacotherapy. 29:503–10.
13. Mehta, J.B., H. Shantaveerapa, R.P. Byrd, S.E. Morton, F. Fountain, and T.M. Roy. 2001. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest. 120:1520–4.
14. Narita, M., M. Hisada, B. Thimmappa, J. Stambaugh, E. Ibrahim, E. Hollender, and D. Ashkin. 2001. Tuberculosis recurrence: multivariate analysis of serum levels of tuberculosis drugs, human immunodeficiency virus status, and other risk factors. Clin Infect Dis. 32(3):515-7.
15. Peloquin, C.A. 2002. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs. 62:2169–83.
16. Peloquin, C.A., R. Namdar, M.D. Singleton, and D.E. Nix. 1999. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest. 115: 12-18.
17. Perez-Guzman, C., M.H. Vargas, L.A. Martinez-Rossler, A. Torres-Cruz, and H. Villarreal-Velarde. 2002. Results of a 12-month regimen for drug-resistant pulmonary tuberculosis. Int J Tuberc Lung Dis. 6:1102-9.
18. Pheiffer, C., N.M. Carroll, N. Beyers, P.R. Donald, K. Duncan, P. Uys, and P. van Helden. 2008. Time to detection of Mycobacterium tuberculosis in BACTEC systems as a viable alternative to colony counting. Int J Tuberc Lung Dis. 12(7): 792-98.
19. Reller, L.B., and C.W. Stratton. 1977. Serum Dilution Test for Bactericidal Activity. II. Standardization and Correlation with Antimicrobial Assays and Susceptibility Tests. J Infect Dis. 136:196-204.
20. Ruslami, R., H. Nijland, R. Aarnoutse, B. Alisjahbana, A.Y. Soeroto, S. Ewalds, and R. van Crevel. 2006. Evaluation of high- versus standard-dose rifampin in Indonesian patients with pulmonary tuberculosis. Antimicrob Agents Chemother. 50:822–33.

21. Rylance, J., M. Pai, C. Lienhardt, P. Garner. 2010. Priorities for tuberculosis research: a systematic review. Lancet Infect Dis. 10:886-92.
22. Seifert, H.I., D.P. Parkin, F.J. Botha, P.R. Donald, and B.J. van der Walt. 2001. Population screening for isoniazid acetylator phenotype. Pharmacoepidemiol Drug Saf. 10: 127-34.
23. Srivastava, S., S. Musuka, C. Sherman, C. Meek, R. Leff, and T. Gumbo. 2010. Efflux-pump–derived multiple drug resistance to ethambutol monotherapy in *Mycobacterium tuberculosis* and the pharmacokinetics and pharmacodynamics of ethambutol. J Infect Dis. 201:1225–31.
24. van Ingen, J., R.E. Aarnoutse, P.R. Donald, A.H. Diacon, R. Dawson, G. Plemper van Balen, S.H. Gillespie, and M.J. Boeree. 2011. Why do we use 600 mg of rifampicin in tuberculosis treatment? Clin Infect Dis. 52:e194–e199.
25. Wallis, R.S., M. Palaci, S. Vinhas, A.G. Hise, F.C. Ribeiro, K. Landen, S.H. Cheon, H.Y. Song, M. Phillips, R. Dietze, and J.J. Ellner. 2001. A whole blood bactericidal assay for tuberculosis. J Infect Dis. 183:1300–3.
26. Wallis, R.S., A.V. Solange, J.L. Johnson, F.C. Ribeiro, M. Palaci, R.L. Peres, R.T. Sa, R. Dietze, A. Chiunda, K. Eisenach, and J.J. Ellner. 2003. Whole blood bactericidal activity during treatment of pulmonary tuberculosis. J Infect Dis. 187:270–8.
27. Wallis, R.S., S. Vinhas, E. Janulionis. 2009. Strain specificity of antimycobacterial immunity in whole blood culture after cure of tuberculosis. Tuberculosis. 89: 221–4.
28. World Health Organization. Global tuberculosis control: a short update to the 2009 report. 2009. http://www.who. int/tb/publications/global_report/2009/update/en/index.html. Accessed 21 July 2010.
29. World Health Organization. 2008. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs.
30. Weiner, M., W. Burman, A. Vernon, D. Benator, C.A. Peloquin, A. Khan, S. Weis, B. King, N. Shah, and T. Hodge. 2003. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am J Respir Crit Care Med. 167:1341–7.
31. Weiner, M., D. Benator, W. Burman, C.A. Peloquin, A. Khan, A. Vernon, B. Jones, C. Silva-Trigo, Z. Zhao, and T. Hodge. 2005. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. Clin Infect Dis. 40:1481–91.
32. Ziglam, H.M., D.R. Baldwin, I. Daniels, J.M. Andrew, and R.G. Finch. 2002. Rifampicin concentrations in bronchial mucosa, epithelial lining fluid, alveolar macrophages and serum following a single 600 mg oral dose in patients undergoing fibre-optic bronchoscopy. J Antimicrob Chemother. 50:1011–15.

CHAPTER 6

The effect of diabetes mellitus on exposure to tuberculosis drugs in Tanzanian patients

Charles M. Mtabho, Hadija H. Semvua, Jossy van den Boogaard, Constantine F. Irongo, Martin J. Boeree, Angela Colbers, David M Burger, Reinout van Crevel, Andre J.A.M. van der Ven, Gibson S. Kibiki, Alma Tostmann, Rob E. Aarnoutse

In final preparation

Abstract

Introduction. Diabetes mellitus (DM) is a well-known risk factor for tuberculosis (TB) and is associated with poor TB treatment outcome and an increased risk of TB relapse. With the current global increase in cases of type 2 DM, attention needs to be increased on optimum treatment of TB in patients with diabetes. Previous studies examining the possible role of altered pharmacokinetic (PK) profiles of TB drugs in DM patients have met with different results, and no studies have been conducted in Africa. We performed a study to describe the PK of TB drugs in Tanzanian TB patients with and without diabetes mellitus.

Methods. We collected blood samples from 20 diabetic TB patients and a control group of 20 non-diabetic TB patients during the intensive phase of TB treatment at a Tanzanian out-patient TB clinic. A full pharmacokinetic profile (0-24 hours) was determined for isoniazid, rifampicin, pyrazinamide and ethambutol, and plasma concentrations were measured using validated HPLC methods. PK parameters were calculated using non-compartmental methods and were compared using the independent samples T-test on log-transformed data. Multiple linear regression analysis was performed to assess the effect of other exploratory variables on the PK of TB drugs.

Results. The geometric mean exposure (AUC_{0-24}) to rifampicin and isoniazid were lower in diabetic than non-diabetic TB patients (39.9 versus 29.9 h*mg/L for rifampicin, p=0.052; 5.4 versus 10.6 h*mg/L for isoniazid, p=0.015). C_{max} of isoniazid was also lower in diabetic TB patients (1.7 versus 2.8 mg/L, p=0.010). 47% versus 35% of patients had C_{max} of rifampicin below the reference range in the diabetic TB group as compared to the non-diabetic TB group, and for isoniazid the proportions were 74% against 55%. Fast and slow acetylators for isoniazid were not statistically different in proportion between diabetic and non-diabetic TB patients. In a multiple linear regression analysis with age, sex, dose per kg, HIV status and acetylator status as additional explanatory variables, DM remained an independent predictor of the PK of isoniazid and rifampicin, next to acetylator status for isoniazid. Fasting blood glucose also predicted exposure to isoniazid and rifampicin. All PK parameters for pyrazinamide and ethambutol were not significantly different between diabetic and non-diabetic TB patients.

Conclusion. Exposure to isoniazid is reduced in Tanzanian diabetic patients with TB and a clear trend to a reduction in the exposure to rifampicin was observed. These effects are most likely explained by the diabetes disease. Increasing the doses of TB drugs in treating diabetic TB patients may be considered in view of accumulating evidence that exposure to TB drugs is related to response.

Introduction

Diabetes mellitus (DM) was a well-known risk factor for tuberculosis (TB) in the past, but this was largely forgotten during the second half of the 20th century, with the advent of widely available treatment for both diseases (1). With the current global increase in cases of type 2 DM, the association between TB and DM is re-emerging(2). The greatest increase in cases of type 2 DM will occur in developing countries, where TB is highly endemic (3). Patients with DM have a higher risk on developing TB(4;5). This is probably because of impaired immunity(6). On top of that, TB is more difficult to treat in diabetic patients. Recent studies have shown that TB patients with DM have a less favourable response to TB treatment as shown by higher failure, relapse, and death rates(7-9).

It has been shown that patients with DM have lower plasma concentrations of certain drugs(10;11). Apparently, absorption, distribution, metabolism and/or excretion of drugs are changed in patients with DM. If this also applies to anti-TB drugs, this may at least partly explain the slower response to TB treatment in patients with DM, as lower plasma concentrations of anti-TB drugs have been associated with clinical failure and acquired drug resistance(12-16). Recently simulations also showed that interindividual variability in pharmacokinetics of TB drugs, rather than non-adherence, causes multidrug resistant TB(17). In addition, a meta-analysis showed that variability in exposure to a single drug is associated with failure of TB therapy and acquired drug resistance(18).

In one study in Indonesia, TB patients with DM had lower rifampicin concentrations than TB patients without DM(19). In that study TB patients with diabetes and age- and sex-matched TB patients without DM were studied during the continuation phase of TB treatment. The exposure to rifampicin up to six hours post dose (area under the curve from 0-6 hours; $AUC_{0-6\ h}$) was 53% lower in patients with TB and DM, compared with patients with TB only; this was attributed to DM per se and to the higher weight of patients with DM. In a follow up study, Indonesian TB patients with and without DM in the intensive phase of treatment were matched for weight and all four TB drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) were analysed; in contrast to previous observations, no differences in drug exposure were found between the two groups of patients(20). Another study in Peruvian patients also did not find a difference in pharmacokinetic parameters between DM and non DM patients taking TB medication(21).

Clearly, these findings need more research. It needs to be explored whether DM affects TB drug concentrations in populations other than Asian and South-American.

Pharmacokinetics can differ between different races and ethnic groups, because of genetic variation in the metabolic and transporter enzymes involved(22). It is important to study this subject also in African TB patients because the African continent has the highest TB incidence and mortality rates(3). Therefore, we designed a pharmacokinetic study in northern Tanzania where we studied the effect of type 2 diabetes on the steady state pharmacokinetics of isoniazid, rifampicin, pyrazinamide and ethambutol. Tanzania is a high burden country for tuberculosis. In 2011, the incidence rate was approximately 169 per 100,000 population per year and there were about 82,000 tuberculosis cases(3). The diabetes prevalence in the Kilimanjaro Region is estimated 1-1.5%, and the prevalence is estimated to be higher in urban Tanzania, namely around 5.5%, which can at least partly be explained by differences in overweight(23).

Methods

Study design

We conducted a prospective observational pharmacokinetic study in which the plasma concentrations of the anti-TB drugs isoniazid, rifampicin, pyrazinamide and ethambutol were compared between Tanzanian TB patients with and without diabetes. The plasma concentrations were measured during the intensive phase of TB treatment. The patients were using TB treatment for drug-sensitive *Mycobacterium tuberculosis*, as prescribed by the Tanzanian National Tuberculosis Guidelines.

Study subjects

Forty adult TB patients were included in this study; 20 TB patients without diabetes were recruited at Mawenzi Hospital, an outpatient TB treatment clinic in Moshi in northern Tanzania, and 20 TB patients with diabetes were recruited from Mawenzi hospital as well as other hospitals around the region which treat TB. TB was diagnosed by these hospitals according to the Tanzanian guideline and practice. The diagnosis was based on clinical symptoms and signs, chest x-ray examination, and sputum smear microscopy. Diabetic patients were included if they had a previously established diagnosis of DM and were attending a diabetic clinic. In addition DM was confirmed at the time of pharmacokinetic sampling using WHO criteria(24) where a fasting blood glucose concentration greater than 7 mmol/L(126mg/dL) was considered to indicate diabetes.

Only patients above 18 years of age were eligible for participation. Participants gave written informed consent and the study was approved by the Tanzanian National Institute of Medical Research.

Drug treatment

Tuberculosis was treated with fixed-dose combination tablets (FDC tablets) manufactured by Sandoz, Mumbai India and donated by Novartis through the WHO Global Drug Facility (GDF) which monitors the quality of TB drugs according to stringent WHO standards. Patients with a body weight above 50 kg daily received four FDC tablets daily (i.e. 300 mg isoniazid, 600 mg rifampicin, 1600 mg pyrazinamide and 900 mg ethambutol), those below 50 kg received three FDC tablets daily (i.e. 225 mg isoniazid, 450 mg rifampicin, 1200 mg pyrazinamide and 675 mg ethambutol). Patients were all under community-based directly observed treatment. Diabetic patients were either on dietary management alone, or were treated with oral hypoglycaemic agents and/or injectable insulin.

Sample collection

The pharmacokinetic sampling took place when patients were on TB treatment for at least two weeks, given the expected steady state of the pharmacokinetic parameters at that point. On the sampling day, serial venous blood samples were collected just before, and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after observed tuberculosis drug intake. Patients fasted at least eight hours (from the preceding dinner to the next morning dose) before drug intake and took a standardised breakfast within 30 minutes after drug intake, which reflected the usual drug intake procedures in this population. The standardized breakfast consisted of a cup (125 ml) of tea with milk and sugar and either a small bowl of porridge or *maandazi*, a typical east African doughnut-like pasty that is rather fat. Plasma was separated immediately and kept frozen at -80°C until transport on dry ice to the Netherlands for bio-analysis.

Bio-analysis

The total (protein bound plus unbound) plasma concentrations of isoniazid, acetyl-isoniazid, rifampicin, desacetylrifampicin, pyrazinamide and ethambutol were assessed by validated high-performance liquid chromatography (HPLC) as described before(25;26).

Pharmacokinetic analysis

Pharmacokinetic evaluations were performed using non-compartimental methods in WinNonLin Version 4.1 (Pharsight Corp., Mountain View, California US). The highest observed plasma concentration was defined as C_{max} with the corresponding sampling time as T_{max}. The area under the plasma concentration-time curve (AUC_{0-24h}) was calculated using the linear/log-trapezoidal rule from zero up to the last concentration at 24h (C_{last}). The terminal log-linear period (log C versus t) was based on the last data points (at least three). The absolute value of the slope (β / 2.303, in which β is the first order elimination rate constant) was calculated using linear regression analysis. The elimination half-life ($t_{1/2}$) was calculated as 0.693/β. The apparent clearance of the

drug (CL/F; in which F is bioavailability) was calculated as dose/AUC_{0-24h} and the apparent volume of distribution (V_d/F) was calculated as (CL/F)/β.

Reference ranges for Cmax values were 3-5 mg/L for isoniazid, 8-24 mg/L for rifampicin, 20-50 mg/L for pyrazinamide and 2-6 mg/L for ethambutol(27).

The acetylator status for isoniazid was determined phenotypically by calculating the metabolic ratio of acetylisoniazid concentration over isoniazid concentration at 3 hours post dose. Patients with a ratio above 1.5 were considered fast or intermediate metabolisers, patients with a ratio below 1.5 were slow metabolisers(28).

Statistical analysis

Demographic data were presented as mean plus standard deviation when normally distributed and as median plus interquartile range when data were not normally distributed. Continuous variables were compared between diabetic and non-diabetic TB patients using a two-sample t-test or Mann-Whitney U test. Associations between numerical variables were determined using parametric correlation (Pearson's correlation) when data were normally distributed, and non-parametric correlation (Spearman's Rho correlation) when not normally distributed.

For pharmacokinetic parameters AUC_{0-24}, C_{max}, volume of distribution, clearance, and elimination half-life, analysis was performed on logarithm transformed data and geometric means were presented. The differences in these parameters between diabetic and non-diabetic TB patients were calculated with an independent sample T-test on the transformed data.

Tmax values were not transformed and were compared with the Mann-Whitney U test. Pearson Chi-square test was used to determine the difference in proportions of patients who reached reference peak plasma concentrations of the TB drugs.

Univariate analyses were performed in the whole group to assess the effects of age, sex, body weight, dose in mg/kg, HIV status, acetylator status, fasting plasma glucose and HbA1con the AUC_{0-24h} and C_{max} of the four first-line TB drugs.

A multivariate analysis with multiple linear regression was used to assess the effects of the explanatory variables DM, age, sex, body weight, dose in mg/kg, , HIV status and acetylator status on the log-transformed AUC_{0-24h} and C_{max} of the TB drugs by using the stepwise method with Probability-of-F-to-enter ffi 0.05 and Probability-of-F-to-remove ffl 0.10. In addition, a second regression analysis was performed in which fasting blood glucose was assessed as independent variable for the log-transformed

AUC_{0-24h} and C_{max} values. The presence of DM and fasting blood glucose cannot be assessed in one model simultaneously as these variables are highly correlated.

All statistical analyses were performed using SPSS version 20 (SPSS Inc, Chicago Il).

Results

Demographics/ baseline characteristics

We enrolled 40 subjects, 20 diabetic and 20 non-diabetic TB patients. For pharmacokinetic (PK) analysis, the data of one subject (with DM) were excluded because the patient had high concentrations for all TB drugs at time zero hour, indicating that the patient had incorrectly taken the drugs at home prior to pharmacokinetic sampling. Patient characteristics of the remaining 39 patients are summarized in table 1. Of these, 78.5% were male, 35% were HIV infected and the median age was 41 yrs. One patient had extrapulmonary TB (TB of the spine), all others had pulmonary TB.

There was no difference in body weight, body mass index (BMI), sex, and dose per kg of the TB drugs between diabetic and non-diabetic TB patients. Diabetic TB patients were older than non-diabetic TB patients (median age 49 versus 38 years). As expected, the median fasting blood glucose (FBG) was higher for diabetic TB patients than non-diabetic TB patients (15.9 versus 6.95 mmol/L). Common presenting symptoms are also shown in table. All diabetic patients had glycosylated haemoglobin (HbA_{1c}; range, 65 to 147 mmol/mol) above the target limit of 53 mmol/mol for good control.

All but one of the diabetic patients knew they had DM, and were enrolled to a diabetic clinic. Three of the diabetic patients were not currently on antidiabetic medications, two were on insulin, 11 on metformin, 10 on chlorpropamide, and six on glibenclamide. Other co-administered drugs were not known to interact with anti-TB drugs (data not shown).

Pharmacokinetic parameters

Table 2 shows the average of PK parameters in diabetic and non-diabetic TB patients and figure 1 compares the plasma concentration-time curves of TB drugs between diabetic and non-diabetic TB patients.

The average AUC_{0-24h} for rifampicin was 25% ($p=0.052$) lower in diabetic TB patients. Rifampicin clearance showed a trend for an increase ($p=0.06$) and its elimination half-life was significantly shorter in diabetic versus non-diabetic TB patients. In

Table 1 Characteristics of 20 tuberculosis (TB) and 20 TB-diabetes (TB-DM) patients in northern Tanzania

Parameter	Measure		
	TBDM	TB	p value
Sex (female, n[%])	5[25%]	4[20%]	0.705**
Age (years; median[IQR])	49(40-56)	38(30-42)	0.001*
Body weight (kg; mean[SD])	58.7(12.3)	55.7(6.4)	0.346
BMI(kg/m² mean[SD])	20.6(4.0)	19.5(2.4)	0.293
TB drugs			
Number of FDC tablets, n[%]			
3FDCs	3[15%]	5[25%]	
4FDCs	17[85%]	15[75%]	0.695**
Dose per kg (mg/kg, mean[SD])			
Rifampicin	9.8(1.4)	10.4(0.9)	0.118
Isoniazid	4.9(0.7)	5.2(0.5)	0.118
Pyrazinamide	25.5(3.8)	27.8(2.4)	0.029
Ethambutol	18(2.5)	19.1(1.7)	0.118
FBG(mmol/L, median(IQR))	15.9(13.1-18)	6.95(5.7-7.4)	<0.001*
HIV positive, n(%)	7(35%)	7(35%)	-
HBA1c(mmol/mol; mean[SD])	106.4[22.7]	-	-
Diabetes treatment			
Dietary only	3(15%)		
Metformin	11(55%)		
Chlorpropamide	10(50%)		
Glibenclamide	6(30%)		
Presenting symptoms, n(%)			
Cough	14(70%)	15(75%)	
Night sweats	14(70%)	17(85%)	
Weight loss	17(85%)	20(100%)	
Chest pain	2(10%)	2(10%)	
Anorexia	2(10%)	4(20%)	
Shortness of breath	2(10%)	-	
Fever	7(35%)	4(20%)	
Hemoptysis	3(15%)	1(5%)	

*test performed is Mann-Whitney U
**test performed is chi square

addition, time to maximum concentration (T_{max}) of rifampicin was longer in diabetic (2.1h; IQR 1-3) than non-diabetic TB patients (1.08h; IQR 0.98-2, p=0.027, table 2).

The average AUC_{0-24h} for isoniazid was 49% (p=0.015) lower in diabetic TB patients. Compared to non-diabetics, diabetic TB patients also had significantly lower values of the isoniazid maximum concentration (C_{max}, p = 0.01). Both clearance and volume of distribution were increased in diabetics compared to non-diabetics (table 2).

AUC_{0-24h} and C_{max} of pyrazinamide showed a trend to lower exposure in diabetic versus non-diabetic TB patients, yet all PK parameters for pyrazinamide as well as for ethambutol were not significantly different between diabetic and non-diabetic TB patients.

With respect to metabolite desacetylrifampicin, there appeared to be no statistically significant difference in average AUC_{0-24h} and C_{max} values or corresponding desacetylrifampicin / rifampicin ratios between diabetics and nondiabetics. Despite the lower exposure to isoniazid in diabetics, the AUC_{0-24h} and C_{max} values for metabolite acetylisoniazid were similar in diabetics and nondiabetics, resulting in nonsignificantly higher acetylisoniazid/isoniazid ratios in diabetics versus nondiabetics.

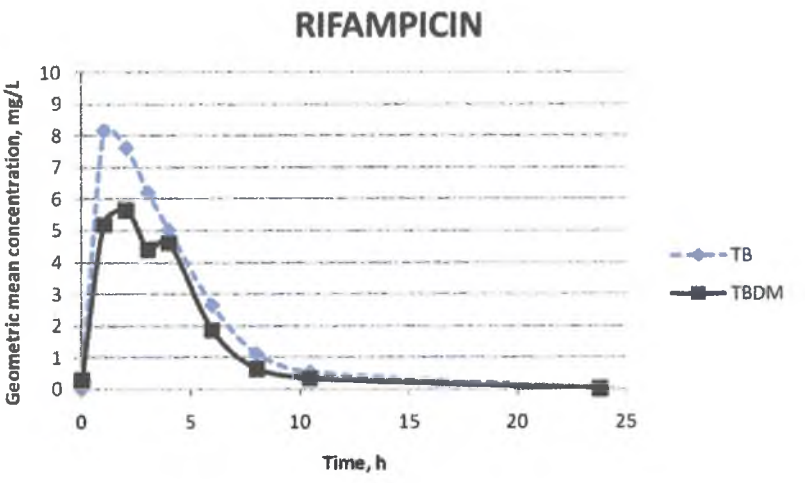
Table 2 Pharmacokinetic parameters of tuberculosis drugs in Tanzanian diabetic and non-diabetic TB patients in northern Tanzania

Drug/PK parameter	Value for group		Ratio of TB-DM to TB value [95%CI]	p-value
	TB-DM	TB		
Rifampicin[a]				
AUC_{0-24}, h*mg/L	29.91[1.77]	39.91[1.26]	0.75[0.56-1.03]	0.052
Cmax, mg/L	7.92[1.71]	8.92[1.28]	0.89[0.67-1.17]	0.384
Proportion with Cmax below reference range, n[%][c]	9[47.4]	7[35.0]	-	0.433
Tmax, h[IQR][b]	2.12[1.03-3.30]	1.08[0.98-2.05]	-	0.027
T1/2, h	1.44[1.30]	1.80 [1.38]	0.80[0.66-0.97]	0.026
Vz, L	38.74[1.79]	37.30 [1.31]	1.04[0.77-1.40]	0.798
CL, L/h	18.60[1.74]	14.40 [1.24]	1.29[0.98-1.71]	0.072
Desacetylrifampin[a]				
AUC_{0-24}, h*mg/L	3.97[1.81]	4.90 [1.45]	0.81[0.57-1.16]	0.240
Cmax, mg/L	0.73[1.97]	0.91[1.32]	0.80[0.56-1.14]	0.206
T1/2, h	1.61[1.44]	2.00[1.30]	0.81[0.64-1.01]	0.066
Desacetylrifampin/rifampin ratio[d]				
AUC	0.125[0.057]	0.133[0.035]	-	0.672
Cmax	0.100 [0.039]	0.107[0.029]	-	0.576
Isoniazid[a]				
AUC_{0-24}, h*mg/L	5.41[2.61]	10.61[1.94]	0.51[0.30-0.87]	0.015
Cmax, mg/L	1.65[2.15]	2.77[1.45]	0.60[0.40-0.89]	0.013
Proportion with Cmax below reference range, n[%][c]	14 [73.7]	11 [55.0]	-	0.224
Tmax, h[IQR] [b]	1.03[0.97-2.08]	1.07[0.98-1.19]	-	0.855
T1/2, h	2.55[1.51]	2.54[1.61]	1.00[0.75-1.34]	0.985
Vz, L	188.78[2.26]	99.19[1.26]	1.90[1.27-2.85]	0.003
CL, L/h	51.31[2.78]	26.89[1.84]	1.91[1.09-3.32]	0.024

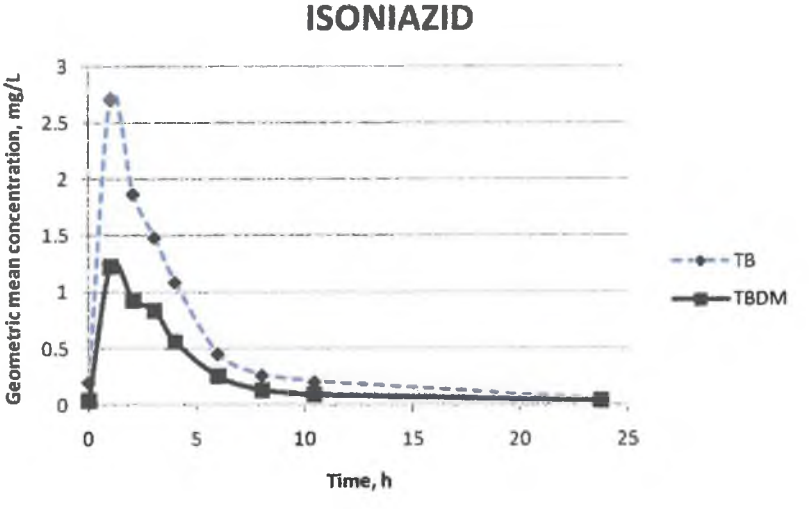
Acetylisoniazid[a]				
AUC_{0-24}, h*mg/L	21.70[2.00]	22.56[1.50]	0.96[0.67-1.39]	0.831
Cmax, mg/L	2.29[2.29]	2.18[1.80]	1.05[0.66-1.67]	0.831
T1/2, h	5.50 [1.76]	5.03[1.49]	1.10[0.80-1.50]	0.562
Acetylisoniazid/Isoniazid ratio				
AUC	4.01 [3.6]	2.125[2.75]	0.53 [0.25-1.12]	0.095
Cmax	1.40[3.36]	0.788[2.3]	0.57 [0.29-1.11]	0.095
Pyrazinamide[a]				
AUC_{0-24}, h*mg/L	289.63[1.41]	344.16[1.29]	0.84[0.69-1.02]	0.083
Cmax, mg/L	34.47[1.23]	38.19[1.17]	0.90[0.80-1.02]	0.090
Proportion with Cmax below reference range, n[%][c]	0	0		-
Tmax, h[IQR] [b]	1.12[1.00-3.00]	1.07[0.97-1.76]	-	0.252
T1/2, h	5.35[1.36]	6.28[1.45]	0.85[0.68-1.07]	0.154
Vz, L	39.54[1.37]	40.35[1.33]	0.98[0.81-1.19]	0.832
CL, L/h	5.12[1.45]	4.45[1.27]	1.15[.094-1.41]	0.170
Ethambutol[a]				
AUC_{0-24}, h*mg/L	19.64[1.57]	20.24[1.22]	0.97[0.77-1.22]	0.789
Cmax, mg/L	3.15[1.56]	3.31[1.31]	0.95[0.75-1.21]	0.672
Proportion with Cmax below reference range, n[%][c]	0	0		-
Tmax, h[IQR] [b]	2.00[1.00-3.00]	1.98[0.99-2.09]	-	0.317
T1/2, h	8.59[1.65]	9.58[1.20]	0.90[0.70-1.16]	0.384
Vz, L	643.74[1.67]	719.03[1.24]	0.90[0.69-1.16]	0.394
CL, L/h	51.92[1.62]	52.04[1.17]	1.00[0.78-1.27]	0.985

[a]Data are presented as geometric mean(standard deviation) unless stated otherwise
[b]By Mann-Whitney U test
[c]By Pearson's chi-square test
[d] Data are presented as mean(standard deviation). An independent-samples T-test was used for testing.

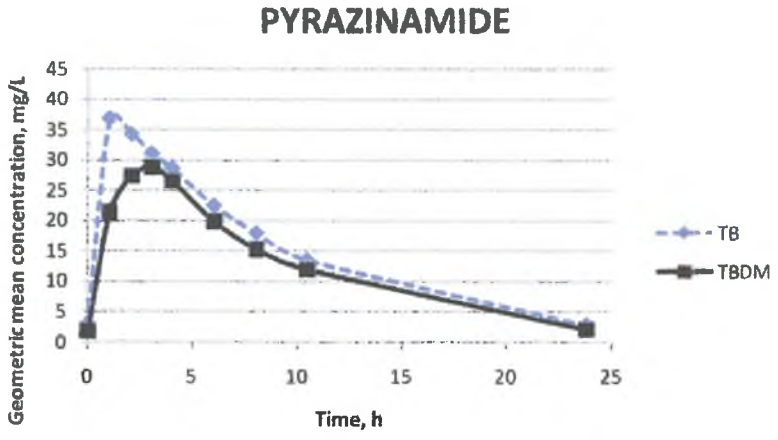
A


RIFAMPICIN
Geometric mean concentration, mg/L
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
Time, h
TB
TBDM

B


ISONIAZID
Geometric mean concentration, mg/L
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Time, h
TB
TBDM

C



D

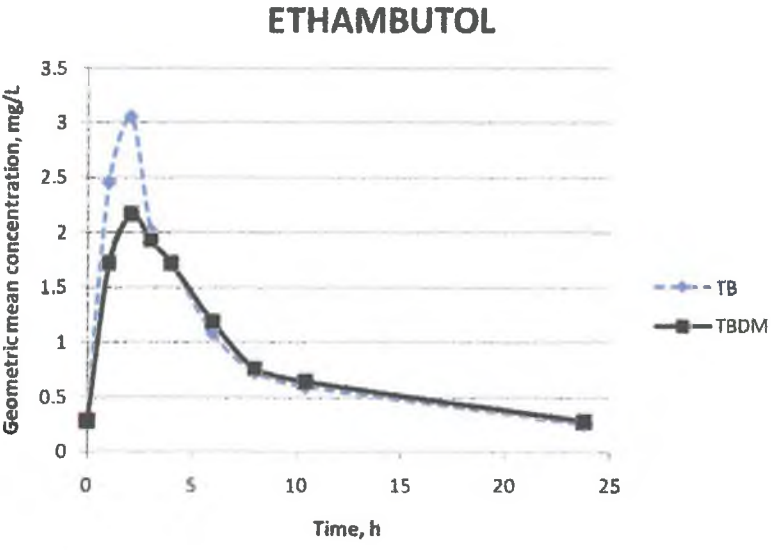


Figure 1 Geometric mean steady-state plasma concentration-time profiles of rifampicin (**A**), isoniazid (**B**), pyrazinamide (**C**), and ethambutol (**D**) in TB-DM patients (solid line graph, n=19) and TB only patients (dotted graph, n=20)

The proportion of patients with maximum concentrations (C_{max}) of rifampicin and isoniazid below reference ranges was higher in diabetic than in non-diabetic TB patients, yet (table 2)the distribution of these proportions was not statistically different between diabetic and non-diabetic TB patients. For pyrazinamide and ethambutol all patients had C_{max} within reference ranges.

Based on the metabolic ratio of acetylisoniazid concentration over isoniazid concentration at 3 hours post dose, acetylator status of 37 patients was determined. Nineteen (51.4%) patients were found to be fast acetylators. Breaking them down by diabetes group, 55.6% of the diabetic versus 47.4% of the non-diabetic TB patients were fast acetylators. The difference was not statistically significant (chi square = 0.248, p value 0.618).

Univariate analysis

Analysis of the association between PK parameters AUC_{0-24} and C_{max} of the TB drugs and other possible explanatory variables (age, sex, body weight, dose per kilogram, HIV status, acetylator status, fasting blood glucose, HbA1c) showed that age significantly correlated only with C_{max} of isoniazid (Spearman's rho = -0.392, p = 0.014). Between men and women, rifampicin C_{max} (geometric means 7.8 versus 10.7 mg/L, p = 0.048) and pyrazinamide C_{max} (35.1 versus 40.7 mg/L, p =0.036) were different. Body weight and dose per kilogram showed no correlations with AUC_{0-24} and C_{max} of the TB drugs. HIV positive and negative patients only differed with respect to the C_{max} of pyrazinamide (geometric means 39.7 versus 34.7 mg/L, p = 0.033). Fast acetylators had significantly lower AUC_{0-24} (geometric means 4.1 versus 16.2 h*mg/L, p = <0.001) and C_{max} (1.6 versus 3.1 mg/L, p = 0.001) values of isoniazid than slow acetylators.

Fasting blood glucose a significant correlation with AUC_{0-24} of rifampicin (Spearman's rho = -0.406, p =0.013) and with AUC_{0-24} (Spearman's rho = -0.376, p =0.022) and C_{max} (Spearman's rho = -0.421, p =0.009) of isoniazid. For the diabetic group, HbA_{1c} did not show a significant correlation with any of the PK parameters of rifampicin, isoniazid, pyrazinamide, and ethambutol.

Multivariate analysis

Multiple regression with the stepwise method and diabetes mellitus, age, sex, dose in mg/kg, HIV status and acetylator status as explanatory variables yielded significant models with regression equations that only contained diabetes mellitus as explanatory variable for log AUC_{0-24} and log C_{max} of rifampicin, as well as for log AUC_{0-24} and log C_{max} of pyrazinamide. Diabetes mellitus and acetylator status were the only variables that remained in regression equations for log AUC_{0-24} and log C_{max} of isoniazid.

Similarly, multiple regression analyses with fasting blood glucose instead of DM yielded fasting blood glucose as a significant predictor for log AUC_{0-24} and log C_{max} of rifampicin, pyrazinamide and isoniazid as well as acetylator status for log AUC_{0-24} and log C_{max} of isniazd.
These results show that the effect of diabetes mellitus and the effect of fasting blood glucose on the pharmacokinetic parameters of TB drugs remained (or even became) significant when other explanatory variables were taken into consideration.

Discussion

This is the first report describing the pharmacokinetic (PK) parameters of tuberculosis (TB) drugs in diabetic and non-diabetic TB patients using intensive PK sampling in an African population. We have found a clear, nearly significant trend for a decrease in AUC_{0-24} of rifampicin in diabetic versus nondiabetic TB patients. Similarly AUC_{0-24} and C_{max} of isoniazid were decreased in diabetic TB patients. The effect of DM correlated with a similar effect of fasting blood glucose on the exposure to these TB drugs. In multivariate analyses, the effects of DM and fasting blood glucose remained significant and acetylator status showed to be another predictor of the AUC_{0-24} and C_{max} of isoniazid.

Similar studies in Indonesia and Peru have shown contradicting results(19-21). The first Indonesian study(19) concluded that DM was associated with a strong decrease in plasma concentrations of rifampicin whereas a follow up study in the same population(20), and a Peruvian study(21) revealed no significant difference in exposure to TB drugs between diabetic and non-diabetic TB patients.

Our study was carried out in a distinctively different ethnic population (Africans) and involved intensive (9 sampling points over 24 hour interval) PK sampling. We analysed all four standard TB drugs in 40 patients with validated methods. In the second Indonesian study the authors implicate the low levels of TB drugs seen in their first study to weight differences between diabetic and non-diabetic TB patients(20). Although this is still a clinically relevant finding, they matched their patients for body weight in their follow-up study to disentangle the effects of weight and DM per se. Although we did not match our patients for body weight, the distribution of body weight (and therefore drug dose per kilogram weight) were the same between diabetic and non-diabetic TB patients (table 1); and besides that body weight was no predictor of exposure to TB drugs in our multiple linear regression analyses. There is no evidence that antidiabetic drugs lower the concentration of TB drugs(29). Therefore we believe the observed differences in PK parameters are due to diabetes

mellitus. This is further substantiated by similar associations that we found between fasting blood glucose levels and exposure to rifampicin and isoniazid in our population of diabetic and non-diabetic patients.

Unlike these previous studies, we have for the first time performed the analysis of isoniazid in diabetic and non-diabetic TB patients and it is actually isoniazid that was found to be most adversely affected in its values for AUC_{0-24} and C_{max}. Next to diabetes mellitus, acetylator status of isoniazid appeared to be a predictor of the AUC_{0-24} and C_{max} of isoniazid. However we realize that we evaluated the effect of DM on the pharmacokinetics of isoniazid and at the same time used a phenotyping method (which is based on relative exposure to acetylisoniazid and isoniazid) to assess the acetylator status. Theoretically, an incidental overrepresentation of (genotypic) fast acetylators among the diabetic TB patients might explain the lower exposure to isoniazid in this group, and this might by concealed by an effect of DM on the phenotypic assessment of acetylator status. Therefore a genotypic assessment of the acetylator status of isoniazid is currently planned.

Although we cannot readily translate suggested diabetes-mediated changes in pharmacokinetics of particular drugs to TB drugs, documented mechanisms suggest that diabetes influences the pharmacokinetics of various drugs by affecting (i) absorption, due to changes in subcutaneous and muscle blood flow and delayed gastric emptying; (ii) distribution, due to non-enzymatic glycation of albumin; (iii) biotransformation, due to differential regulation of enzymes involved in drug biotransformation and drug transporters; and (iv) excretion, due to nephropathy(10;11;30;31). As to rifampicin, the current study suggests that the clearance of rifampicin may be increased by DM, yet this appears not to be mediated by an effect on the metabolism of rifampicin into desacetylrifampicin (table 2). For isoniazid, both clearance and volume of distribution were increased in diabetic TB-patients, ultimately resulting in a relative increase in the formation of metabolite acetylisoniazd.

Evidence has shown that diabetic patients who get TB have poor treatment response to TB treatment and poor treatment outcome(7-9). Moreover accumulating evidence suggests that total exposure (AUC) and maximum concentration (C_{max}) of TB drugs are very important for efficacy of the drugs(7;12-17;32). Since increasing the dose of TB drugs results in increased plasma concentration of the drugs and improved treatment outcome(33), individualization of the dosages and therapeutic drug monitoring in diabetic TB patients are necessary. However because therapeutic drug monitoring is not feasible in developing countries, increasing the doses of TB drugs (especially rifampicin and isoniazid) in the whole population of diabetic patients seems to be a better strategy so as attain average plasma concentrations that are associated with

good treatment outcome in the majority of patients(27;33). To this end, higher doses of TB drugs have been shown to be safe and tolerable(25;33-35). Besides our findings and conclusions, further studies in African and other ethnic groups are required to confirm our hypothesis.

Our study findings may be limited by unequal age distribution between the groups, the diabetic TB group having on average older patients than the TB group. Age has been found not to be associated with pharmacokinetics of TB drugs in many studies(19;20;25;36;37). Similarly we didn't match the groups for sex and weight; however the distribution of these parameters was the same in the two groups. In addition, age, gender and dose/kg were no significant predictors in multiple regression analyses. The number of patients (n=39) may be high for a PK study with intensive PK sampling, yet this number is relatively low to perform multiple regression analyses with several possible explanatory variables.

In summary, we have shown a reduction in isoniazid plasma concentrations and a clear trend towards a reduction in exposure to rifampicin in Tanzanian diabetic patients with TB. We conclude that diabetes disease is likely to be responsible for the observed effects. More African and other studies are needed to confirm our findings, and higher doses of the drugs need to be considered.

Acknowledgements

We wish to thank all the patients for participating in this study; the staff of the TB clinic at Mawenzi Hospital in Moshi and the research nurses at KCMC for their cooperation and effort; the laboratory technicians at KCMC and at the Department of Pharmacy of the Radboud University Medical Center, The Netherlands for their technical support.

Financial support

This study was co-supported by the African Poverty Related Infection Oriented Research Initiative (APRIORI), a research network granted by the Netherlands-African partnership for Capacity development and Clinical interventions Against Poverty-related diseases (NACCAP) and by a funding from the *UNESCO/L'Oreal For Young Women in Science Fellowship 2008* which was awarded to A. Tostmann.

References

(1) BOUCOT KR. Diabetes mellitus and pulmonary tuberculosis. J Chronic Dis 1957 Sep;6(3):256-79.
(2) Restrepo BI. Convergence of the tuberculosis and diabetes epidemics: renewal of old acquaintances. Clin Infect Dis 2007 Aug 15;45(4):436-8.
(3) World Health Organisation. Global tuberculosis report 2012. 2012.
(4) Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med 2008 Jul 15;5(7):e152.
(5) Mugusi F, Swai AB, Alberti KG, McLarty DG. Increased prevalence of diabetes mellitus in patients with pulmonary tuberculosis in Tanzania. Tubercle 1990 Dec;71(4):271-6.
(6) Stalenhoef JE, Alisjahbana B, Nelwan EJ, Van d, V, Ottenhoff TH, van der Meer JW, et al. The role of interferon-gamma in the increased tuberculosis risk in type 2 diabetes mellitus. Eur J Clin Microbiol Infect Dis 2008 Feb;27(2):97-103.
(7) Harries AD, Lin Y, Satyanarayana S, Lonnroth K, Li L, Wilson N, et al. The looming epidemic of diabetes-associated tuberculosis: learning lessons from HIV-associated tuberculosis. Int J Tuberc Lung Dis 2011 Nov;15(11):1436-44, i.
(8) Baker MA, Harries AD, Jeon CY, Hart JE, Kapur A, Lonnroth K, et al. The impact of diabetes on tuberculosis treatment outcomes: a systematic review. BMC Med 2011;9:81.
(9) Syed Sulaiman SA, Ishaq Aweis DM, Mohamed AJ, Razakmuttalif A, Moussa MA. Role of diabetes in the prognosis and therapeutic outcome of tuberculosis. Int J Endocrinol 2012;2012:645362.
(10) Gwilt PR, Nahhas RR, Tracewell WG. The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. Clin Pharmacokinet 1991 Jun;20(6):477-90.
(11) Dostalek M, Akhlaghi F, Puzanovova M. Effect of diabetes mellitus on pharmacokinetic and pharmacodynamic properties of drugs. Clin Pharmacokinet 2012 Aug 1;51(8):481-99.
(12) Kimerling ME, Phillips P, Patterson P, Hall M, Robinson CA, Dunlap NE. Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. Chest 1998 May;113(5):1178-83.
(13) Mehta JB, Shantaveerapa H, Byrd RP, Jr., Morton SE, Fountain F, Roy TM. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest 2001 Nov;120(5):1520-4.
(14) Weiner M, Burman W, Vernon A, Benator D, Peloquin CA, Khan A, et al. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am J Respir Crit Care Med 2003 May 15;167(10):1341-7.
(15) Weiner M, Benator D, Burman W, Peloquin CA, Khan A, Vernon A, et al. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. Clin Infect Dis 2005 May 15;40(10):1481-91.
(16) Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, Wells CD, et al. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin Infect Dis 2009 Jun 15;48(12):1685-94.
(17) Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis 2011 Dec 15;204(12):1951-9.
(18) Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis 2012 Jul;55(2):169-77.
(19) Nijland HM, Ruslami R, Stalenhoef JE, Nelwan EJ, Alisjahbana B, Nelwan RH, et al. Exposure to rifampicin is strongly reduced in patients with tuberculosis and type 2 diabetes. Clin Infect Dis 2006 Oct 1;43(7):848-54.
(20) Ruslami R, Nijland HM, Adhiarta IG, Kariadi SH, Alisjahbana B, Aarnoutse RE, et al. Pharmacokinetics of antituberculosis drugs in pulmonary tuberculosis patients with type 2 diabetes. Antimicrob Agents Chemother 2010 Mar;54(3):1068-74.
(21) Requena-Mendez A, Davies G, Ardrey A, Jave O, Lopez-Romero SL, Ward SA, et al. Pharmacokinetics of rifampin in Peruvian tuberculosis patients with and without comorbid diabetes or HIV. Antimicrob Agents Chemother 2012 May;56(5):2357-63.

(22) Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med 2005 May 26;352(21):2211-21.
(23) Aspray TJ, Mugusi F, Rashid S, Whiting D, Edwards R, Alberti KG, et al. Rural and urban differences in diabetes prevalence in Tanzania: the role of obesity, physical inactivity and urban living. Trans R Soc Trop Med Hyg 2000 Nov;94(6):637-44.
(24) World Health Organization. Definition, diagnosis, and classification of diabetes mellitus and its complications. World Health Organization, Geneva, Switzerland. 1999.
(25) Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van CR, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. Antimicrob Agents Chemother 2007 Jul;51(7):2546-51.
(26) Tostmann A, Mtabho CM, Semvua HH, van den BJ, Kibiki GS, Boeree MJ, et al. Pharmacokinetics of first-line tuberculosis drugs in Tanzanian patients. Antimicrob Agents Chemother 2013 Jul;57(7):3208-13.
(27) Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 2002;62(15):2169-83.
(28) Hutchings A, Routledge PA. A simple method for determining acetylator phenotype using isoniazid. Br J Clin Pharmacol 1986 Sep;22(3):343-5.
(29) Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin : clinical relevance. Clin Pharmacokinet 2003;42(9):819-50.
(30) Okabe N, Hashizume N. Drug binding properties of glycosylated human serum albumin as measured by fluorescence and circular dichroism. Biol Pharm Bull 1994 Jan;17(1):16-21.
(31) Cashion AK, Holmes SL, Hathaway DK, Gaber AO. Gastroparesis following kidney/pancreas transplant. Clin Transplant 2004 Jun;18(3):306-11.
(32) Hall RG, Leff RD, Gumbo T. Treatment of active pulmonary tuberculosis in adults: current standards and recent advances. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy 2009 Dec;29(12):1468-81.
(33) Magis-Escurra C, van den BJ, Ijdema D, Boeree M, Aarnoutse R. Therapeutic drug monitoring in the treatment of tuberculosis patients. Pulm Pharmacol Ther 2012 Feb;25(1):83-6.
(34) Steingart KR, Jotblad S, Robsky K, Deck D, Hopewell PC, Huang D, et al. Higher-dose rifampin for the treatment of pulmonary tuberculosis: a systematic review. Int J Tuberc Lung Dis 2011 Mar;15(3):305-16.
(35) Katiyar SK, Bihari S, Prakash S, Mamtani M, Kulkarni H. A randomised controlled trial of high-dose isoniazid adjuvant therapy for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2008 Feb;12(2):139-45.
(36) McIlleron H, Rustomjee R, Vahedi M, Mthiyane T, Denti P, Connolly C, et al. Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: implications for international dosing guidelines. Antimicrob Agents Chemother 2012 Jun;56(6):3232-8.
(37) Jonsson S, Davidse A, Wilkins J, Van der Walt JS, Simonsson US, Karlsson MO, et al. Population pharmacokinetics of ethambutol in South African tuberculosis patients. Antimicrob Agents Chemother 2011 Sep;55(9):4230-7.

CHAPTER 7

Efavirenz, tenofovir and emtricitabine combined with first line tuberculosis treatment in tuberculosis-HIV-co-infected Tanzania patients: a pharmacokinetic and safety study

Hadija H Semvua, Charles M Mtabho, Quirine Fillekes, Jossy van den Boogaard, Riziki M Kisonga, Liberate Mleoh, Arnold Ndaro, Elton R Kisanga, Andre van der Ven, Rob E Aarnoutse, Gibson S Kibiki, Martin J Boeree, David M Burger

Antiviral Therapy 2013;18(1):105-13

Abstract

Objective. To evaluate the effect of rifampicin-based tuberculosis (TB) treatment on the pharmacokinetics of efavirenz/tenofovir/emtricitabine in a fixed-dose combination tablet, and vice versa, in Tanzanian TB-HIV-coinfected patients.

Method. This was a Phase II open-label multiple dose pharmacokinetic and safety study. This study was conducted in TB-HIV-coinfected Tanzanian patients who started TB treatment (rifampicin/isoniazid/pyrazinamide/ethambutol) at week 1 to week 8 and continued with rifampicin and isoniazid for another 16 weeks. Antiretroviral treatment (ART) of efavirenz/tenofovir/emtricitabine in a fixed-dose combination tablet was started at week 4 after initiation of TB treatment. A 24-h pharmacokinetic sampling curve was recorded at week 8 (with TB treatment) and week 28 (ART alone). For TB drugs, blood samples at 2 and 5 h post-dose were taken at week 3 (TB treatment alone) and week 8 (with ART).

Results. A total of 25 patients (56% male) completed the study; 21 had evaluable pharmacokinetic profiles. The area under the concentration-time curve 0-24 h post-dose of efavirenz, tenofovir and emtricitabine were slightly higher when these drugs were coadministered with TB drugs; geometric mean ratios (90% CI) were 1.08 (0.90, 1.30), 1.13 (0.93, 1.38) and 1.05 (0.85, 1.29), respectively. For TB drugs, equivalence was suggested for peak plasma concentrations when administered with and without efavirenz/tenofovir/emtricitabine. Adverse events were mostly mild and no serious adverse events or drug discontinuations were reported.

Conclusion. Coadministration of efavirenz, tenofovir and emtricitabine with a standard first-line TB treatment regimen did not significantly alter the pharmacokinetic parameters of these drugs and was tolerated well by Tanzanian TB patients who are coinfected with HIV.

Introduction

Co-infection with tuberculosis (TB) and HIV is an important public health problem. Nearly 40 million people are living with HIV worldwide and one-third is co-infected with TB [1-3]. The majority of TB cases in people living with HIV/AIDS occur in Africa, where 39% of people who develop TB are HIV-positive. The continent accounts for 82% of new TB cases living with HIV worldwide [1]. Management of the dual infection requires combining TB treatment and antiretroviral treatment (ART) which markedly decrease the risk of morbidity and mortality to those patients [4].

Co-administering TB drugs with ART precipitates pharmacokinetic interactions, mostly caused by rifampicin. Rifampicin is a well-known inducer of many iso-enzymes of the hepatic cytochrome P450 (CYP) enzyme system, which metabolizes a large number of drugs [5]. In addition, it stimulates the expression of the multi-drug resistance transporter, P-glycoprotein [6, 7]. Current guidelines for ART in patients also using rifampicin recommend the use of efavirenz plus two nucleoside reverse transcriptase inhibitors (NRTIs) [8, 9]. Although combined use of rifampicin and efavirenz leads to an average decrease of about 18 – 26% in efavirenz exposure due to induction of the CYP*2B6* enzyme[10, 11], several studies have demonstrated adequate virological response in patients using both drugs[4, 12].

A fixed-dose combination (FDC) tablet of efavirenz, tenofovir and emtricitabinehas been approved by the Food and Drug Administration in the USA in 2006. This combination tablet is widely used as it is highly effective and can be administered as a single tablet once-daily [13]. There are no data available on the combined use of a rifampicin-based TB regimen and this FDC in TB/HIV co-infected patients. The aim of this study was to evaluate the effect of rifampicin-based TB treatment on the pharmacokinetics (PK) and safety of the FDC of efavirenz, tenofovir and emtricitabine. The secondary objective was to evaluate the effect of this FDC on the PK of the TB medication.

Methods

Study design and population

This was a multiple-dose, open-label, three-period, phase II PK and safety study. The enrollment started on November 2008 and ended in February 2010. Patients included were 18-65 years and TB/HIV co-infected with CD4 counts of 50-350 cells/mm^3 (later amended to 0-350 cells/mm^3). Patients were excluded if they were already using ART, they were pregnant or breastfeeding, they had liver dysfunction (objectified by biochemistry results, and hepatitis B and C antigen tests) and if they were hypersensitive to either of the regimens. Another exclusion criterion was a Karnofsky

score <40 based on the activities a patient could perform. The study protocol was approved by the Institutional Review Board of the Kilimanjaro Christian Medical University Centre, Moshi, Tanzania, and the National Institute for Medical Research, Dar-es-salaam, Tanzania. Written informed consent was obtained from each patient. Eligible patients were admitted at Kibong'oto National Tuberculosis Hospital, Kilimanjaro, Tanzania for the first eight weeks of TB treatment (intensive phase). Patients <50kg were given TB treatment consisting of rifampicin 450mg, isoniazid 225mg, pyrazinamide 1200mg and ethambutol 825mg, all administered once-daily for eight weeks. If patients were >50kg, they received rifampicin 600mg, isoniazid 300mg, pyrazinamide 1600mg and ethambutol 1100mg once-daily for eight weeks. After a negative sputum culture at week 8, TB treatment was continued with rifampicin and isoniazid for another 16 weeks. TB drugs were administered 30 minutes after a light breakfast. TB drugs were provided by the Tanzanian National TB program, and they were manufactured by Sandoz, Mumbai India and donated by Novartis. The ART regimen of once-daily one FDC tablet of efavirenz 600mg, tenofovir 300mg and emtricitabine 200mg (Atripla®, Merck & Co. Inc. USA) was initiated at week 4 after starting TB treatment and was administered together with the TB drugs. The study was divided in three phases (Figure 1): From week 1 to the end of week 4 patients were exposed to TB drugs alone (phase I). From week 5 to the end of week 24 they were exposed to concomitant use of TB drugs and a FDC tablet containing efavirenz, tenofovir, and emtricitabine (phase II). After 24 weeks, when TB treatment was ended, patients were exposed to ART alone (phase III). Both TB and HIV treatment were administered under supervised care including directly observed treatment for 8 weeks. After 8 weeks, patients were discharged. A one month dose of efavirenz, tenofovir and emtricitabine and standard TB treatment was dispensed and patients were asked to come for follow-up visits at the clinic every month.

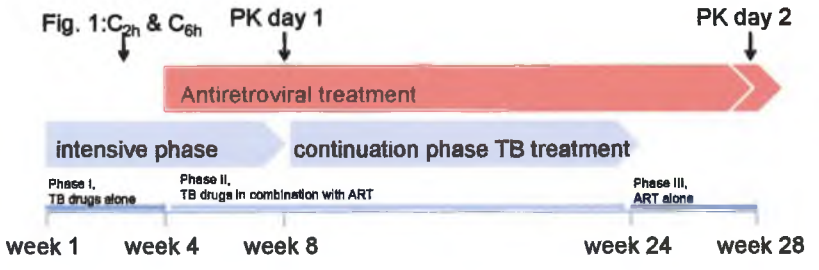


Figure 1 Trial study design.

Blood sampling and bio-analysis procedures

Eight weeks after starting TB treatment, a 24-hour PK sampling session was done. Samples were taken 5-20 minutes before directly observed intake of efavirenz, tenofovir and emtricitabine (t=0) and 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hours later. At 28 weeks, four weeks after cessation of TB treatment, intensive plasma PK sampling was repeated. In addition, for the determination of rifampicin, isoniazid, pyrazinamide and ethambutol plasma concentrations, blood samples were taken after initiation of TB treatment at three weeks (without ART) and eight weeks (with ART). These blood samples were drawn at two and six hours after observed medication intake to be able to assess ('catch') peak plasma concentrations of TB drugs, which was taken as highest of the two. Breakfast (non-milky tea and/or porridge) was provided 5-20 minutes after pre-dose sampling, together with TB and/or HIV medication. Six mL of blood was collected per time point and centrifuged at 2800rpm for 10min. Within one hour plasma was stored at -20°C for one day and then shifted to a -80°C freezer until transportation on dry-ice to Radboud University, The Netherlands, for bio-analysis. Plasma concentrations of all drugs except isoniazid were assayed by validated high-performance liquid chromatography methods [14-17].

Isoniazid was measured with liquid/liquid extraction followed by UPLC with ultraviolet (UV) detection. Accuracy was between 98% and 107%, dependent on the concentration level. The intra- and interassay coefficients of variation were less than 13% over the ranges of 0.051 to 15.1 mg/L. The lower limit of quantitation was 0.051 mg/l. Isoniazid containing samples were stable (<5% loss) for at least 12 months at -80°C.

Safety and tolerability monitoring

Baseline clinical, hematology and biochemistry parameters were taken and, when applicable, a pregnancy test was done. Safety and tolerability of the trial treatment were assessed by a medical doctor on the basis of the occurrence of adverse events (AEs) about which patients were interviewed every two weeks during the first eight weeks, and every one month after hospital discharge. The onset, severity, and potential relationship of any AE to the study medication were recorded. Severity was rated according to the Common Terminology Criteria for AEs (v4, 2009) and the Division of AIDS table for grading the severity of Adult and Paediatrics AEs (DAIDS, 2004). In addition, the use of concomitant medication was reported, as were the results of a physical examination including measurement of vital signs. Clinical laboratory testing (hematology and biochemistry parameters) was performed at baseline, week 2, 4, 6, 8, 12, 16, 24 and 28. Follow-up sputum collection, CD4 lymphocyte count and plasma HIV-1 RNA quantification (using Abbott Real Time HIV-1 assay) was done at baseline, week 4, 8, 16, and 28.

PK and statistical analyses

PK parameters (area under the concentration-time curve 0-24 hours post-dose [AUC_{0-24h}], C_{min}, C_{max} of efavirenz, tenofovir and emtricitabine were calculated by non-compartmental analysis using WinNonlin (version 5.2; Pharsight, CA). AUC_{0-24h} was calculated using the linear-log trapezoidal rule. PK parameters, geometric means (GM) with standard deviation (SD), geometric mean ratios (GMRs) for AUC_{0-24h}, C_{max} and C_{min} for the antiretroviral drugs with and without TB treatment and the corresponding 90% confidence intervals (CIs) were calculated. PK parameters of the antiretroviral drugs with vs. without TB drugs were considered bioequivalent if the GMR and corresponding 90%CI fell completely within the 0.80-1.25 range. Equivalence was 'suggested' in case the GMR is within the 0.80-1.25 range, but with one confidence interval limit outside this range. Inequivalence was to be concluded if the GMR and corresponding 90%CI fell completely outside the 0.80-1.25 range. Inequivalence was 'suggested' if the GMR is outside the 0.80-1.25 range, with one confidence interval inside this range [18]. PK analysis included only those patients with two evaluable PK curves (at week 8 and week 28). All statistical analyses were done in SPSS, version 18.0. To ensure an evaluable PK dataset of at least 20 patients, a total of 30 patients were included (based on an expected maximum drop-out of 33% during the trial after ART initiation).

Results

Study population

We screened 66 HIV-infected, patients with confirmed smear-positive pulmonary TB. Thirty-eight (58%) patients were not eligible: 19 had a CD4 count> 350cells/mm^3, eight had a CD4 count <50 cells/mm^3 (before protocol amendment), five were already on ART, two had a positive hepatitis antigen test, three had a Karnofsky score <40 and one had relapse TB. Of the 28 patients (42%) enrolled in the trial three withdrew consent; the remaining 25 patients completed follow-up and were evaluable for analysis. For PK evaluation four patients were excluded due to non-adherence or incorrect dosing, as indicated by undetectable plasma concentrations for efavirenz, tenofovir and emtricitabine prior to observed intake during the intensive PK sampling day. Hence, 21 subjects were qualified for the PK evaluation. In the patients who completed follow-up, 14 patients (56%) were male, the median (interquartile range, IQR) baseline age and body weight were 32 (27.5-42.5) years and 48.4 (43.5-52.8) kg, respectively. in our study population body weight (mean+SD, kg) increased significantly from 53.4 (8.0) at week 8 to 56.8 (6.5) at week 28. The median (IQR) CD4 count at baseline was 155 (71-208) cells/mm^3, the median (IQR) viral load (VL) was 129,779 (71,214-310,141) copies/mL. Baseline characteristics are in Table 1.

Table 1 Enrolment characteristics of the study participants (N=25)

Characteristics	Total
Number of patients followed up	25
Male, n (%)	14 (56%)
Age, years	32 (27.5-42.5)
Weight, kg	48.4 (43.5-52.8)
CD4 count, cells/mm^3	155 (71-208)
HIV viral load, copies/ml	129,779 (71,214 -310,141)

Note: Values are N (%) for categorical variables and median (IQR) for continuous variables.

PK of ART

Figure 2 illustrates the effect of the TB treatment on the geometric mean concentration-time profiles of the ART. The average efavirenz AUC_{0-24h}, C_{max} and C_{min} were slightly higher when efavirenz was co-administered with rifampicin-based TB treatment. The GMRs lie within the range of 0.80-1.25 with the upper limit of the 90% CIs for AUC_{0-24h} and C_{24h} just above 1.25 (Table 2). GMRs (90%CI) were 1.08 (0.90-1.30), 1.02 (0.8-1.23) and 1.11 (0.90-1.37) for AUC_{0-24h}, C_{max} and C_{24h}, respectively, which means that equivalence was suggested (i.e. no effect of rifampicin on the efavirenz PK) Three of the 21 (14%) patients had subtherapeutic efavirenz plasma concentrations (<1.0 mg/L [19]) after observed intake at one or both PK sessions: one patient at week 8 only, one patient at week 28 only and one at both PK days. Potentially toxic plasma levels (>4.0 mg/L [19]) after observed intake were found in ten (48%) of the patients. Eight of these patients had potentially toxic levels at both PK sessions. Two patients had a Cmin and Cmax both >10mg/ml. . The time to reach the maximum concentration (T_{max}) was observed to be higher during co-administration with TB drugs.

Similarly, for tenofovir and emtricitabine the GMRs and 90% CIs of the PK parameters suggest a slightly higher exposure in combination with TB drugs. GMRs (90%CIs) of AUC_{0-24h}, C_{max} and C_{24h} for tenofovir were 1.1 (0.93-1.4), 0.98 (0.78-1.4) and 1.09 (0.86-1.4), respectively, while for emtricitabine they were 1.05 (0.85-1.3), 0.97 (0.75-1.3) and 1.3 (1.04-1.5), respectively. The PK parameters (AUC_{0-24h}, C_{max}, C_{24h}, T_{max}, elimination half-life ($t_{1/2}$), volume of distribution (V/F) and clearance (CL/F)) for efavirenz, tenofovir and emtricitabine are summarized in Table 2.

PK of TB drugs

The plasma concentrations of all TB drugs tended to be slightly lower during co-administration in combination with ART. The bio-equivalence approach applied to the C_{max} values for TB drugs suggested bioequivalence of the TB drugs when given alone versus in combination with ART (Table 3)

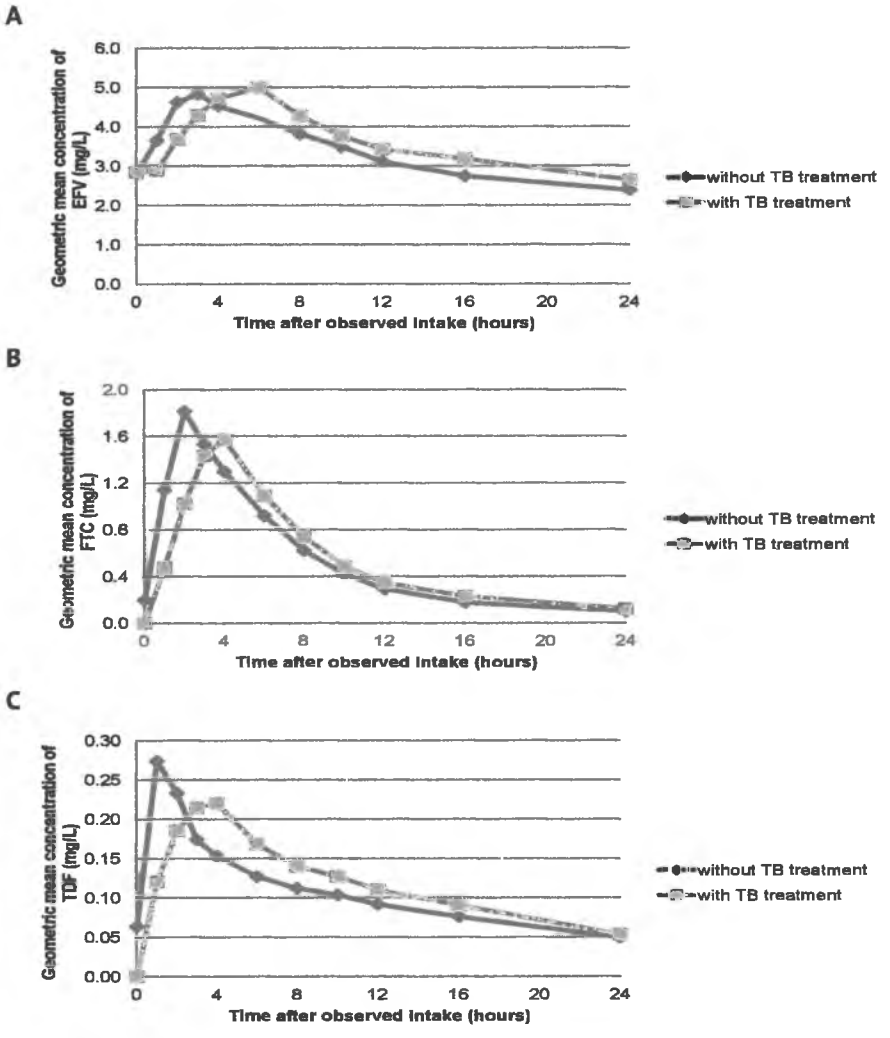


Figure 2 Curves of mean plasma concentration versus time for efavirenz (**A**), emtricitabine (**B**) and tenofovir (**C**) *(order from above downwards)* after observed intake of FDC tablet efavirenz/tenofovir/emtricitabine alone (grey curve) and in combination with TB treatment (black curve)

Table 2 Pharmacokinetic parameters of efavirenz, tenofovir and emtricitabine alone and in combination with TB treatment

	PK parameter	Week 28 (ART alone; geometric mean (95% CI); mg/L)	Week 8 (ART with TB treatment; geometric mean (95% CI); mg/L)	GMR (90% CI)
Efavirenz	AUC_{0-24h}, h.mg/L	81 (58-114)	88 (60-129)	1.08 (0.90-1.30)
	C_{max}, mg/L	5.7 (4.2-7.5)	5.7 (4.3-7.7)	1.02 (0.80-1.23)
	C_{min}, mg/L	2.4 (1.6-3.5)	2.6 (1.6-4.2)	1.11 (0.90-1.37)
	T_{max}, h	3.1 (1.0-10.1)	4.0 (1.1-8.0)	-
	$t_{1/2}$, h	27 (25-35)	29 (20-47)	1.08 (0.77-1.41)
	Cl/F, L/h	7.4 (5.3-10.4)	6.8 (4.6-10.1)	0.92 (0.77-1.11)
	V/F, L	313 (232-421)	301 (236-383)	0.96 (0.72-1.29)
Tenofovir	AUC_{0-24h}, h.mg/L	2.6 (2.1-3.2)	2.9 (2.4-3.5)	1.13 (0.93-1.38)
	C_{max}, mg/L	0.33 (0.26-0.41)	0.32 (0.26-0.40)	0.98 (0.78-1.38)
	C_{min}, mg/L	0.05 (0.04-0.06)	0.05 (0.04-0.07)	1.09 (0.86-1.38)
	T_{max}, h	1.0 (0.9-8.0)	2.1 (0.0-6.2)	-
	$t_{1/2}$, h	14 (12-15)	11 (10-12)	0.81 (0.75-0.86)
	Cl/F, L/h	94 (76-117)	83 (69-100)	0.88 (0.73-1.08)
	V/F, L	1852 (1438-2385)	1319 (1099-1584)	0.71 (0.59-0.86)
Emtricitabine	AUC_{0-24h}, h.mg/L	14 (12-16)	15 (11-18)	1.05 (0.85-1.29)
	C_{max}, mg/L	2.1 (1.8-2.3)	2.0 (1.5-2.7)	0.97 (0.75-1.25)
	C_{min}, mg/L	0.10 (0.09-0.12)	0.12 (0.10-0.16)	1.26 (1.04-1.53)
	T_{max}, h	2.0 (1.0-8.0)	3.0 (0.0-6.0)	-
	$t_{1/2}$, h	6.6 (5.8-7.5)	7.3 (6.1-8.6)	1.10 (0.93-1.31)
	Cl/F, L/h	18 (15-21)	17 (13-22)	0.96 (0.77-1.18)
	V/F, L	171 (139-210)	180 (124-261)	1.05 (0.75-1.48)

Note: T_{max} is presented as median (IQR)

Efficacy

Twenty-five patients completed follow-up to the end of week 28. At week 4 of the study (prior to ART) they had a median (IQR) VL of 134,204 (71,414-401,004) copies/mL. At week 28 (after starting ART), 17 (68%) patients had an undetectable VL (<40 copies/ml). The remaining eight patients (32%) had a median (IQR) VL of 51(40-532) copies/mL. From 23 of the patients (92%), CD4 counts were available both at baseline and at the end of the study. Their median (IQR) CD4 count increased from 119 (69-203) cells/mm^3 to 238 (147-424) cells/mm^3 ($p<0.01$; Signed Rank).

Table 3 Peak plasma concentrations (Cmax) of rifampicin, isoniazid, pyrazinamide and ethambutol alone and in combination with ART, based on sampling at 2 h and 6 h post dose

Drug	GM Week 2 (without HIVdrug; mg/L)	GM Week 8 (with HIV drug; mg/L)	GMR (90% CI)
Rifampicin	4.60	3.97	1.16(0.88-1.53)
Isoniazid	1.24	1.18	1.06(0.85-1.31)
Pyrazinamide	31.64	35.95	0.88(0.78-0.99)
Ethambutol	2.09	2.11	0.99(0.78-1.26)

By the end of eight weeks of TB treatment, 19 of 25 patients (76%) had negative sputum smears (confirmed by negative sputum cultures) and continued with rifampicin and isoniazid for 16 weeks. Six patients (24%) had positive sputum smear results after 8 weeks of treatment, of which two had negative and four had a positive cultures for *Mycobacterium tuberculosis* (MTB). Drug susceptibility testing of the isolates obtained from these four patients showed normal susceptibility patterns and therefore, those four patients continued with intensive phase TB treatment for another four weeks. At week 12, sputum smear results of all four patients were negative. Of the four, two had an undetectable VL at week 16, the remaining two had had 659 copies/mL and 8,032 copies/mL each. All 25 patients had negative sputum culture results at the end of TB treatment.

Safety

Twelve patients (48%) developed elevated biochemistry parameters. These included elevated alkaline phosphatase in 11 patients (all grade 1 except for two patients who had grade 2), elevated creatinine in three patients (two of which were grade 2 and one was grade 3), and elevated pancreatic amylase and ALT in one patient each (both grade 1). The patient with grade 3 elevation of creatinine recovered spontaneously. Nineteen patients (76%) developed decreased hematological parameters. These were mostly leucopenia and neutropenia (12 subjects each), and were all grade 1. Four patients had anemia, of which two were grade 1, one was grade 2 and the remaining one was grade 3. Two patients had thrombocytopenia (one grade 1 and the other grade 2). The laboratory abnormalities had all resolved at completion of the study.

Tolerability

Before administration of ART (Phase 1), 24 of 25 (96%) patients had experienced one or more clinical AEs; with a total of 99 events (65% grade 1, 34% grade 2, 1% grade 3). After starting ART (Phase 2), 23 of the 25 (92%) subjects experienced one or more new

or aggravated AEs at some time during follow-up. These were in total 95 AEs, mostly mild disturbances (64% grade 1, 33% grade 2, 3% grade 3). There were no serious AEs reported and no modifications or discontinuations of treatment were done. Thirteen percent of the clinical events were judged to have no relationship to the drugs, 83% had either a doubtful or possible relationship and 4% had a possible relationship. At the end of follow-up, all events were recovered except for four patients who still had either loss of appetite, body rash, general body weakness or constipation.
Fifty percent of the 10 patients who had toxic levels of efavirenz experienced a clinical AE compared to 40% among patients without toxic levels (p=0.70) and 30% of the events were grade 2 for the group with toxic levels versus 38.5% for the sub therapeutic group (p=0.23). Lab events were reported in 90% of the patients with toxic levels of efavirenz and 93% of patients without toxic levels. Two patients with efavirenz concentration above 10 mg/L throughout showed only mild (grade 1) AEs.

Discussion

This study is the first to report on the interactions of efavirenz, tenofovir emtricitabine with first line rifampicin-based TB treatment. No interactions were observed in the pharmacokinetic analysis. The observed slightly higher AUC_{0-24h}, C_{max} and C_{24h} of efavirenz when co-administered with the rifampicin-based TB treatment in our study is consistent with a recent study that showed the absence of an effect of rifampicin on efavirenz PK when patients are poor metabolizers of *CYP2B6[20]*. Our data are also in agreement with recent studies in South Africa, Tanzania and India showing that efavirenz concentrations do not decrease when it is co-administered with a rifampicin-based TB treatment regimen [21-23]. The metabolism of efavirenz is extensively influenced by pharmacogenetic factors [21,24-26]. A single nucleotide polymorphism at position 516 on the *CYP2B6* gene has been widely reported to play an important role in the metabolism of efavirenz and nevirapine [27-29].Genetic polymorphisms with *CYP2B6* occur in all populations, and this may affect the exposure to efavirenz and its susceptibility to rifampicin-based enzyme induction [30-32]. The slightly higher efavirenz levels during combined TB and HIV treatment in our study could be explained by genetic differences [20, 23, 25].

A once-daily dose of efavirenz 600mg during TB therapy is reported to be adequate [22, 33, 34]. However, in our study potentially toxic plasma levels (>4.0 mg/L) were observed in 48% of the patients. Also sub-therapeutic levels of efavirenz (<1.0mg/L) were found in 14% of the patients. The observed high intervariability of drug levels suggests inter-patient differences in the expression of the drug metabolizing enzymes [11]. For observed potentially toxic levels, no further action was taken as the

patients were already discharged in a good condition. The levels occurred both before and during co-administration with TB treatment. Nevertheless the frequent presence of high levels in our African patient population may warrant the use of therapeutic drug monitoring of efavirenz plasma concentrations to improve the safe use of ART during TB treatment, and the need for individual dose adjustment [10].
Comparison of exposure to tenofovir with and without rifampicin-based TB treatment did not suggest any effect of rifampicin, although PK equivalence (lack of interaction) could not be concluded. Studies elsewhere have also shown no difference in tenofovir concentration while observing bioequivalence [15]. However, participants in these studies were healthy volunteer subjects who received tenofovir only shortly, which implicate that less potential factor may have affected the PK of tenofovir in those subjects. PK parameters may be different in patients as compared to healthy volunteers due to changes brought by both HIV and TB infection [35]. It is known that tenofovir is eliminated unchanged by glomerular filtration and active tubular secretion [36], while rifampicin is extensively metabolized in intestinal and hepatic metabolism [7] which minimizes the interactions. Emtricitabine has also been reported to have no PK drug-drug interactions with other antiretroviral drugs and rifampicin.

The time to reach maximum concentrations (T_{max}) of emtricitabine, tenofovir and efavirenz was longer when co-administered with rifampicin than without rifampicin, although the median difference was only one hour for all three drugs. The reason for one of these drugs may be delayed absorption upon co-administration of rifampicin. Clearance of efavirenz could be affected in patients with genotype CYP2*B6*. We did not analyze genetic aspects of these patients, hence further studies are warranted. No large differences were found in clearance and volume of distribution.

This study showed a small decrease in levels of TB drugs with versus without co-administration of efavirenz, tenofovir and emtricitabine. We do not consider this to be clinically significant. Studies elsewhere have also found no statistically significant effect of efavirenz on rifampicin [5, 7, 22, 37]. In our study, rifampicin and isoniazid peak plasma concentrations were lower than the reference peak values (8-24 mg/L rifampicin; 3-5 mg/L isoniazid) [38]. One possible explanation could be that the values in this study, obtained from either two or six hours after intake, were not the actual peak values. Secondly, our patients took medications just after food intake which is known to lower concentrations of rifampicin and isoniazid[39].
All patients had a positive response to treatment in terms of sputum culture and decrease of VL. An important finding of our study is that we detected non-adherence in four patients included in the trial when pre-dose samples were analyzed. This can be explained by the fact that the patients were discharged home after eight weeks,

and subjects were asked to come for follow-up visits at the clinic every month. We recommend strong coordination between the national TB; community DOT system and the AIDS control programs.

The main shortcoming of this study is that we did not look into genetic factors of the patients, which could have provided an explanation of high efavirenz drug levels and for the extent of the interaction between efavirenz and rifampicin-based TB treatment. Also, the study did not examine the effect of anti-TB treatment on intracellular PK of tenofovir and emtricitabine, as intracellular diphosphate or triphosphate levels are associated with clinical effects. It is possible that rifampin-containing TB treatment may not influence plasma PK but could affect intracellular PK through induction or inhibition of drug transporters.

In conclusion, our findings suggest that co-administration of the standard first line TB treatment regimen with efavirenz, tenofovir, and emtricitabine does not alter pharmacokinetic parameters. The combination is tolerated well by Tanzanian TB/HIV co-infected patients. Hence, a combination of efavirenz, tenofovir and emtricitabine may be considered in managing HIV infection in African patients who are co-infected with TB.

References

1. World Health Organization. Global tuberculosis control: WHO report 2011. In. 2011 ed. Geneva: World Health Organization; 2011.
2. Corbett EL, Marston B, Churchyard GJ, De Cock KM. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet 2006,367:926-937.
3. Lawn SD, Churchyard G. Epidemiology of HIV-associated tuberculosis. Curr Opin HIV AIDS 2009,4:325-333.
4. Manosuthi W, Kiertiburanakul S, Sungkanuparph S, Ruxrungtham K, Vibhagool A, Rattanasiri S, et al. Efavirenz 600 mg/day versus efavirenz 800 mg/day in HIV-infected patients with tuberculosis receiving rifampicin: 48 weeks results. AIDS 2006,20:131-132.
5. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin : clinical relevance. Clin.Pharmacokinet. 2003,42:819-850.
6. Finch CK, Chrisman CR, Baciewicz AM, Self TH. Rifampin and rifabutin drug interactions: an update. Arch. Intern.Med. 2002,162:985-992.
7. Burman WJ, Gallicano K, Peloquin C. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin.Pharmacokinet. 2001,40:327-341.
8. World Health Organisation. Treatment of Tuberculosis: Guidelines. In. Fourth Edition ed. Geneva: World Health Organisation; 2010.
9. World Health Organisation. Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach. In. 2010 Revision ed. Geneva: World Health Organisation; 2010.
10. Kwara A, Tashima KT, Dumond JB, Poethke P, Kurpewski J, Kashuba AD, et al. Modest but variable effect of rifampin on steady-state plasma pharmacokinetics of efavirenz in healthy African-American and Caucasian volunteers. Antimicrob Agents Chemother 2011,55:3527-3533.
11. Lopez-Cortes LF, Ruiz-Valderas R, Viciana P, Alarcon-Gonzalez A, Gomez-Mateos J, Leon-Jimenez E, et al. Pharmacokinetic interactions between efavirenz and rifampicin in HIV-infected patients with tuberculosis. Clin.Pharmacokinet. 2002,41:681-690.
12. Friedland G, Khoo S, Jack C, Lalloo U. Administration of efavirenz (600 mg/day) with rifampicin results in highly variable levels but excellent clinical outcomes in patients treated for tuberculosis and HIV. J. Antimicrob.Chemother. 2006,58:1299-1302.
13. Semvua HH, Kibiki GS. AtriplaR/anti-TB combination in TB/HIV patients. Drug in focus. BMC Res Notes 2011,4:511.
14. Aarnoutse RE, Grintjes KJ, Telgt DS, Stek M, Jr., Hugen PW, Reiss P, et al.The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther. 2002,71:57-67.
15. Droste JAH, Verweij-van Wissen CPWGM, Kearney BP, Buffels R, vanHorssen P, Hekster YA, et al. Pharmacokinetic Study of Tenofovir Disoproxil Fumarate Combined with Rifampin in Healthy Volunteers. Antimicrob.Agents Chemother. 2005,49:680-684.
16. la Porte CJ, Colbers EP, Bertz R, Voncken DS, Wikstrom K, Boeree MJ, et al. Pharmacokinetics of Adjusted-Dose Lopinavir-Ritonavir Combined with Rifampin in Healthy Volunteers. Antimicrob.Agents Chemother. 2004,48:1553-1560.
17. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampicin dose versus the standard dose in pulmonary tuberculois patients. Antimicrob.Agents Chemother. 2007.
18. Williams RL, Chen ML, Hauck WW. Equivalence approaches. Clin.Pharmacol.Ther. 2002,72:229-237.
19. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 2001,15:71-75.
20. Cohen K, Grant A, Dandara C, Mcilleron H, Pemba L, Fielding K, et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. Antivir.Ther. 2009,14:687-695.
21. Ngaimisi E, Mugusi S, Minzi O, Sasi P, Riedel KD, Suda A, et al.Effect of rifampicin and CYP2B6 genotype on long-term efavirenz autoinduction and plasma exposure in HIV patients with or without tuberculosis. Clin Pharmacol Ther 2011,90:406-413.

22. Orrell C, Cohen K, Conradie F, Zeinecker J, Ive P, Sanne I, et al. Efavirenz and rifampicin in the South African context: is there a need to dose-increase efavirenz with concurrent rifampicin therapy? Antivir Ther 2011,16:527-534.
23. Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Kumar P, Ramesh K, Anitha S, et al. CYP2B6 G516T polymorphism but not rifampin coadministration influences steady-state pharmacokinetics of efavirenz in human immunodeficiency virus-infected patients in South India. Antimicrob.Agents Chemother. 2009,53:863-868.
24. Kwara A, Lartey M, Sagoe KW, Xexemeku F, Kenu E, Oliver-Commey J, et al. Pharmacokinetics of efavirenz when co-administered with rifampin in TB/HIV co-infected patients: pharmacogenetic effect of CYP2B6 variation. J.Clin.Pharmacol. 2008,48:1032-1040.
25. Uttayamakul S, Likanonsakul S, Manosuthi W, Wichukchinda N, Kalambaheti T, Nakayama EE, et al. Effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected Thai adults. AIDS Res Ther 2010,7:8.
26. di Iulio J, Fayet A, Arab-Alameddine M, Rotger M, Lubomirov R, Cavassini M, et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. Pharmacogenet Genomics 2009,19:300-309.
27. Haas DW, Gebretsadik T, Mayo G, Menon UN, Acosta EP, Shintani A, et al. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African americans. J.Infect.Dis. 2009,199:872-880.
28. Powers V, Ward J, Gompels M. CYP2B6 G516T genotyping in a UK cohort of HIV-positive patients: polymorphism frequency and influence on efavirenz discontinuation. HIV Med 2009,10:520-523.
29. King J, Aberg JA. Clinical impact of patient population differences and genomic variation in efavirenz therapy. AIDS 2008,22:1709-1717.
30. Nyakutira C, Roshammar D, Chigutsa E, Chonzi P, Ashton M, Nhachi C, et al. High prevalence of the CYP2B6 516G-->T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharmacol 2008,64:357-365.
31. Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am.J.Respir.Crit Care Med. 2003,167:603-662.
32. Klein K, Lang T, Saussele T, Barbosa-Sicard E, Schunck WH, Eichelbaum M, et al. Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. Pharmacogenet.Genomics 2005,15:861-873.
33. Friedland G, Churchyard GJ, Nardell E. Tuberculosis and HIV coinfection: current state of knowledge and research priorities. J Infect Dis 2007,196 Suppl 1:S1-3.
34. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray A, et al. Timing of initiation of antiretroviral drugs during tuberculosis therapy. N Engl J Med 2010,362:697-706.
35. Lee BL, Wong D, Benowitz NL, Sullam PM. Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. Clin.Pharmacol.Ther. 1993,53:529-535.
36. Barditch-Crovo P, Deeks SG, Collier A, Safrin S, Coakley DF, Miller M, et al. Phase i/ii trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. Antimicrob.Agents Chemother. 2001,45:2733-2739.
37. Kwara A, Lartey M, Sagoe KW, Court MH. Paradoxically elevated efavirenz concentrations in HIV/tuberculosis-coinfected patients with CYP2B6 516TT genotype on rifampin-containing antituberculous therapy. AIDS 2011,25:388-390.
38. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. DRUGS 2002,62:2169-2183.
39. Lin MY, Lin SJ, Chan LC, Lu YC. Impact of food and antacids on the pharmacokinetics of anti-tuberculosis drugs: systematic review and meta-analysis. Int J Tuberc Lung Dis 2010,14:806-818.

CHAPTER 8

General discussion

Introduction

Tuberculosis (TB) is a major public health problem worldwide, including in Sub-Sahara African countries like Tanzania where it causes a significant burden of disease[1, 2]. A number of knowledge gaps are still present that limit optimal TB management and hamper the fight against this disease.

In order to improve the management of TB in Tanzania, a number of studies were designed and subsequently executed. Our focus was to carefully describe the burden of disease in north-eastern Tanzania, as well as the clinical and pharmacological aspects of the disease so as to give insight on how to optimize its treatment. The results of these studies can be summarized as follows: 1) the burden of TB among children in Tanzania is substantial; 2) a significant proportion of Tanzanian TB patients achieve low plasma drug levels of key TB agents; 3) saliva can only be used for a semi-quantitative assessment of rifampicin plasma concentrations; 4) a TB Drug activity Assay (TDA) may serve as a tool for monitoring treatment response and early identification of treatment failure, in settings where more sophisticated tools are not available; 5) diabetes mellitus adversely alters the exposure to the key TB drugs rifampicin and isoniazid; and 6) concomitant treatment of TB/HIV co-infected patients with standard TB drugs and combination anti-retroviral treatment using emtricitabine/tenofovir/efavirenz is tolerable and efficacious without clinically relevant changes in antiretroviral plasma drug concentrations induced by rifampicin.
An outline and discussion about our results can be found below as well as their implications.

Burden of TB in children

Chapter 2 provided evidence that the burden of childhood TB in the Kilimanjaro region of Tanzania is significant, contributing 13% of all TB cases. Children (0-14 years) had an annual prevalence of 147 in urban and 42 (per 100,000 children's population) in rural areas, compared with 166.6/100,000 in the general Kilimanjaro population and 164/100,000 for Tanzania in 2006[3]. We also showed that children have a high mortality rate (10.9%) and relatively low treatment success rate (72.9%), which is worse than the WHO estimates[4, 5] and other reports from other countries like Malawi, Thailand, and the United States[6-8]. We also observed that younger children (0-4 years) are more affected than older children, which is in line with previous studies[4, 9, 10]. Furthermore, it was found that 24.9% of the children that were treated for TB were tested for acid-fast bacilli (AFB) by Ziehl-Neelsen staining showing that only 5.8% of all patients with TB were AFB smear positive. This means that 94.2% were presumptively treated

for TB. Our findings indicates that there is an urgent need to improve the diagnostic process, such as the introduction and evaluation of new tools such as induced sputum, laryngeal swabs and nasopharyngeal aspiration, molecular tests and active case finding. in line with the recommendations of the World Health Organization[11-13], we also urge the Tanzanian TB program to give special consideration to TB in children and to take up the stipulated research agenda and pioneer regular studies together with other stakeholders.
The formulated priorities in the research agenda[11, 14, 15] are summarized in the table below:

Proposed research priorities regarding the epidemiology and clinical aspects of childhood TB

1. to prospectively evaluate the incidence of childhood TB and the program performance with regard to childhood TB
2. to study the criteria when to suspect and diagnose childhood TB using uniform criteria and to evaluate new methodologies for this purpose
3. to study the pharmacokinetics and toxicity of anti-tuberculosis drugs in children and the long-term outcome of the treatment of children
4. to determine how many childhood contacts of adult pulmonary TB qualify for chemoprophylaxis in different communities
5. to study chemoprophylaxis for drug-resistant TB and chemoprophylaxis among certain groups of adolescents
6. to document at what level children enter the National TB programs, the availability of qualified staff and their effectiveness in performing diagnostic investigations and ensuring quality care
7. to study the role of families as agents for DOTS, evaluate private sector participation in childhood TB management
8. to document Bacille Calmette-Guérin (BCG) immunization complications and study its management strategies

WHO has recently changed the dosing guidelines for children. The doses on a mg/kg base are increased in order to achieve that children have similar exposures to TB drugs as adults[16]. This recognizes that children especially those under the age of five have faster clearance and larger volumes of distribution for TB drugs[17, 18]. Unfortunately, the drug exposures achieved with the new higher doses have not been validated yet. In addition, corresponding drug formulations are not available yet, hindering the implementation of the revised dosing guidelines in practice.

Practical considerations to perform pharmacokinetic studies of TB drugs in children need to be considered as well. Blood sampling in young children may be challenging and patient unfriendly and the possibility of using saliva as opposed to plasma samples was therefore explored for the pivotal TB drug rifampicin (chapter 4). The prediction of rifampicin plasma concentrations based on salivary concentrations was sufficiently accurate, but precision was insufficient. This means that salivary concentrations of rifampicin in individual patients can only be used for a semi-quantitative assessment of rifampicin plasma concentrations or to differentiate between low, intermediate or high rifampicin plasma concentrations. This is not useful in pharmacokinetic studies, but may be sufficient for treatment monitoring in patient care.

Vulnerable groups with increased risk of sub-optimal treatment response

Low plasma levels of TB drugs is an important cause of poor treatment outcome and a reason for development of resistance[19-27] which is caused by poor adherence to medication and large inter-individual variability in exposure to TB drugs[25, 26]. Furthermore, a suboptimal functioning of the immune system also predisposes to treatment failure. For instance, compared to adults, the innate pulmonary defenses are often impaired in neonates and young children, allowing mycobacteria to grow. Adults have an increased risk to develop active TB disease when a medical condition develops that weakens the immune system such as HIV infection, substance abuse, silicosis, diabetes mellitus, severe kidney disease, low body weight, cancer, or the use of immune suppressive treatments such as corticosteroids or specialized treatment for organ transplants or autoimmune diseases like rheumatoid arthritis or Crohn's disease[1].

The Tanzanian National Tuberculosis and Leprosy Control Program (NTLP) generally has been successful as high rates of adherence and successful treatment outcomes have been reported[28-31]. In the present thesis, the focus is therefore on vulnerable groups that may profit from a better insight into different aspects of treatment. A comparison with data obtained from TB patients from the general populations was also done.

Chapter 2 focused on the childhood TB treatment whereby we have demonstrated a relatively low percentage (79.9%) of treatment success among the pediatric population in the Kilimanjaro region in Tanzania. The WHO target of at least 85% needs to be strived through prioritizing pediatric TB by the national TB program, active case finding, evaluating new diagnostic tools and closely supervising treatment.

Moreover, WHO revised dosing guidelines for children[16] should be followed which result in exposures to TB drugs in plasma similar to those in adults.

Chapter 6 focused on patients with TB and diabetes. Concomitant TB and diabetes mellitus is a growing and challenging co-morbidity in developing countries. The co-morbidity is of public health importance because 1) diabetes mellitus is increasing in developing countries, 2) TB is endemic in these countries, and 3) diabetes strongly predisposes to TB[32-36]. Having few studies in Africa that try to explain the increased risk of TB in diabetic patients and the consequent poor response to treatment among TB/diabetes co-morbid patients, we were prompted to design a study to assess whether diabetes disease affects the pharmacokinetics of TB drugs in African patients. We set up the first comparative pharmacokinetic study in Africans in this respect, involving intensive (24hr) sampling to evaluate the pharmacokinetics of rifampicin, isoniazid, pyrazinamide, and ethambutol in two TB groups, with and without diabetes mellitus.

We found that the total exposure to rifampicin and isoniazid was lower in diabetic versus non-diabetic TB patients. A large effect was seen for isoniazid for which the maximum concentration was also significantly decreased. Previous studies have indeed demonstrated that diabetes may reduce the absorption and distribution of drugs[37-40]. Increasing the doses of the TB drugs is therefore advocated for diabetic patients, as well as treatment response monitoring. Although more studies in diabetic TB patients are needed, available evidence suggests higher doses of rifampicin and isoniazid have been shown to be safe and tolerable in TB patients[41-45]. The Kilimanjaro Clinical Research Institute is one of the trial sites for two studies that aimed to evaluate higher doses of rifampicin. In the first study, higher doses of 900mg (circa 15 mg/kg) and 1200mg (20 mg/kg) rifampicin were evaluated for efficacy and tolerability. The second study evaluated 35 mg/kg of rifampicin in combination with other TB drugs. Preliminary data of these studies indicate the higher doses were well tolerated by Tanzanian patients.

It is equally important to optimize treatment of diabetes alongside that for TB. Drug interactions are known to occur between rifampicin and all sulphonylurea derivatives, which are widely used in treating diabetes patients worldwide. On the other hand the biguanide metformin was thought not to be affected by rifampicin as it is not metabolized by the hepatic cytochrome P450 system[25, 26]. However, its exposure may still be influenced, as it is a substrate for human organic cation and other transporters. In healthy volunteers, rifampicin appeared to increase organic cation transporter (OCT1) expression and hepatic uptake of metformin, leading to an enhanced (rather than reduced) glucose lowering effect in healthy individuals[46]. Clearly, a follow-up

study in actual TB patients is needed. Metformin is also widely used in Tanzania. In view of the strong effect of rifampicin on the exposure to sulphonylurea antidiabetics and pending more data on the combination with rifampicin, this drug is recommended in diabetic patients who are also undergoing TB treatment. Besides this, optimal glycemic control need to be ensured as it, in itself, enhances efficacy of TB treatment and prevents complications from diabetes disease.

In chapter 7, HIV-TB co-infected patients were studied. HIV is a well known risk factor for TB and it alters the clinical course of TB disease by lowering CD4+ T-lymphocytes that play an important role in the body's defense against tubercle bacilli[1]. The concomitant treatment of these two infections poses various challenges due to drug-drug interactions, immune reconstitution syndrome (IRIS) and high pill burden[47, 48]. This challenge has compelled clinicians to recommend the delay of HIV treatment until the TB has been controlled, or to even stopping or changing HIV medication if the person develops TB while already taking HIV drugs.
A fixed dose of emtricitabine/tenofovir/efavirenz (Atripla) that has been approved in the USA and EU is very effective, administered only once a day and thought to be potentially useful in TB infected HIV patients. We therefore carried out a clinical trial to evaluate its use in TB patients, in particular focus on drug-drug interactions and efficacy of its concomitant administration with first line TB drugs.

A number of observations were made during this clinical trial. At first, IRIS was not observed in any of our patients in the clinical setup of that study and the combination of the first line TB drugs with emtricitabine/tenofovir/efavirenz was well tolerated. There were no excess adverse events attributed to the combined use of the two regimens, as the frequency of adverse events before and after anti-retroviral therapy (ART) were essentially the same (96% of patients experienced at least an adverse event before ART compared to 92% after) and these were mild to moderate. There were no serious adverse events and no modification or discontinuation of treatment was made. This makes it apparently safe to start TB/HIV patients on Atripla early on during the course of TB treatment.
Secondly, there were no relevant interactions observed in the pharmacokinetic analysis. Our study was the first to report on the interaction of first line TB drugs with the combination emtricitabine/tenofovir/efavirenz in the fixed dose combination-Atripla. The pharmacokinetic parameters (AUC, C_{max}, and C_{min}) of emtricitabine, tenofovir, and efavirenz were not significantly altered upon co-administration with rifampicin and other TB drugs, as based on geometric mean ratios. The other way around, the pharmacokinetic parameter C_{max} of the TB drugs rifampicin, isoniazid, pyrazinamide, and ethambutol were not significantly altered too (Table 2 and 3, chapter 7). This is contrary to observations in other studies showing that exposure to

TB drugs may be decreased in HIV infected patients[49-51]. Interestingly, in our study, we observed slightly higher levels of efavirenz when co-administered with rifampicin based treatment, contrary to the expected induction by rifampicin[27]. Although we did not perform pharmacogenetic analysis in our study, we think genetic polymorphism of the cytochrome P450 (CYP) system would explain the observed slight increase in efavirenz concentration. CYP2B6, the main metabolic enzyme for efavirenz, is highly polymorphic. Certain CYP2B6 polymorphysms are known to be associated with reduced enzyme activity and hence elevated plasma levels of efavirenz[52-57]. Although we did not perform pharmacogenetic analysis in our study, we think genetic polymorphism of the cytochrome P450 (CYP) system would explain the observed slight increase in efavirenz concentration. CYP2B6, the main metabolic enzyme for efavirenz, is highly polymorphic. Certain CYP2B6 polymorphysms are known to be associated with reduced enzyme activity and hence elevated plasma levels of efavirenz[52-57]. Thus an increase in efavirenz dose during rifampicin containing therapy may not be necessary in individuals with this slower metabolizing genotype. In addition, the slower metabolizing genotype may be less susceptible to induction by rifampicin. Of note, the polymorphisms differ greatly among populations of different ethnic backgrounds[58, 59]. The incidence of the slow metabolizing genotype is about 25% in African and Indian populations. Consequently, dosage increases of efavirenz during co-administration with rifampicin as based on studies in Caucasians are not directly applicable to African populations.

Thirdly, the efficacy of both antiretroviral therapy and anti-TB therapy was not compromised by the co-administration. As it would be expected with Atripla alone, there was a significant and acceptable increase in CD4 count among the patients after 28 weeks of ART. Similarly, for anti-TB efficacy, 76% of the patients had converted to smear negative by the end of eight weeks of TB treatment, and at 12 weeks all patients had converted to smear negative and culture negative at the end of TB treatment.

The general population of patients with pulmonary TB

Evaluating the pharmacokinetics of TB drugs in the general Tanzanian population (chapter 3), we found that the plasma levels of key TB drugs in the majority of these patients was low. More specifically, we found that half of study participants had isoniazid peak plasma (C_{max}) levels and a third had rifampicin peak plasma concentrations below the reference ranges. A second group of TB patients from the general population was studied whereby a plasma drug assay (chapter 5) was evaluated. Also in that study, low mean peak levels (estimated by C_{2hr}) of rifampicin and isoniazid (2.5 and 1.85 mg/L respectively) were recorded.

These observed widespread low peak levels in our studies do not correspond directly to the recorded adverse treatment outcome in Tanzanian TB patients. As an explanation, it should be noted that the reference ranges for peak plasma concentrations of TB drugs represent the normal (average) concentrations that can be expected in adults with standard doses of TB drugs[60]. These reference values may not necessarily represent the therapeutic range or optimal concentrations.

In line with this is the concept that the total exposure (AUC) is a more relevant predictor of efficacy than peak plasma concentrations for all first line TB drugs, as evidenced by preclinical studies[23, 61-65]. The relevance of AUC has also been demonstrated by a recent clinical study[66], showing that AUC values of pyrazinamide, rifampicin and isoniazid were the top 3 predictors of long-term response to TB drugs. This study described a cut-off therapeutic value for total exposure or AUC (to pyrazinamide of 363 h*mg/L. This exposure cut-off value is high and suggests that the dose of pyrazinamide should be increased to achieve maximum response, as even the average exposure to pyrazinamide in our study (344 h*mg/L) was below this cut-off value. Our patients also had slightly lower average values of pyrazinamide (344 h*mg/L) and rifampicin (39.9 h*mg/L) compared to an Indonesian (473 and 48.5 respectively)[41] and mixed population in the Netherlands (480 and 41.1 respectively)[67]. The dose and exposure to rifampicin can be strongly and safely increased based on studies performed at the Kilimanjaro Clinical Research Institute and other sites (see before) but the same may not apply to pyrazinamide. A large number of reports in the past have shown a significant relationship between pyrazinamide dose and hepatotoxicity. Based on this, the currently recommended dose (15-30 mg/kg daily) was proposed and it has significantly less hepatotoxicity. A recent meta-analysis of 29 studies (some dating back to 1952), however, did not show a significant relationship between pyrazinamide dose and hepatotoxicity although the data seemed to show a trend[68].

We postulated that the simultaneous administration of TB drugs and food could partly explain the low plasma levels of key TB drugs in our studies. It is common practice in Tanzania that patients take TB treatment in the morning on an empty stomach but that breakfast is consumed soon thereafter. However, it is well known that fat or carbohydrate content of food decreases the rate of isoniazid absorption and therefore significantly reduces the isoniazid C_{max} with 20% to 51%[69-73]. Furthermore, rifampicin C_{max} can be reduced up to 36% when it is taken directly after a meal. We therefore recommend the following: at first, patients are advised to take the TB medication on empty stomach and consume their breakfast after at least 2 hours of time. If nausea or vomiting develops, the TB medication can be taken together with a light meal. Similarly if such intake of drugs on an empty stomach is difficult in the patient's daily life, the TB medication may be administered with breakfast as well as a somewhat lower exposure to TB drugs seems preferable than

poor compliance. The low exposure to TB drugs may also be prevented by increasing the dose of rifampicin and isoniazid when administered with food, as evidence shows that increasing the dose of these TB drugs may result in increased plasma concentrations of the drugs and improved treatment outcome[42], and higher doses of rifampicin and isoniazid have been shown to be safe and tolerable[41-44]. It has not been studied whether an increase in dose compensates for the effect of concurrent food intake on the exposure to rifampicin and isoniazid. This is a relevant topic to be addressed in a future pharmacokinetic study.

Improving management of TB

Successful management and control of TB relies on an appropriate diagnosis, adequate chemotherapy, and treatment monitoring so that treatment failure is rapidly diagnosed and managed.
In resource-limited settings, tuberculosis diagnosis relies mostly on sputum smear microscopy[74, 75]. In Tanzania, all level laboratories perform smear microscopy to diagnose TB. Currently, diagnostic facilities are present in all regions, districts and other primary health care centres. Sputum microscopy has been improved by introducing LED fluorescent microscopy in all 26 regions and 133 districts[29]. Culture is performed in six intermediate laboratories (either public or private), and drug-susceptibility testing is available at the Central Tuberculosis/Leprosy Reference Laboratory in Dar-es-Salaam. Diagnosis in children is challenging due to the atypical nature of the disease in children and difficulty in obtaining adequate clinical samples such as sputum[15, 76, 77]. In chapter 2 we reported that the sputum smear testing rate among children was very low (25%) and that nearly 95% of the children were treated empirically. We strongly recommend the NTLP that the improvement of TB diagnosis and treatment in Tanzanian children is prioritized.

Therapeutic drug monitoring (TDM) with measurements of serum TB drug levels can be used to monitor treatment and adherence[60, 78]. Furthermore, microbiological techniques can be used to monitor treatment response; however, mycobacterial cultures often only become positive after weeks of culture while smear microscopy is inferior. However, TDM involves blood drawing while HPLC assays to analyze serum drug levels are expensive, laborious and logistically challenging in use and maintenance. To avoid blood drawing, we assessed whether saliva could be used as an alternative matrix for TDM and pharmacokinetic studies (Chapter 4) by comparing the pharmacokinetics of rifampicin in saliva and plasma. This would be potentially advantageous for PK studies especially in children, where multiple blood drawing is unwanted. Other advantages of saliva specimen include easy and inexpensive sample

collection, reduction of infection risk and discomfort, and it may be used as a possible tool to measure compliance. In addition, because only the free or unbound fraction of the drug diffuses in saliva, measuring saliva may reflect the active, free fraction of the drug in plasma. It should be noted that measuring drug concentration in serum yields the total concentration, i.e. combination of bound and free drug that are in equilibrium with each other; and that only the free or unbound fraction is active.
As expected, measurable rifampicin concentrations were not detected in all plasma and saliva samples, as rifampicin is having a short elimination half-life. Pharmacokinetic parameters of rifampicin both in plasma and saliva and their ratios are shown in chapter 4. Our main findings were that the exposure to salivary rifampicin was significantly lower than the protein –unbound exposure in plasma. Furthermore, it was evident from the study that the salivary rifampicin was able to predict total or protein-unbound plasma concentrations without bias, but imprecision was too large. We conclude therefore that salivary concentrations of rifampicin cannot be used as a substitute for plasma or serum concentrations in therapeutic drug monitoring or pharmacokinetic studies. Saliva may however be used in patient care, as semi-quantitative estimation can be made, e.g. whether a drug level is estimated low - intermediate - high.

Finally, because of the high cost of routine therapeutic drug monitoring with plasma drug measurement, we investigated the possibility of developing a less expensive but feasible approach with a plasma drug assay using patient's own plasma and organisms (chapter 5). More specifically, plasma TB drug activity assay (TDA) measures the time to positivity of organisms cultured in plasma relative to control organisms cultured in plain media. Apart from its use in assessing treatment response, TDA is a possible biomarker of long term treatment outcome[79-81]. A TDA value of 1 means no relative difference in killing and a TDA of more than 1, more killing in plasma than control. For this purpose, MTB was isolated from each patient from sputum collected prior to initiation of treatment. After two weeks of treatment, the previously isolated MTB was re-cultured with patients' own plasma (which contains drugs taken by the patient) and the killing of the bacteria was subsequently assessed with the MGIT 960 system. Calibration of the assay was done with known organisms and serial drug concentrations. The performance of the assay was compared to plasma drug measurement with HPLC. We found that TDA correlated with plasma levels, MIC and short-term treatment response. MDR-TB strains were also assessed using the assay, whereby TDA values close to 1 were documented. Interestingly, as mentioned before, HPLC analysis showed that the peak plasma levels of rifampicin and isoniazid were low in most of the patients, with 88% and 69% of patients respectively having concentrations below expected ranges.

In general, whole-blood and plasma bactericidal assays, and other biomarkers are important for the evaluation of new drugs that can be used to shorten TB treatment, and in monitoring treatment response. Other biomarkers include 2-month culture conversion, serial colony counts or time to culture positivity, measurement of mycobacterial RNA (mRNA and rRNA, the so called mycobacterial load assay (MBL) in sputum), and measurement of human cytokines responses[81-86]. The only biomarker that has been shown to correlate with long-term treatment outcome is the 2-month culture conversion, however, the results of this assay are only available after a minimum of 2-3 months of treatment. Serial sputum colony forming unit (CFU) count measurement (SSCC) constitutes the Early Bactericidal Activity (EBA; a biomarker that evaluates the fall in CFUs during the first two days of treatment), or an extended-EBA (e.g. two weeks or two months). Actually, EBA probably does not detect the sterilizing activity of anti-TB drugs, while the performance to detect the sterilizing activity of an extended-EBA or a SSCC still needs to be established. mRNA and cytokine measurement may be done faster but unlike whole-blood and plasma bactericidal assays, they are complex, expensive and may need to wait until after two months of treatment[85, 86]. Whole-blood and plasma bactericidal assays have demonstrated the advantage of being relatively cheap, simple to perform, and can be carried out as early as two weeks after initiation of treatment. The results from our study suggest that, given further validation, whole-blood and plasma bactericidal assays may be useful adjunct tools to optimize TB therapy in resource-limited settings where plasma drug measurements with HPLC is not feasible.

In conclusion, Pediatric TB is a public health concern in Tanzania, underscoring the national TB program and other stakeholders to prioritize interventions addressing this vulnerable group. A considerable proportion of the general population of TB patients achieve low plasma concentrations of TB drugs probably due to interpersonal variability or concurrent drug and food intake; moreover, diabetic TB patients achieve low plasma concentrations of TB drugs possibly due to the effect of the diabetes disease. These groups may need optimization of treatment and increasing the doses of key TB drugs such as rifampicin and isoniazid. HIV co-infected TB patients can safely be treated with the fixed dose combination of emtricitabine/tenofovir/efavirenz early on the course of TB treatment.

WHO launched an ambitious "Stop TB" campaign to eliminate TB as a public health problem, defined as a global incidence of active TB of less than one case per 1 million population per year, by 2050[87]. The Millennium Development Goal for TB is to reverse TB incidence by 2015. WHO reports that the world is reaching that target, however incidence is falling very slowly. In addition, all regions, except Africa and Europe, are on track to achieve the Stop TB Partnership target of 50% decline in mortality by 2015.

Many actions still need to be taken therefore which involves both public and private institutions. Steps that we need to execute include commitment (professional, political and financial) by authorities, active surveillance and quest into the burden and into ways of improving diagnosis and rigorous treatment of TB.

Directions for future research

Our results have enlightened on the burden of TB and approaches to improving its management, but also stimulated areas of further research:

1. Optimization of TB diagnosis in children as diagnosis of TB in children is challenging due to the nature of the disease, investigating the best clinical criteria to diagnosing the disease, along with studying the novel laboratory diagnostic approaches is crucial.
2. Optimization of the dose of the currently available TB drugs in order to achieve higher exposures and improved efficacy. The dose of rifampicin is currently being investigated, also at the Kilimanjaro Clinical Research Institute, but the efficacy and toxicity of higher doses of pyrazinamide warrants more study as well. The dose of newly developed TB drugs should also be based upon pharmacokinetic-pharmacodynamic relationships assessed for these drugs.
3. Plasma TB drug activity assay (TDA) - from our study on the Plasma TB drug activity assay, TDA is shown to correlate with short-term treatment response that we were able to document. Further validation of this assay is required with larger number of patients and as capacity building to clinical laboratories in the general practice.
4. Optimization of diabetes and TB medication when prescribed concurrently.
5. Genetics and drug metabolism of efavirenz, as efavirenz has been demonstrated to be metabolized by CYP2B6. These enzymes exhibit ethnic and inter-individual differences in the expression or activity levels. Studies on genotyping of the gene variants associated with altered expression of CYP2B6 are recommended.

References

1. S. D. Lawn and A. I. Zumla, "Tuberculosis," *Lancet* 378, no. 9785 (2011): 57-72.
2. World Health Organization. Global tuberculosis report 2013, WHO, available at http://www.who.int/tb/publications/global_report/en/. 2013.
3. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2006. 2007.
4. B. Rekha and S. Swaminathan, "Childhood Tuberculosis - Global Epidemiology and the Impact of HIV," *Paediatr.Respir.Rev.* 8, no. 2 (2007): 99-106.
5. World Health Organization. *Global tuberculosis control: surveillance, planning, financing. WHO report 2006.* Geneva. WHO/HTM/TB/2006.362. 2006.
6. A. D. Harries et al., "Childhood Tuberculosis in Malawi: Nationwide Case-Finding and Treatment Outcomes," *Int.J.Tuberc.Lung Dis.* 6, no. 5 (2002): 424-431.
7. L. J. Nelson and C. D. Wells, "Global Epidemiology of Childhood Tuberculosis", *Int.J.Tuberc.Lung Dis.* 8, no. 5 (2004): 636-647.
8. R. Lolekha et al., "Childhood TB Epidemiology and Treatment Outcomes in Thailand: a TB Active Surveillance Network, 2004 to 2006," *BMC Infect.Dis* 8 (2008): 94.
9. Rie A. van et al., "Childhood Tuberculosis in an Urban Population in South Africa: Burden and Risk Factor," *Arch.Dis Child* 80, no. 5 (1999): 433-437.
10. L. J. Nelson et al., "Epidemiology of Childhood Tuberculosis in the United States, 1993-2001: the Need for Continued Vigilance," *Pediatrics* 114, no. 2 (2004): 333-341.
11. World Health Organization. A research agenda for childhood tuberculosis: improving the management of childhood tuberculosis within national tuberculosis programmes: research priorities based on a literature review.Geneva, Switzerland. 2007.
12. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children.WHO/HTM/TB/2006.371. Geneva, Switzerland. 2006.
13. World Health Organization. WHO Tuberculosis programme.Framework for effective tuberculosis control. WHO/TB/94.179.Geneva, Switzerland. 1994.
14. P. R. Donald, D. Maher, and S. Qazi, "A Research Agenda to Promote the Management of Childhood Tuberculosis Within National Tuberculosis Programmes," *Int.J.Tuberc.Lung Dis.* 11, no. 4 (2007): 370-380.
15. S. M. Graham et al., "Childhood Tuberculosis: Clinical Research Needs," *Int.J.Tuberc.Lung Dis.* 8, no. 5 (2004): 648-657.
16. World Health Organization. Rapid advice: Treatment of tuberculosis in children. Available at http:apps.who.int/medicinedocs/documents/s19925en/s19925en.pdf. 2010.
17. M. Zhu et al., "Pharmacokinetics of Ethambutol in Children and Adults With Tuberculosis," *Int.J.Tuberc.Lung Dis.* 8, no. 11 (2004): 1360-1367.
18. M. Zhu et al., "Population Pharmacokinetic Modeling of Pyrazinamide in Children and Adults With Tuberculosis," *Pharmacotherapy* 22, no. 6 (2002): 686-695.
19. M. E. Kimerling et al., "Low Serum Antimycobacterial Drug Levels in Non-HIV-Infected Tuberculosis Patients," *Chest* 113, no. 5 (1998): 1178-1183.
20. J. B. Mehta et al., "Utility of Rifampin Blood Levels in the Treatment and Follow-Up of Active Pulmonary Tuberculosis in Patients Who Were Slow to Respond to Routine Directly Observed Therapy," *Chest* 120, no. 5 (2001): 1520-1524.
21. M. Weiner et al., "Low Isoniazid Concentrations and Outcome of Tuberculosis Treatment With Once-Weekly Isoniazid and Rifapentine," *Am.J.Respir.Crit Care Med.* 167, no. 10 (2003): 1341-1347.
22. M. Weiner et al., "Association Between Acquired Rifamycin Resistance and the Pharmacokinetics of Rifabutin and Isoniazid Among Patients With HIV and Tuberculosis," *Clin.Infect.Dis.* 40, no. 10 (2005): 1481-1491.
23. S. Srivastava et al., "Multidrug-Resistant Tuberculosis Not Due to Noncompliance but to Between-Patient Pharmacokinetic Variability," *J.Infect.Dis.* 204, no. 12 (2011): 1951-1959.
24. S. Chideya et al., "Isoniazid, Rifampin, Ethambutol, and Pyrazinamide Pharmacokinetics and Treatment Outcomes Among a Predominantly HIV-Infected Cohort of Adults With Tuberculosis From Botswana," *Clin.Infect.Dis.* 48, no. 12 (2009): 1685-1694.

25. A. Tornio et al., "Drug Interactions With Oral Antidiabetic Agents: Pharmacokinetic Mechanisms and Clinical Implications," *Trends Pharmacol.Sci.* 33, no. 6 (2012): 312-322.
26. H. Sun and D. O. Scott, "Impact of Genetic Polymorphisms of Cytochrome P450 2 C (CYP2C) Enzymes on the Drug Metabolism and Design of Antidiabetics," *Chem.Biol.Interact.* 194, no. 2-3 (2011): 159-167.
27. A. Kwara et al., "Paradoxically Elevated Efavirenz Concentrations In HIV/Tuberculosis-Coinfected Patients With CYP2B6 516TT Genotype on Rifampin-Containing Antituberculous Therapy," *AIDS* 25, no. 3 (2011): 388-390.
28. A. Mkopi et al., "Adherence to Tuberculosis Therapy Among Patients Receiving Home-Based Directly Observed Treatment: Evidence From the United Republic of Tanzania," *PLoS.ONE.* 7, no. 12 (2012): e51828.
29. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2011. 2012.
30. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2010. 2011.
31. Ministry of Health and Social Welfare. National tuberculosis and Leprosy control Programme-Annual report-2009. 2010.
32. C. Y. Jeon and M. B. Murray, "Diabetes Mellitus Increases the Risk of Active Tuberculosis: a Systematic Review of 13 Observational Studies," *PLoS.Med.* 5, no. 7 (2008): e152.
33. F. Young et al., "A Review of Co-Morbidity Between Infectious and Chronic Disease in Sub Saharan Africa: TB and Diabetes Mellitus, HIV and Metabolic Syndrome, and the Impact of Globalization," *Global.Health* 5 (2009): 9.
34. K. E. Dooley and R. E. Chaisson, "Tuberculosis and Diabetes Mellitus: Convergence of Two Epidemics," *Lancet Infect.Dis.* 9, no. 12 (2009): 737-746.
35. R. Ruslami et al., "Implications of the Global Increase of Diabetes for Tuberculosis Control and Patient Care," *Trop.Med.Int.Health* 15, no. 11 (2010): 1289-1299.
36. M. A. Baker et al., "The Impact of Diabetes on Tuberculosis Treatment Outcomes: a Systematic Review," *BMC.Med.* 9 (2011): 81.
37. M. Dostalek, F. Akhlaghi, and M. Puzanovova, "Effect of Diabetes Mellitus on Pharmacokinetic and Pharmacodynamic Properties of Drugs," *Clin.Pharmacokinet.* 51, no. 8 (2012): 481-499.
38. P. R. Gwilt, R. R. Nahhas, and W. G. Tracewell, "The Effects of Diabetes Mellitus on Pharmacokinetics and Pharmacodynamics in Humans," *Clin.Pharmacokinet.* 20, no. 6 (1991): 477-490.
39. A. K. Cashion et al., "Gastroparesis Following Kidney/Pancreas Transplant," *Clin.Transplant.* 18, no. 3 (2004): 306-311.
40. N. Okabe and N. Hashizume, "Drug Binding Properties of Glycosylated Human Serum Albumin As Measured by Fluorescence and Circular Dichroism," *Biol.Pharm.Bull.* 17, no. 1 (1994): 16-21.
41. R. Ruslami et al., "Pharmacokinetics and Tolerability of a Higher Rifampin Dose Versus the Standard Dose in Pulmonary Tuberculosis Patients," *Antimicrob.Agents Chemother.* 51, no. 7 (2007): 2546-2551.
42. C. Magis-Escurra et al., "Therapeutic Drug Monitoring in the Treatment of Tuberculosis Patients," *Pulm. Pharmacol.Ther.* 25, no. 1 (2012): 83-86.
43. K. R. Steingart et al., "Higher-Dose Rifampin for the Treatment of Pulmonary Tuberculosis: a Systematic Review," *Int.J.Tuberc.Lung Dis.* 15, no. 3 (2011): 305-316.
44. S. K. Katiyar et al., "A Randomised Controlled Trial of High-Dose Isoniazid Adjuvant Therapy for Multidrug-Resistant Tuberculosis," *Int.J.Tuberc.Lung Dis.* 12, no. 2 (2008): 139-145.
45. A. H. Diacon et al., "Early Bactericidal Activity of High-Dose Rifampin in Patients With Pulmonary Tuberculosis Evidenced by Positive Sputum Smears," *Antimicrob.Agents Chemother.* 51, no. 8 (2007): 2994-2996.
46. S. K. Cho et al., "Rifampin Enhances the Glucose-Lowering Effect of Metformin and Increases OCT1 MRNA Levels in Healthy Participants," *Clin.Pharmacol.Ther.* 89, no. 3 (2011): 416-421.
47. M. Muller et al., "Immune Reconstitution Inflammatory Syndrome in Patients Starting Antiretroviral Therapy for HIV Infection: a Systematic Review and Meta-Analysis," *Lancet Infect.Dis.* 10, no. 4 (2010): 251-261.
48. S. D. Lawn et al., "Tuberculosis-Associated Immune Reconstitution Disease: Incidence, Risk Factors and Impact in an Antiretroviral Treatment Service in South Africa," *AIDS* 21, no. 3 (2007): 335-341.

49. H. McIlleron et al., "Determinants of Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Pharmacokinetics in a Cohort of Tuberculosis Patients," *Antimicrob.Agents Chemother.* 50, no. 4 (2006): 1170-1177.
50. Gurumurthy et al., "Decreased Bioavailability of Rifampin and OtherAntituberculosis Drugs in Patients With Advanced Human Immunodeficiency Virus Disease," *Antimicrob.Agents Chemother.* 48, no. 11 (2004): 4473-4475.
51. J. Sahai et al., "Reduced Plasma Concentrations of Antituberculosis Drugs in Patients With HIV Infection," *Ann.Intern.Med.* 127, no. 4 (1997): 289-293.
52. Z. Desta et al., "Impact of CYP2B6 Polymorphism on Hepatic Efavirenz Metabolism in Vitro," *Pharmacogenomics.* 8, no. 6 (2007): 547-558.
53. D. W. Haas et al., "Associations Between CYP2B6 Polymorphisms and Pharmacokinetics After a Single Dose of Nevirapine or Efavirenz in African Americans," *J.Infect.Dis.* 199, no. 6 (2009): 872-880.
54. V. Powers, J. Ward, and M. Gompels, "CYP2B6 G516T Genotyping in a UK Cohort of HIV-Positive Patients: Polymorphism Frequency and Influence on Efavirenz Discontinuation," *HIV.Med.* 10, no. 8 (2009): 520-523.
55. J. King and J. A. Aberg, "Clinical Impact of Patient Population Differences and Genomic Variation in Efavirenz Therapy," *AIDS* 22, no. 14 (2008): 1709-1717.
56. M. Swart et al., "High Predictive Value of CYP2B6 SNPs for Steady-State Plasma Efavirenz Levels in South African HIV/AIDS Patients," *Pharmacogenet.Genomics* 23, no. 8 (2013): 415-427.
57. J. Bertrand et al., "Dependence of Efavirenz- and Rifampicin-Isoniazid-Based Antituberculosis Treatment Drug-Drug Interaction on CYP2B6 and NAT2 Genetic Polymorphisms: ANRS 12154 Study in Cambodia," *J.Infect.Dis.* 209, no. 3 (2014): 399-408.
58. V. Lamba et al., "Hepatic CYP2B6 Expression: Gender and Ethnic Differences and Relationship to CYP2B6 Genotype and CAR (Constitutive Androstane Receptor) Expression," *J.Pharmacol.Exp.Ther.* 307, no. 3 (2003): 906-922.
59. P. Arnaldo et al., "Frequencies of Cytochrome P450 2B6 and 2C8 Allelic Variants in the Mozambican Population," *Malays.J.Med.Sci.* 20, no. 4 (2013): 13-23.
60. C. A. Peloquin, "Therapeutic Drug Monitoring in the Treatment of Tuberculosis," *Drugs* 62, no. 15 (2002): 2169-2183.
61. R. Jayaram et al., "Pharmacokinetics-Pharmacodynamics of Rifampin in an Aerosol Infection Model of Tuberculosis," *Antimicrob.Agents Chemother.* 47, no. 7 (2003): 2118-2124.
62. R. Jayaram et al., "Isoniazid Pharmacokinetics-Pharmacodynamics in an Aerosol Infection Model of Tuberculosis," *Antimicrob.Agents Chemother.* 48, no. 8 (2004): 2951-2957.
63. T. Gumbo et al., "Concentration-Dependent Mycobacterium Tuberculosis Killing and Prevention of Resistance by Rifampin," *Antimicrob.Agents Chemother.* 51, no. 11 (2007): 3781-3788.
64. T. Gumbo et al., "Isoniazid Bactericidal Activity and Resistance Emergence: Integrating Pharmacodynamics and Pharmacogenomics to Predict Efficacy in Different Ethnic Populations," *Antimicrob.Agents Chemother.* 51, no. 7 (2007): 2329-2336.
65. T. Gumbo et al., "Pharmacokinetics-Pharmacodynamics of Pyrazinamide in a Novel in Vitro Model of Tuberculosis for Sterilizing Effect: a Paradigm for Faster Assessment of New Antituberculosis Drugs," *Antimicrob.Agents Chemother.* 53, no. 8 (2009): 3197-3204.
66. J. G. Pasipanodya et al., "Serum Drug Concentrations Predictive of Pulmonary Tuberculosis Outcomes," *J.Infect.Dis.* 208, no. 9 (2013): 1464-1473.
67. C. Magis-Escurra et al., "Population Pharmacokinetics and Limited Sampling Strategy for First-Line Tuberculosis Drugs and Moxifloxacin," *Int.J.Antimicrob.Agents* (2014).
68. J. G. Pasipanodya and T. Gumbo, "Clinical and Toxicodynamic Evidence That High-Dose Pyrazinamide Is Not More Hepatotoxic Than the Low Doses Currently Used," *Antimicrob.Agents Chemother.* 54, no. 7 (2010): 2847-2854.
69. M. V. Joshi et al., "Food Reduces Isoniazid Bioavailability in Normal Volunteers," *J.Assoc.Physicians India* 39, no. 6 (1991): 470-471.
70. C. Zent and P. Smith, "Study of the Effect of Concomitant Food on the Bioavailability of Rifampicin, Isoniazid and Pyrazinamide," *Tuber.Lung Dis.* 76, no. 2 (1995): 109-113.
71. C. A. Peloquin et al., "Pharmacokinetics of Isoniazid Under Fasting Conditions, With Food, and With Antacids," *Int.J.Tuberc.Lung Dis.* 3, no. 8 (1999): 703-710.

72. M. Y. Lin et al., "Impact of Food and Antacids on the Pharmacokinetics of Anti-Tuberculosis Drugs: Systematic Review and Meta-Analysis," *Int.J.Tuberc.Lung Dis.* 14, no. 7 (2010): 806-818.
73. C. A. Peloquin et al., "Pharmacokinetics of Rifampin Under Fasting Conditions, With Food, and With Antacids," *Chest* 115, no. 1 (1999): 12-18.
74. K. R. Steingart, A. Ramsay, and M. Pai, "Optimizing Sputum Smear Microscopy for the Diagnosis of Pulmonary Tuberculosis," *Expert.Rev.Anti.Infect.Ther.* 5, no. 3 (2007): 327-331.
75. L. M. Parsons et al., "Laboratory Diagnosis of Tuberculosis in Resource-Poor Countries: Challenges and Opportunities," *Clin.Microbiol.Rev.* 24, no. 2 (2011): 314-350.
76. H. J. Zar, "Diagnosis of Pulmonary Tuberculosis in Children--What's New?," *S.Afr.Med.J.* 97, no. 10 Pt 2 (2007): 983-985.
77. A. C. Hesseling et al., "A Critical Review of Diagnostic Approaches Used in the Diagnosis of Childhood Tuberculosis," *Int J Tuberc Lung Dis* 6, no. 12 (2002): 1038-1045.
78. C. A. Peloquin, "Pharmacological Issues in the Treatment of Tuberculosis," *Ann.N.Y.Acad.Sci.* 953 (2001): 157-164.
79. R. S. Wallis et al., "A Whole Blood Bactericidal Assay for Tuberculosis," *J.Infect.Dis.* 183, no. 8 (2001): 1300-1303.
80. R. S. Wallis et al., "Whole Blood Bactericidal Activity During Treatment of Pulmonary Tuberculosis," *J.Infect.Dis.* 187, no. 2 (2003): 270-278.
81. R. S. Wallis et al., "Biomarkers and Diagnostics for Tuberculosis: Progress, Needs, and Translation into Practice," *Lancet* 375, no. 9729 (2010): 1920-1937.
82. R. Brindle, J. Odhiambo, and D. Mitchison, "Serial Counts of Mycobacterium Tuberculosis in Sputum As Surrogate Markers of the Sterilising Activity of Rifampicin and Pyrazinamide in Treating Pulmonary Tuberculosis," *BMC.Pulm.Med.* 1 (2001): 2.
83. D. A. Mitchison, "Assessment of New Sterilizing Drugs for Treating Pulmonary Tuberculosis by Culture at 2 Months," *Am.Rev.Respir.Dis.* 147, no. 4 (1993): 1062-1063.
84. D. A. Mitchison, "Modern Methods for Assessing the Drugs Used in the Chemotherapy of Mycobacterial Disease," *Soc.Appl.Bacteriol.Symp.Ser.* 25 (1996): 72S-80S.
85. L. E. Desjardin et al., "Measurement of Sputum Mycobacterium Tuberculosis Messenger RNA As a Surrogate for Response to Chemotherapy," *Am.J.Respir.Crit Care Med.* 160, no. 1 (1999): 203-210.
86. R. Ribeiro-Rodrigues et al., "Sputum Cytokine Levels in Patients With Pulmonary Tuberculosis As Early Markers of Mycobacterial Clearance," *Clin.Diagn.Lab Immunol.* 9, no. 4 (2002): 818-823.
87. World Health Organization. The global plan to stop TB. 2011-2015. transforming the fight towards elimination of tuberculosis. Geneva, Switzerland:WHO, 2010. 2010.

CHAPTER 9A

Summary
Samenvatting
Muhtasari

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* that affects mostly the lungs (pulmonary TB -PTB) but also other organs (extra-pulmonary TB) such as meninges, bone, pericardium, kidney, and lymphnodes. It is a global public health problem affecting approximately 9 million people every year. TB is a leading cause of death from a curable infectious disease despite availability of efficacious treatment for decades. About 1.3 million people die from the disease every year. Apart from its direct health related effects, TB also causes a significant loss of productive workforce and the costs of treating it are very high. The burden of TB increased with the spread of HIV because of its negative effects of HIV on the immune system. TB is therefore also a major cause of morbidity and mortality in persons with HIV infection. TB is generally considered a Poverty Related Disease as over 80% of the incident TB cases occur in 22 high-burden countries that are mostly low- or middle-income countries in Africa or South-East Asia. Nine of these countries are in Africa and Tanzania is among them. In 2012 Tanzania had an estimated incidence of 169 per 100,000 inhabitants.

TB treatment is not only challenged by the increases in TB burden and the dual infection with HIV, but also by increasingly resistance formation as well. Multidrug-resistant TB (MDR-TB), defined as resistance to isoniazid and rifampicin has emerged as a major and growing challenge to global TB control efforts. In 2012 there were an estimated 450,000 new cases of MDR-TB worldwide and approximately 170,000 of them died. In that year 1.1% of TB cases in Tanzania were estimated to be MDR-TB. The current treatment of drug susceptible TB comprises a six-month regimen whereby four first line drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) are given for 2 months and dual therapy with isoniazid, rifampicin is given for another 4 month thereafter. Treatment of MDR-TB is however much more complex and lengthy, moreover, the drugs required to treat MDR-TB are expensive and more toxic than the standard TB drugs. Recommended drugs for MDR-TB include Amikacin/Kanamycin, Ofloxacin/Levofloxacin, Pyrazinamide, Ethionamide, Cycloserine and Ethambutol.

A number of knowledge gaps are still present that limit optimal TB management and hamper the fight against this disease. These include aspects of management and epidemiology of TB in children, management of TB in the growing co-morbidities such as diabetes mellitus and HIV. In order to improve the management of TB in Tanzania, a number of studies were designed and subsequently executed. Our focus was to carefully describe the burden of disease in North Tanzania as well as the clinical and pharmacological aspects of the disease that gives insight on how to optimize its treatment. All studies presented in this thesis were conducted in the Kilimanjaro region in Tanzania.

In chapter 2, the burden of TB is described for the pediatric population in the Kilimanjaro region. The epidemiology of TB in this vulnerable and forgotten population is described. We have shown that the burden of childhood TB in the Kilimanjaro region of Tanzania is significant; contributing 13% of all TB cases, and that children have a high mortality rate (10.9%) and relatively low treatment success rate (72.9%) which is worse than the WHO estimates and other reports from other countries like Malawi, Thailand and the United States. We also observed that younger children (0-4 years) are more affected than older children and that testing for acid-fast bacilli (AFB) was very low as only a quarter of the children that were treated for TB were tested by Ziehl-Neelsen staining. We indicated an urgent need of improve the diagnostic process, such as the introduction and evaluation of new tools such as induced sputum, laryngeal swabs and nasopharyngeal aspiration, molecular tests and active case finding.

In Chapter 3 the pharmacokinetics of first line TB drugs is described in Tanzanian patients. Besides poor adherence to treatment, low blood levels of TB drugs are another possible cause for unfavorable treatment outcome and reason for development of resistance. In this study, we found that the plasma levels of key TB drugs in the majority of these patients was low. Specifically, half of study participants had isoniazid peak plasma levels and a third had rifampicin peak plasma concentrations below the reference ranges. Moreover exposure to the drugs were lower compared to other study populations studied outside Africa. We postulated that the simultaneous administration of TB drugs and food could partly explain the low plasma levels of key TB drugs in our studies and recommended further analysis of this association. We also recommended consideration of higher doses of the drugs in order to achieve higher exposures and peak plasma concentrations.

Chapter 4 investigates whether saliva can be used as an alternative matrix in pharmacokinetic studies and therapeutic drug monitoring. This would be potentially advantageous for PK studies especially in children, where extensive blood drawing is unwanted, and also because of the easy and inexpensive sample collection and of saliva specimen. We compared the pharmacokinetics of rifampicin in saliva and plasma. We found out the exposure to salivary rifampicin was significantly lower than the protein –unbound exposure in plasma. Furthermore, it was evident from the study that the salivary rifampicin was not able to predict total or protein-unbound plasma concentration. We concluded that salivary concentrations cannot be used as a substitute for plasma or serum concentrations in therapeutic drug monitoring or pharmacokinetic studies but saliva may however be used in patient care, as semi-quantitative estimation can be made, e.g. whether a drug level is estimated low - intermediate - high.

In Chapter 5 we investigated and set up a plasma culture assay (plasma TB drug activity assay- TDA) for *Mycobacterium tuberculosis* to assess whether it can be an alternative to plasma drug measurement in assessing treatment response and early diagnosis of treatment failure. Therapeutic drug levels monitoring with High-Performance Liquid Chromatography (HPLC) is very expensive whereas blood and plasma drug assays are cheap and easy to perform. Blood/plasma TB drug activity assays measure the time to positivity of organisms cultured in plasma, relative to control (organism cultured in plain media). In the study, patients collected sputum prior to initiation of treatment and this was cultured to isolate the organisms for each patient. At two weeks of treatment, the isolated organisms were re-cultured with patients' own plasma (which contain drugs taken by patient) and killing of the bacteria assessed with the MGIT 960 system. We found out that TDA correlated with plasma levels, MIC and short-term treatment response. From this study it we concluded that, plasma bactericidal assays may be promising tools for optimization of TB therapy in resource-limited settings where plasma drug measurements with HPLC is not feasible. It was recommended to conduct further studies to validate the tooi

In Chapter 6, the effect of type 2 diabetes on the pharmacokinetics of first line TB drugs was analyzed. Concomitant TB and diabetes mellitus is a growing co-morbidity in developing countries as diabetes mellitus is increasing in these countries, where TB is endemic. Diabetes predisposes to TB and diabetic TB patients have poor response to treatment.

We recruited 40 Tanzanian adult TB patients (20 TB only, 20 TB and diabetes) and took blood samples at pre-dose, 1, 2, 3, 4, 6, 8, 10, and 24 hours after observed drug intake. We found out that both exposure and maximum concentration of rifampicin and isoniazid are lower in diabetic than non-diabetic TB patients. Much effect was seen in isoniazid in which clearance and volume of distribution were also adversely affected. The study concluded that these effects are likely explained by the diabetes disease. The recommendation was given to increase the doses of these drugs in treating TB-Diaetes patients as it results in improved exposure.

In chapter 7, HIV-TB co-infected patients were studied where the efficacy, tolerability, and pharmacokinetics of a fixed dose combination of emtricitabine/tenofovir/efavirenz (Atripla) when co-administered with first line TB drugs were investigated. HIV is a well known risk for TB and it alters the clinical course of TB disease. The concomitant treatment of these two infections poses challenge due to drug-drug interactions, immune reconstitution syndrome (IRIS), and high pill burden. The drug-drug interactions are mainly due to the induction effect of the cytochrome P450 enzymes by rifampicin.

We conducted a phase II clinical trial to evaluate the efficacy, tolerability, and pharmacokinetics of a fixed dose combination of emtricitabine/tenofovir/efavirenz when co-administered with first line TB drugs. A number of observations were made during this clinical trial: IRIS was not observed and the combination of the first line TB drugs with emtricitabine/tenofovir/efavirenz was well tolerated. There were no interactions observed. The pharmacokinetic parameters (AUC, C_{max}, and C_{min}) of emtricitabine, tenofovir, and efavirenz were not significantly altered although the levels of efavirenz were slightly higher when co-administered with rifampicin based treatment, contrary to the expected induction by rifampicin. Likewise the pharmacokinetic parameter C_{max} of the TB drugs rifampicin, isoniazid, pyrazinamide, and ethambutol were not significantly altered. The efficacy of both antiretroviral therapy and anti-TB therapy were not compromised by this combination. We concluded that co-administration of the standard fist line TB treatment regimen with emtricitabine, tenofovir, and efavirenz does not alter pharmacokinetic parameters of either drugs to a clinically relevant extent. We recommended future studies to look into patient genetic factors because the metabolism of efavirenz is known to be extensively influenced by pharmacogenetic factors.

In Chapter 8, findings of the all studies are discussed in summary and identification of future steps that need to be taken as well as recommendations to improve the management of TB in Tanzania are provided. It is made clear that the success of the fight needs the joining of forces between all parties; governments, institutions, programmes, organizations, and individuals.

CHAPTER 9B

Samenvatting

Tuberculose (TBC) is een infectieziekte die veroorzaakt wordt door *Mycobacterium tuberculosis* en tast vooral de longen (pulmonale TBC) maar ook andere organen (extra-pulmonale TBC) aan, zoals de hersenvliezen, botten, pericardium, nieren en lymfeklieren. Het is een wereldwijd volksgezondheidsprobleem met ongeveer 9 miljoen nieuwe gevallen per jaar. TBC is de grootste oorzaak van overlijden door een te genezen ziekte, ondanks de beschikbaarheid van effectieve behandeling sinds decennia. Ongeveer 1.3 miljoen mensen per jaar overlijden door door TBC. Naast directe gezondheidseffecten veroorzaakt TBC ook een significant verlies in de productieve werksector en de kosten van behandeling zijn erg hoog. De ziektelast van TBC nam toe met de verspreiding van HIV vanwege de negatieve effecten van HIV op het immuunsysteem. Daarom is TBC een grote veroorzaker van morbiditeit en mortaliteit onder HIV-geïnfecteerde personen. TBC wordt over het algemeen gerelateerd aan armoede omdat 80% van de incidente TB gevallen zich bevindt in 22 landen met een hoge ziektelast. Die landen zijn vooral laag of midden inkomen landen in Afrika of Zuid-Oost Azië. Negen van deze landen liggen in Afrika en Tanzania is een van hen. In 2012 had Tanzania een geschatte incidentie van 169 per 100.000 inwoners.

TBC behandeling is niet alleen een uitdaging vanwege de toename in ziektelast en co-infectie met HIV, maar ook door de toenemende resistentie. Multidrug-resistente TBC (MDR-TBC), gedefinieerd als resistentie tegen isoniazide en rifampicine, is een grote en toenemende uitdaging binnen de wereldwijde TBC controlemaatregelen. In 2012 werden globaal 450.000 nieuwe gevallen van MDR-TBC geschat en ongeveer 170.000 gevallen overleden daaraan. Datzelfde jaar werd geschat dat 1,1% van de TBC gevallen MDR-TBC patiënten in Tanzania waren.
De huidige behandeling van medicatie-gevoelige TBC omvat een zes maanden durend regime waarin de vier eerstelijns geneesmiddelen (isoniazide, rifampicine, pyrazinamide en ethambutol) twee maanden worden gegeven, waarna de behandeling wordt voortgezet met isoniazide en rifampicin gedurende vier maanden. Behandeling van MDR-TBC is echter complexer en duurt langer. Bovendien zijn de medicijnen voor behandeling van MDR-TB duurder en toxischer dan de standaard TBC medicijnen. Aanbevolen medicijnen voor MDR-TBC zijn onder andere Amikacine/Kanamycine, Ofloxacine/Levofloxacine, Pyrazinamide, Ethionamide, Cycloserine en Ethambutol.

Er zijn nog steeds hiaten in de kennis die een optimale behandeling van TBC beperken en de strijd ertegen belemmeren. Deze omvatten aspecten van behandeling en epidemiologie van TBC in kinderen en behandeling van TBC in steeds meer voorkomende co-morbiditeiten zoals diabetes mellitus en HIV-infectie. Om de behandeling van TBC in Tanzania te verbeteren, hebben we een aantal studies opgezet en vervolgens uitgevoerd. Onze focus lag op het zorgvuldig beschrijven van

de ziektelast in Noord-Tanzania alsook de klinische en farmacologische aspecten van de ziekte die inzicht geven in hoe de behandeling kan worden geoptimaliseerd. Alle onderzoeken die in dit proefschrift worden gepresenteerd zijn uitgevoerd in de Kilimanjaro regio.

in hoofdstuk 2 wordt de ziektelast van TBC beschreven in de kinderpopulatie in de Kilimanjaro regio. De epidemiologie van TBC in deze kwetsbare en vergeten groep wordt beschreven. We hebben aangetoond dat de ziektelast van TBC in kinderen in Kilimanjaro in Tanzania significant is; zij bedraagt 13% van alle TBC gevallen, en kinderen hebben een hoge mortaliteit (10.9%) en een laag behandelingssucces (72.9%), hetgeen slechter is dan de WHO-schattingen en rapporten vanuit landen als Malawi, Thailand en de Verenigde Staten. We zagen ook dat jonge kinderen (0-4 jaar) vaker aangedaan zijn dan oudere kinderen en dat testen op zuurvaste bacillen (AFB) weinig plaatsvindt; een kwart van de behandelde kinderen werd getest met Ziehl-Neelsen kleuring. We stelden vast dat het diagnostisch proces met hoge urgentie moet worden verbeterd, zoals middels de introductie en evaluatie van nieuwe middelen zoals geïnduceerd sputum, laryngeale swabs en nasopharyngeale aspiratie, moleculaire testen en actieve bron en contactopsporing.

In hoofdstuk 3 wordt de farmacokinetiek van eerstelijns TBC medicatie beschreven in Tanzaniaanse patiënten. Naast slechte therapietrouw, zijn lage bloedwaarden van TBC medicatie een andere mogelijke oorzaak van slechte behandeluitkomsten en dragen zij bij aan het ontwikkelen van resistentie. In deze studie vonden we dat de plasmaconcentraties van de belangrijkste TBC middelen in de meeste patiënten laag zijn. Meer specifiek had de helft van de studiedeelnemers isoniazide 'piek'plasmaconcentraties en één derde rifampicine piekplasma concentraties beneden de referentiewaarden. Bovendien was de blootstelling aan de medicatie lager dan in andere onderzochte populaties in Afrika. We denken dat de gelijktijdige toediening van TBC medicatie en voedsel deels de lage plasmawaarden van TBC medicatie verklaart en bevelen verder onderzoek van de relatie aan. We bevelen ook aan een hogere dosis te gebruiken om zo hogere blootstelling en piekplasmaconcentraties te kunnen behalen. Hoofdstuk 4 onderzoekt of speeksel gebruikt kan worden als een alternatieve matrix voor farmacokinetische studies en voor het meten van geneesmiddelconcentraties (therapeutische medicatie monitoring). Dit is potentieel voordelig voor farmacokinetisch onderzoek, vooral in kinderen, waar bloedafname minder gewenst is. Daarnaast is speekselafname makkelijk en goedkoop. We vergeleken concentraties van rifampicine in speeksel en plasma. We vonden dat de blootstelling aan speekselrifampicine significant lager was dan de eiwit-ongebonden blootstelling in plasma. Bovendien was het evident in deze studie dat speekselrifampicine niet in staat is om de totale (eiwit-ongebonden plus gebonden) plasma-concentraties van rifampcine te

voorspellen. We concludeerden dat speekselconcentraties niet gebruikt kunnen worden als vervanger van plasma of serumconcentraties in therapeutische medicatie monitoring of in farmacokinetische studies. Echter, speeksel kan gebruikt worden in de patiëntenzorg als semi-kwantitatieve schatting, bijvoorbeeld om na te gaan of de medicatie niveaus laag, middelmatig of hoog zijn.

In hoofdstuk 5 onderzochten en zetten we een zogenaamde 'plasma TBC geneesmiddel activiteit test- TDA) op voor *Mycobacterium tuberculosis* om na te gaan of het een alternatief kan zijn voor plasma medicatiemetingen voor het nagaan van behandelingsresultaat en vroege diagnose van behandelingsfalen. Meting van geneesmiddelconcentraties met High Performance Vloeistof Chromatografie (HPLC) is erg duur terwijl bloed en plasma geneesmiddeltesten goedkoop zijn en makkelijk uit te voeren. Bloed/plasma TBC geneesmiddel activiteit testen meten de tijd tot positiviteit (zichtbare groei) van tuberculosebacteriën gecultiveerd in plasma, vergeleken met controles (bacteriënn gecultiveerd in gewone media). In deze studie verzamelden patiënten sputum voor start van behandeling en dat werd gecultiveerd om bacteriën te isoleren voor iedere patiënt. Na twee weken behandeling werden de geïsoleerde bacteriën gerecultiveerd met het eigen plasma van de patiënten (dat de medicatie bevat die de patiënt heeft genomen). Het doden van de bacteriën werd gemeten met het MGIT 960 systeem. We vonden dat TDA correleerde met plasmaconcentraties, MIC en korte termijns behandelingsresultaat. Op basis van deze studie concludeerden we dat plasma bactericidale testen een veelbelovend middel zijn voor optimalisatie van TBC behandeling in laag-inkomen landen waar plasma geneesmiddelmetingen met HPLC niet bruikbaar zijn. Het werd aanbevolen om verdere studies te doen om de test te valideren.

In hoofdstuk 6 werd het effect van type 2 diabetes op de farmacokinetiek van eerstelijns TBC medicatie geanalyseerd. Gelijktijdige TBC en diabetes mellitus is een toenemende co-morbiditeit in ontwikkelingslanden omdat diabetes mellitus toeneemt in deze landen waar TBC endemisch is. Diabetes maakt iemand vatbaar voor TBC en diabetische TBC patiënten reageren slechter op TB-behandeling. We recruteerden 40 Tanzaniaanse volwassen TBC patiënten (20 alleen TBC, 20 TBC en diabetes) en namen bloed af voor inname van de TBC-geneesmiddelen en op 1, 2, 3, 4, 6, 8, 10, en 24 uur na geobserveerde medicatie-inname. We vonden dat zowel de blootstelling als de maximale (piek) concentratie van rifampicine en isoniazide lager zijn in diabetische dan in non-diabetische TBC patiënten. Een groot effect werd met name gezien voor isoniazide. De studie concludeerde dat deze effecten waarschijnlijk verklaard worden door de diabetes ziekte. De aanbeveling werd gegeven om de dosis van deze medicatie te verhogen in de behandeling van TBC-diabetes patiënten omdat het resulteert in verbeterde blootstelling.

In hoofdstuk 7 werden HIV-TBC co-geïnfecteerde patiënten bestudeerd. We onderzochten de werkzaamheid, verdraagbaarheid en farmacokinetiek van een vaste-dosis-combinatie van emtricitabine/tenofovir/efavirenz (Atripla) dat werd gegeven samen met eerstelijns TBC medicatie. HIV-infectie is een bekend risico voor TBC en het verandert het klinisch beloop van de TBC ziekte. De gelijktijdige behandeling van deze twee infecties is uitdagend door de wisselwerkingen tussen de HIV en TBC-geneesmiddelen, het 'immuun reconstitutie syndroom' (IRIS) en de grote pillenlast. De wisselwerkingen komen vooral door inductie van cytochroom P450 enzymen door rifampicine. We voerden een fase II klinische trial uit om de werkzaamheid, verdraagbaarheid en farmacokinetiek van een vaste-dosis-combinatie van emtricitabine/tenofovir/efavirenz in combinatie met eerstelijns TBC medicatie te onderzoeken. Een aantal observaties werden gedaan tijdens de klinische trial: IRIS werd niet gezien en de combinatie van eerstelijns TBC medicatie met emtricitabine/tenofovir/efavirenz werd goed getolereerd. Er werden geen relevante wisselwerkingen waargenomen. De farmacokinetische parameters (AUC, C_{max}, and C_{min}) van emtricitabine, tenofovir, en efavirenz waren niet significant veranderd, hoewel de niveaus van efavirenz iets hoger waren wanneer toegediend met rifampicinebehandeling, in tegenstelling tot de verwachte inductie door rifampicine. Eveneens was de farmacokinetische parameter C_{max} van de TBC-geneesmiddelen rifampicine, isoniazide, pyrazinamide en ethambutol niet significant veranderd door de geneesmiddelen tegen HIV-infectie. De werkzaamheid van zowel antiretrovirale therapie als anti-TBC therapie werd niet verminderd door het gecombineerd geven van deze middelen. We concludeerden dat gezamenlijke toepassing van het standaard eerstelijns anti-TB regime met de anti-HIV-combinatie emtricitabine, tenofovir, en efavirenz de farmacokinetische parameters van beide geneesmiddelcombinaties niet verandert tot een klinisch relevant niveau. We bevelen verder onderzoek aan naar de genetische factoren van de patiënt omdat het metabolisme van efavirenz sterk wordt beïnvloed door farmacogenetische factoren.

In hoofdstuk 8 worden de resultaten van alles studies samenvattend bediscussieerd en worden mogelijkheden voor vervolgonderzoek vastgesteld. Daarnaast worden aanbevelingen om TBC behandeling te verbeteren gegeven. Het wordt duidelijk gemaakt dat het succes in de strijd tegen TBC vraagt om samenwerking tussen alle betrokken partijen: overheden, instituten, programma's, organisaties en individuen.

CHAPTER 9C

Muhtasari

Ugonjwa wa kifua kikuu (TB) ni maradhi ya kuambukiza yanayosababishwa na vimelea aina ya bacteria viitwavyo *Mycobacterium tuberculosis*. Ugonjwa huu huathiri zaidi mapafu (huitwa kifua kikuu, au kifua kikuu cha mapafu), lakini pia unaweza kuathiri viungo vingine kama vile utando unaofunika ubongo, mifupa, utando unaofunika moyo, figo, na pia matezi. Kifua kikuu ni tatizo kubwa la afya ya jamii duniani kote, ambapo huathiri takriban watu milioni tisa kila mwaka. Ugonjwa wa kifua kikuu unaongoza kwa kusababisha vifo vitokanavyo na ugonjwa wa kuambukiza unaotibika, licha ya kuwepo kwa dawa zinazoutibu kwa miongo kadhaa. Takriban watu milioni 1.3 hufa kila mwaka kutokana na ugonjwa huu. Mbali na madhara yake ya moja kwa moja kiafya, ugonjwa wa kifua kikuu pia husababisha hasara kubwa ya nguvukazi ya uzalishaji mali, na gharama za kuutibu ni kubwa mno. Tatizo la kifua kikuu liliongezeka kufuatia kusambaa kwa ugonjwa wa UKIMWI kwa sababu ya athari ya UKIMWI kwenye mfumo wa kinga wa mwili. Kwa hiyo, kifua kikuu pia ni sababu kuu ya maradhi na vifo kwa watu wenye maambukizi ya VVU. Kwa ujumla kifua kikuu unachukuliwa kuwa ni ugonjwa unaohusiana na umaskini kwa maana zaidi ya asilimia 80 ya wagonjwa wanatoka katika nchi 22 zilizoathirika zaidi, ambazo ni nchi za kipato cha chini au cha kati, zilizoko Afrika au Asia ya Kusini-Mashariki. Kati ya nchi hizo, tisa ziko Afrika, na Tanzania ni miongoni mwao. Mwaka 2012 Tanzania ilikuwa na kiwango cha kifua kikuu cha wastani wa matukio 169 kwa kila watu 100,000.

Tiba ya kifua kikuu inakabiliwa na changamoto si tu ya kuongezeka kwa ukubwa wa tatizo la ugonjwa huo na uwepo wa maambukizi ya kifua kikuu na VVU kwa pamoja, bali pia ongezeko kubwa la usugu dhidi ya tiba yake. Ugonjwa wa kifua kikuu sugu (MDR-TB), unaofafanuliwa kama usugu dhidi ya dawa za isoniazid na rifampicin, umeibuka kuwa changamoto kuu na inayokua, dhidi ya jitihada za kimataifa za kudhibiti kifua kikuu. Mwaka 2012 kulikuwa na wastani wa wagonjwa wapya 450,000 wa kifua kikuu sugu duniani kote, na takriban 170,000 kati yao walifariki dunia. Katika mwaka huo huo, asilimia 1.1 ya wagonjwa wa kifua kikuu nchini Tanzania walikadiriwa kuwa na kifua kikuu sugu. Tiba ya sasa ya kifua kikuu kisicho sugu inahusisha matibabu ya miezi sita, ambapo dawa nne za mstari wa kwanza (isoniazid, rifampicin, pyrazinamide, na ethambutol) hutolewa kwa miezi miwili ya kwanza. Baada ya hapo, dawa mbili za isoniazid na rifampicin, hutolewa kwa miezi minne iliyobaki. Matibabu ya kifua kikuu sugu ni magumu zaidi na huhitaji muda mrefu. Aidha, dawa za kutibu kifua kikuu sugu ni aghali na zina madhara ya pembeni makubwa kuliko zile za kifua kikuu cha kawaida. Dawa zinazopendekezwa kwa ajili ya kutibu kifua kikuu sugu ni pamoja na Amikacin / Kanamycin, ofloxacin / Levofloxacin, pyrazinamide, Ethionamide, Cycloserine na Ethambutol.

Bado kuna maeneo kadhaa ya uelewa duni, jambo linaloathiri tiba bora ya kifua kikuu na kudhoofisha mapambano dhidi ya ugonjwa huu. Maeneo hayo ni pamoja na

masuala ya tiba na epidemiolojia ya kifua kikuu kwa watoto, tiba ya kifua kikuu katika magonjwa-ambatano kama vile kisukari na VVU. Ili kuboresha tiba ya kifua kikuu nchini Tanzania, tuliandaa na kufanya tafiti kadhaa. Lengo letu lilikuwa ni kupambanua kwa umakini: ukubwa wa tatizo la ugonjwa wa kifua kikuu kaskazini mwa Tanzania, pamoja na masuala ya kitabibu na kifamakolojia yatakayosaidia katika kuboresha tiba. Tafiti zote zilizowasilishwa katika kitabu hiki zilifanyika katika mkoa wa Kilimanjaro nchini Tanzania.

Katika sura ya 2, ukubwa wa tatizo la kifua kikuu kwa kundi la watoto mkoani Kilimanjaro umepambanuliwa. Picha kamili ya kiwango na mtawanyiko wa kifua kikuu katika kundi hili hatarishi na lililosahaulika imepambanuliwa. Tumeonesha kuwa tatizo la kifua kikuu kwa watoto mkoani Kilimanjaro nchini Tanzania ni kubwa; wakichangia asilimia 13 ya wagonjwa wote, na kwamba watoto wana kiwango cha juu cha vifo (10.9%) na pia kiwango duni cha mafanikio ya tiba (72.9%). Kiwango hiki cha mafanikio ya tiba kiko chini ukilinganisha na makadirio ya Shirika la Afya Duniani (WHO) na pia ukilinganisha na ripoti za tafiti toka nchi nyingine kama Malawi, Thailand na Marekani. Tulibaini pia kuwa watoto wadogo (miaka 0-4) wanaathirika zaidi kuliko watoto wakubwa, na kwamba upimaji wa vimelea vya kifua kikuu (AFB testing) ulikuwa wa kiwango cha chini sana; kwa maana ni robo tu ya watoto waliotibiwa na dawa za kifua kikuu ndio walikuwa wamepimwa kwa kipimo hicho. Tumeonesha na kusisitiza haja na uhitaji wa haraka wa kuboresha mchakato wa uchunguzi, kama vile kuanzisha na kutathmini mbinu mpya mfano usisimuaji wa makohozi (induced sputum), laryngeal swabs na nasopharyngeal aspiration, vipimo vya molecular na utafutaji wa wagonjwa.

Katika Sura ya 3 pharmacokinetics ya dawa za kifua kikuu za mstari wa kwanza imepambanuliwa kwa wagonjwa wa kitanzania. Mbali na uzingatiaji duni wa matibabu, viwango vya chini vya dawa za kifua kikuu kwenye damu ni sababu nyingine inayoweza kupelekea matokeo mabaya ya matibabu na pia kutokea kwa usugu. Katika utafiti huu, tuligundua kwamba kiwango cha dawa muhimu za kifua kikuu kwenye damu kilikuwa cha chini katika idadi kubwa ya wagonjwa. Nusu ya washiriki wa utafiti walikuwa na kiwango cha kilele (C_{max}) kwenye damu cha dawa ya isoniazid kilicho chini ya kiwango kinachotakiwa, na theluthi moja walikuwa na kiwango cha dawa ya rifampicin kilicho chini. Aidha kiwango cha mjumuisho (exposure) cha dawa hizi kilikuwa chini ikilinganishwa na tafiti zingine nje ya Afrika. Tuliweka dhana kuwa unywaji wa dawa pamoja na chakula umechangia kwa kiasi fulani udogo wa kiwango cha dawa kwenye damu katika utafiti wetu. Tulipendekeza pia kuwa ifikiriwe kutoa dozi kubwa ya dawa hizo ili kupata kiwango kikubwa cha dawa kwenye damu.

Sura ya 4 inachunguza iwapo mate yanaweza kutumika kama sampuli mbadala katika tafiti za pharmacokinetics (PK) na katika kufuatilia tiba kwa upimaji wa dawa. Hii yaweza kuwa na manufaa kwa ajili ya tafiti za PK hasa kwa watoto, ambapo utoaji wa sampuli nyingi za damu haufai, na pia kwa sababu ya urahisi na unafuu wa ukusanyaji wa sampuli za mate. Tulilinganisha PK za dawa ya rifampicin katika mate, na katika damu. Tuligundua kuwa kiwango cha rifampicin kwenye mate kilikuwa chini zaidi kuliko kwenye damu. Aidha, utafiti huo ulidhihirisha kwamba kiwango cha rifampicin kwenye mate hakiwezi kutabiri kwa usahihi kiwango cha dawa hiyo kwenye damu. Tulihitimisha kuwa upimaji wa viwango vya dawa kwenye mate hauwezi kutumika kama mbadala wa upimaji kwenye damu na katika kufuatilia tiba kwa upimaji wa dawa, au katika tafiti za pharmacokinetics, lakini mate yanaweza kutumika kama makadirio tu katika tiba ya wagonjwa kliniki mfano kubashiri kama kiwango cha dawa kiko chini, kati, au juu.

Katika sura ya 5 tulichunguza na kuandaa kipimo cha kubaini ufanyaji kazi wa dawa kwa njia ya kuotesha vimelea vya kifua kikuu kwenye sampuli ya damu (plasma TB drug activity assay- TDA) na kutathmini kama TDA inaweza kuwa mbadala wa upimaji wa dawa kwenye damu katika kutathmini maendeleo ya matibabu na utambuzi wa mapema wa usugu kwenye matibabu. Ufuatiliaji wa tiba kwa kupima kiwango cha dawa kwenye damu kwa kutumia njia ya HPLC ni aghali sana, lakini kipimo cha TDA ni nafuu na ni rahisi kukiandaa. TDA hupima muda wa vimelea vilivyovundikwa kwenye damu au plasma kuota ikilinganishwa na control (vimelea vilivyovundikwa sehemu isiyokuwa na dawa). Katika utafiti huo, makohozi ya wagonjwa yalichukuliwa kwa ajili ya kuotesha na kuopoa vimelea kabla hawajaanza tiba. Katika wiki ya pili ya matibabu, vimelea vilivyoopolewa vilivundikwa tena kwenye damu (damu ambayo ina dawa inayotumiwa na mgonjwa) na maangamizi ya bakteria yalitathminiwa kwa kifaa cha MGIT 960. Tulibaini kwamba TDA inalandana na viwango vya dawa kwenye damu pamoja na matokeo ya muda mfupi ya matibabu. Kutokana na utafiti huu tulihitimisha kuwa, TDA inaweza kuwa zana na yenye matumaini kwa ajili ya kuboresha matibabu ya kifua kikuu katika mazingira ya rasilimali haba na ambapo upimaji wa kiwango cha dawa mwenye damu kwa kutumia HPLC hauwezi kufanyika. Ilipendekezwa kuwa tafiti zaidi zifanyike ili kuthibitisha zana hiyo.

Katika Sura ya 6, athari za ugonjwa wa kisukari kwenye PK za dawa za mstari wa kwanza za kifua kikuu zilichambuliwa. Kifua kikuu kilichoambatana na ugonjwa wa kisukari ni tatizo linaloongezeka kwa kasi katika nchi zinazoendelea kwa maana ugonjwa wa kisukari unaongeza katika nchi hizi, ambako pia ugonjwa wa kifua kikuu upo kwa wingi. Ugonjwa wa kisukari huongeza uwezekano wa kupata kifua kikuu, na wagonjwa wa kisukari wenye kifua kikuu wana matokeo duni ya matibabu ya kifua kikuu.

Katika utafiti huu tulisajili watanzania 40 wenye kifua kikuu (20 kati yao walikuwa na kifua kikuu tu, na 20 walikuwa na kifua kikuu pamoja na kisukari). Tulichukua sampuli za damu kabla ya dozi, na katika dozi ya saa ya 1, 2, 3, 4, 6, 8, 10, na 24. Tuligundua kwamba, kwa dawa za isoniazid na rifampicin, kiwango cha mjumuisho (exposure) na kiwango cha kilele (C_{max}) kwenye damu vilikuwa chini katika wagonjwa wa kifua kikuu wenye kisukari kuliko wasiokuwa na kisukari. Athari kubwa zaidi ilionekana katika dawa ya isoniazid. Utafiti alihitimisha kuwa athari hizi kwa sehemu kubwa zinasababishwa na ugonjwa wa kisukari. Pendekezo lilitolewa kuwa dozi za dawa hizi ziongezwe kwa wagonjwa wa kifua kikuu wenye kisukari kwa maana kwa kufanya hivi kiwango cha dawa kwenye damu huongezeka.

Katika sura ya 7, wagonjwa wenye maambukizi ya VVU na kufua kikuu walifanyiwa utafiti ambapo ufanisi, ustahimilivu/madhara, pamoja na PK ya mchanganyiko wa emtricitabine / tenofovir / efavirenz (Atripla) zikitumiwa kwa wakati mmoja na dawa za mstari wa kwanza madawa za kifua kikuu, zilichunguzwa. Inafahamika kuwa maambukizi ya VVU huhatarisha na kupelekea kifua kikuu, na hubadilisha tabia na mwenendo wa ugonjwa wa kifua kikuu. Matibabu ya sambamba ya magonjwa haya mawili huleta changamoto kutokana na mwingiliano wa madawa, immune reconstitution syndrome (IRIS), na idadi kubwa ya kidonge. Mwingiliano huo wa madawa husababishwa hasa na uchochewaji wa vimeng'enyo vya saitokromu P450 unaofanywa na dawa ya rifampicin.

Hivyo tulifanya utafiti wa majaribio kutathmini ufanisi, ustahimilivu, na PK ya mchanganyiko wa emtricitabine / tenofovir / efavirenz zikitumiwa kwa wakati mmoja na dawa za mstari wa kwanza za kifua kikuu. Tuligundua mambo kadhaa kwenye utafiti huu: Hapakuwepo na tatizo la IRIS, na matumizi ya sambamba ya dawa hizo hayakuleta madhara yoyote kwa wagonjwa, wala muingiliano. Vipimo vya ki-pharmacokinetic (AUC, Cmax, na Cmin) vya emtricitabine, tenofovir, na efavirenz havikuathiriwa japokuwa viwango vya efavirenz vilikuwa juu kidogo tofauti na matarajio. Hali kadhalika kipimo cha ki-pharmacokinetic (C_{max}) cha dawa za rifampicin, isoniazid, pyrazinamide, na ethambutol hakikuathiriwa. Ufanisi wa dawa za kurefusha maisha na dawa za kifua kikuu havikuathirika kutokana na mchanganyiko huu. Tulihitimisha kwamba utoaji sambamba wa dawa za kifua kikuu za mstari wa kwanza, na dawa za kurefusha maisha (emtricitabine, tenofovir, na efavirenz) hauathiri vigezo vya ki- pharmacokinetic vya dawa zote hizo. Tulipendekeza tafiti zaidi kwa baadaye kuangalia masuala ya kinasaba dhidi ya dawa ya efavirenz kwa maana metaboli ya dawa hii hutegemeana na sababu za kinasaba.

Katika sura ya 8, matokeo ya tafiti zote yamejadiliwa kwa ufupi, pamoja na kubaini hatua stahiki zinazofuata, na mapendekezo: mambo ambayo yatasaidia kuboresha

matibabu ya kifua kikuu Tanzania. Ikawekwa wazi kuwa mafanikio ya kupambana na kifua kikuu yanahitaji kuunganisha nguvu na juhudi baina ya wadau wote: serikali, taasisi, mipango, mashirika na watu binafsi.

Acknowledgements
Curriculum Vitae
List of publications

Acknowledgements

First and foremost, I thank the almighty God for granting me protection and guidance in my life and in all my undertakings.

Secondly, I acknowledge with humility the significant individuals upon whose contribution, the pursuit and successful completion of my PhD has been possible:

My parents:
My father Christopher Nyakibhatare Mtabho and my mother Specioza Ndabacha Makungu Mtabho, for their encouragement, patience and prayers. Not only during my PhD studies, but my parents have always supported me and born with me throughout my milestones and my academic life. Asanteni sana!

My Supervisors:
Prof. Andre van der Ven. I thank you for your lighthearted material and moral support. As my promotor, your advices and guidance have been very positive and instrumental to the success of this work.
Dr. Rob Aarnoutse- Rob, you have been an important entity behind the success of my PhD. You have guided me marvelously through the field of pharmacology and pharmacokinetics. Your support in my PhD is unforgettable; you have been the fuel for my success. During difficult moments your interventions always revived hope.
I thank Prof. Gibson Kibiki for his constant concern for my future career. I will not forget your tireless endeavors to make sure as many young Africans as possible get PhDs. You always showed me the way; you suggested new ideas when the focus of my PhD was initially not yet prominent.
Dr. Martin Boeree has been equally useful. I harvested a lot from your vast experience in pulmonary medicine and research. Your wonderful advices and comments ultimately shaped my works to international standards.

My Family:
My wife Penina Mbuke Masanja always supported me, endured with me when I was away, and took the responsibilities of care to our beloved children. She tirelessly encouraged me and always reminded me to accomplish obligations before deadlines. I remember the way you gave me company even though not very familiar with the field of studies I was undertaking. It sufficed giving a hand with such things as proofreading data and giving a chat as I was writing down manuscripts. Most encouraging were the prayers to the almighty God you always made. Thank you!
My lovely kids Dorinda and Ndabacha for their endurance when they missed me. You had to miss my company a lot. You certainly motivated me to work hard!

I dedicate this PhD to my family.

I acknowledge the *staff from institutions that I worked with*; they have actually contributed to the accomplishment of my PhD:
The KCMC administration for accepting me to KCMC. I thank the former director Prof. John Shao, the Executive director Prof. Moshi Ntabaye, and the former director of KCRI Prof. Franklin Mosha.
The staff members of KCRI in entirety deserve my appreciation, including Marion Sumari who joined KCRI in 2010. Thank you Marion for your encouragement and inputs into my PhD.
The Kibong'oto National TB Hospital (KNTH): I thank Dr. Liberate Mleoh, Dr. Riziki Kisonga, Dr. Stella Mpagama, Batuli Mono, Taji Mzava, Tumaini Mmnari, Leonard Kachalla, Juliana Nkya, Ruwaichi Uisso, and all others for all your contribution into some of my research works.
Staff from the Kilimanjaro region TB program: At the Mawenzi Hospital, I thank the former Regional TB Coordinator (RTLC) for Kilimanjaro Dr. Constantine Irongo, Sr. Martha Lema and other TB clinic staff. I also thank Mr. Cliff Mushi (DTLC Moshi Rural district), the late Goodluck Mosha (former DTLC Moshi Urban district), Juliana Mpwata, Paul Sekibojo (DTLC Same district), John Shayo (DTLC Mwanga district), Alphonce Shirima (DTLC Hai district), Rundiel Mmari (DTLC Siha district), and Ludovick Mashelle (DTLC Rombo district).
In the Netherlands, I acknowledge the laboratory staff at the pharmacy department of the Radboud University Medical Center, for performing all the sample analyses. I remember the company of Mr. Martijn who showed me the HPLC procedures and we used to meet in the lab even on the weekends. I thank Angela Colbers for assistance in all pharmacokinetic data analyses.
I am grateful to my colleague PhD candidates Hadija Semvua, Jossy van den Boogard, Alma Tostmann, Quirine Fillekes, and Cecile Magis-Escurra with whom we worked together in some projects.
I thank APRIORI staff Henri van Asten, Mieke Daalderop and Carla Ysenbrandt, who made my visits and stay to the Netherlands smooth.
I sincerely thank all co-authors in my publications for their contribution while working with them.

Lastly I acknowledge my employer, the District Executive Director for Meatu district council, for granting me permission to attend my studies.

Curriculum Vitae

Charles Michael Mtabho was born on the 17th of June 1976 in Magu, Tanzania. He obtained his primary school education at Nyamisingisi Primary School in Isenye, Serengeti in Mara region in 1989. He then attended ordinary level secondary education at Musoma Alliance Secondary School in Musoma and advanced level secondary school in Ilboru High School in Arusha, Tanzania between 1990 and 1996.

In September 1997 he joined the University of Dar es salaam at the Muhimbili University College of Health Sciences where he attained his bachelors' degree of Doctor of Medicine (MD) in 2002. From May 2003 to April 2004 he worked as an intern doctor at the Kilimanjaro Christian Medical Center (KCMC). in June 2004 he was posted by the Ministry of health to his first appointment as a medical officer in Meatu district, Simiyu region (by then Shinyanga region). Since July 2004 he has been working with the Meatu district council and has held different posts including district malaria and IMCI coordinator, Medical Officer In-charge of the district hospital, and later District Medical Officer. While in the hospital he also attended several short courses including minilap skill training for health providers and diagnostic ultrasound.

In September 2006 he was selected to join a master course in Public Health at Tumaini University KCM College in Moshi. In November 2007 he was awarded a Master of Public health (MPH) degree and the best performer for the year 2006-2007 intake.

In October 2008 he started his PhD training at the Kilimanjaro Clinical Research Institute (KCRI) and later registered with Radboud University Nijmegen, The Netherlands. He has attended several short courses of epidemiology and biostatistics, public health research, research methodology, clinical research ethics, clinical trial monitoring, good clinical practice, grant writing and management, and writing biomedical papers.

As for research experience, Charles has been involved in epidemiological research on the burden of tuberculosis, malnutrition, and schistosomiasis in children, and also in clinical trials. At KCRI he has been a clinical coordinator for clinical trials that aim at shortening TB treatment duration and optimizing antiretroviral therapy in patients with concomitant TB and HIV. Moreover, he conducted a pharmacokinetic study on the effects of diabetes mellitus on exposure to TB drugs in TB/DM co-morbid patients. He has experience in conducting both field work, making different source documents, and internal monitoring for the trials. His current research work is on improving management of tuberculosis with and without accompanying co-morbidities. Different epidemiological, pharmacokinetic, pharmacodynamic, safety, and tolerability studies were conducted from 2008 to 2012.

Charles is married to Penina Mbuke Masanja and they have two children.

List of Publications

Publications in peer reviewed journals

1. Mtabho, C. M., Irongo, C. F., Boeree, M. J., Aarnoutse, R. E., & Kibiki, G. S. 2010, "Childhood tuberculosis in the Kilimanjaro region: lessons from and for the TB programme", *Trop.Med.Int.Health*, vol. 15, no. 5, pp. 496-501.
2. Scott K. Heysell, Charles Mtabho, Stellah Mpagama, Solomon Mwaigwisya, Suporn Pholwat, Norah Ndusilo, Jean Gratz, Rob E. Aarnoutse, Gibson S. Kibiki and Eric R Houpt. 2011, "Plasma Drug Activity Assay for Treatment Optimization in Tuberculosis Patients". *Antimicrob. Agents Chemother.* 55(12):5819-25.
3. Stellah G. Mpagama, Charles Mtabho, Solomon Mwaigwisya, Liberate J. Mleoh, I Marion Sumari-de Boer, Scott K. Heysell, Eric R. Houpt, and Gibson S. Kibik, 2012. "Comparison of Overnight Pooled and Standard Sputum Collection Method for Patients with Suspected Pulmonary Tuberculosis in Northern Tanzania," *Tuberculosis Research and Treatment*, vol. 2012, Article ID 128057.
4. Hadija H Semvua, Charles M Mtabho, Quirine Fillekes, Jossy van den Boogaard, Riziki M Kisonga, Liberate Mleoh, Arnold Ndaro, Elton R Kisanga, Andre van der Ven, Rob E Aarnoutse, Gibson S Kibiki, Martin J Boeree, David M Burger, 2013. "Efavirenz, tenofovir and emtricitabine combined with first line tuberculosis treatment in TB–HIV-coinfected Tanzania patients: a pharmacokinetic and safety study. " *Antiviral Therapy*; 18(1):105-13.
5. Alma Tostmann, Charles M. Mtabho, Hadija H. Semvua, Jossy van den Boogaard, Gibson S. Kibiki, Martin J. Boeree, Rob E. Aarnoutse, 2013. "Pharmacokinetics of first line tuberculosis drugs in Tanzanian patients. " *Antimicrob Agents Chemother.* 57(7):3208-13.
6. Suba,M.R, Ayana, S.M, Mtabho, Charles.M, Kibiki, G.S, 2010. "The aetiology, management and clinical outcome of upper gastrointestinal bleeding among patients admitted at the Kilimanjaro Christian Medical Centre in Moshi, Tanzania", *Tanzania Journal of Health Research*;12(4):302-5.
7. Charles E. Mwanziva, Jovin Kitau, Patrick K. Tungu, Clement N. Mweya, Humphrey Mkali, Chacha M. Ndege, Alex Sanga, Charles Mtabho, Charles Lukwaro, Joseph Myamba, Salum Abdulalazizi, Stephen M. Magesa, Jaffu Chilongola, Seif Shekalaghe, Franklin W. Mosha, 2011. "Transmission intensity and malaria vector population structure in Magugu, Babati District in northern Tanzania". *Tanzania Journal of Health Research*; 13(1):54-61.
8. Charles M. Mtabho, Hadija H. Semvua, , Marga Teulen, Angela Colbers, Rogate Machange, Arnold Ndaro, André van der Ven, David Burger, Gibson S. Kibiki, Rob E. Aarnoutse. Associations between salivary, protein-unbound and total plasma concentrations of rifampicin. *Submitted.*

9. Charles M. Mtabho, Hadija H. Semvua, Jossy van den Boogaard, Constantine F. Irongo, Martin J. Boeree, Angela Colbers, David M Burger, van Crevel R, van der ven AJAM, Gibson S. Kibiki, Alma Tostmann, Rob E. Aarnoutse. The effect of diabetes mellitus on exposure to tuberculosis drugs in Tanzanian patients. *In preparation.*

Abstracts at conferences

1. The magnitude of childhood tuberculosis in the Kilimanjaro region, northern Tanzania: a retrospective study from regional TB programme registry. *Fifth EDCTP forum, October 12-14 2009, Arusha, Tanzania*
2. Childhood tuberculosis in the Kilimanjaro region: lessons from the TB program. The 24th Annual Joint Scientific Conference and 30th Anniversary of the National Institute for Medical Research, *Arusha International Conference Centre, Arusha, Tanzania, 15-18th March 2010*
3. Plasma levels of tuberculosis drugs in tuberculosis patients in northern Tanzania. *Sixth EDCTP forum, October 9-11 2011, Addis Ababa, Ethiopia*
4. Plasma levels of tuberculosis drugs in tuberculosis patients in northern Tanzania. *NIMR Annual Joint Scientific Conference, April16-20 2012, Arusha, Tanzania*
5. The effect of first line anti-tubercular drugs on the pharmacokinetics and tolerability of emtricitabine/tenofovir/efavirenz in patients with TB/HIV co-infection. *Keystone symposia-Drug Resistance and Persistence in Tuberculosis, May 13-18 2012, Kampala Uganda*
6. Plasma levels of tuberculosis drugs in tuberculosis patients in northern Tanzania. *Keystone symposia-Drug Resistance and Persistence in Tuberculosis, May 13-18-2012, Kampala Uganda*
7. The effect of diabetes mellitus on the pharmacokinetics of tuberculosis drugs in Tanzanian patients. *The 7th EDCTP Forum, 30thJune-2nd July 2014. Berlin, Germany*

PROPOSITIONS

belonging to the thesis:

IMPROVING THE MANAGEMENT OF TUBERCULOSIS IN TANZANIA: CLINICAL AND EPIDEMIOLOGICAL STUDIES

1. Diabetes mellitus significantly lowers exposure to isoniazid and rifampicin, therefore higher doses of these drugs should be considered in patients with diabetes and TB. *(This thesis)*
2. A considerable proportion of Tanzanian TB patients achieve lower plasma concentrations of isoniazid and rifampicin attributable to concurrent food and drug intake and interpersonal variability. These factors need careful consideration. *(This thesis)*
3. Higher doses (15mg/kg/day and 20mg/kg/day) of rifampicin are safe and tolerated by Tanzanian TB patients. (*High-Rif trial conducted at KCRI*)
4. Tanzanian children constitute a significant burden of TB disease by 13% and exhibit poor treatment outcome. *(This thesis)*
5. Salivary concentrations of rifampicin exhibit too low precision to be able to predict plasma drug levels, but may be used in patient care as semi-quantitative estimation of plasma levels. *(This thesis)*
6. The fixed dose combination emtricitabine/tenofovir/efavirenz can be safely administered simultaneously with first-line TB drugs in TB/HIV patients without compromising efficacy of either group of drugs. *(This thesis)*
7. AUC values (rather than C_{max}) of pyrazinamide, rifampicin and isoniazid are better predictors of long-term treatment response to TB drugs. (*J. G. Pasipanodya et al., J.Infect.Dis. 208, no. 9 (2013): 1464-1473*)
8. Whole-blood and plasma activity assays are feasible in resource limited settings and are beneficial compared to plasma drug measurement; they can help treatment response monitoring and predict outcome. *(This thesis)*
9. Tuungane pamoja kupambana na maadui watatu wa maendeleo: umaskini, ujinga, na maradhi. (Let's join forces to fight the three enemies of development namely poverty, ignorance, and diseases)
   (Baba wa Taifa Mwalimu Julius Kambarage Nyerere)
10. Kazi ni dawa. (Work is like a medicine. Work cures). (*Swahili proverb*)

Charles Michael Mtabho- December 2014