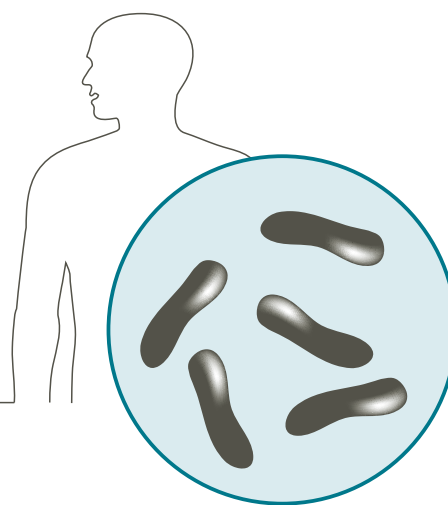




4th International Workshop on



CLINICAL PHARMACOLOGY OF TUBERCULOSIS DRUGS



CHICAGO IL , USA

| 16 September 2011

Program & Abstracts



4th International Workshop on Clinical Pharmacology of Tuberculosis Drugs

16 September 2011, Chicago IL, USA

Program & Abstracts

Acknowledgements

This workshop has been made possible by our sponsors.

Financial support for the 4th International Workshop on Clinical Pharmacology of Tuberculosis Drugs has been provided by:

Silver Level Sponsor

**BILL & MELINDA
GATES foundation**

Principal Supporter

**National Institutes of Health
Office of AIDS Research**



Supporter

sanofi aventis
Because health matters

Contributor

TB Alliance

Scholarship Provider

Novartis

Scientific Supporters

UMC  St Radboud

 UNIVERSITY OF
LIVERPOOL

 UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

 TB ALLIANCE
GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT

 JOHNS HOPKINS
MEDICINE

 UNIVERSITY OF
FLORIDA

Content

- Acknowledgement Workshop sponsors *p. ii*
- Content Index *p. iii*
- General Information *p. iii*
- Organizing Committee *p. iv*
- Scientific Committee *p. v*
- Program *p. vi*
- Invited Speakers *p. ix*
- Abstracts *p. 01*
- Author Index *p. 20*

General Information

Badge Policy

All registered delegates are provided with an identity badge.
Please wear it at all times to ensure admission to the meeting.

Certificate of Attendance

The certificates can be picked up during lunch.

Conference Materials

Presentations will be posted on www.virology-education.com shortly after the workshop.

Date and Venue

The date of the meeting is Friday 16 September, 2011
The meeting is held in The Congress Plaza Hotel, 520 South Michigan Avenue, Chicago, IL.

Language

The official language of the workshop is English.

Social Program

A workshop dinner is scheduled for Friday evening 16 September for those delegates who have pre-registered.

Workshop Secretariat

The registration desk is located near the plenary meeting room.
The conference organizers can be addressed for all questions concerning the logistics of the meeting.

Organizing Committee



Rob Aarnoutse, PharmD, PhD.

Senior Hospital Pharmacist
Radboud University Nijmegen Medical Centre
Nijmegen, The Netherlands



Geraint Davies, MD.

Senior Lecturer and Honorary Consultant in Infectious Diseases
University of Liverpool
Liverpool, United Kingdom



Helen McIlleron, MBChB, PhD.

Head of the Pharmacokinetic Research group, Division of Clinical Pharmacology
University of Cape Town
Cape Town, South Africa



Khisi Mdluli, PhD.

Senior Project Leader, Biology
Global Alliance for TB Drug Development
New York NY, USA



Eric Nuermberger, MD.

Associate Professor of Medicine and International Health
Johns Hopkins School of Medicine
Baltimore MD, USA



Charles Peloquin, PharmD.

Professor of Pharmacy and Medicine
University of Florida
Gainesville FL, USA

Scientific Committee

Bill Burman MD

Jan Gheuens MD, PhD

Stephen Gillespie PhD

Saye Khoo MD, PhD

Christian Lienhardt MD, PhD

William MacKenzie MD

David McNeeley MD

Andrew Vernon MD

Denver Public Health, Denver CO, USA

Bill & Melinda Gates Foundation, Seattle WA, USA

University of St Andrews, Fife, Scotland, United Kingdom

University of Liverpool, Liverpool, United Kingdom

WHO - Stop TB Department, Geneva, Switzerland

CDC/CCID/NCHHSTP, Atlanta GA, USA

Tibotec, Yardley PA, USA

CDC TBTC, Atlanta GA, USA

This Workshop is organized by



Biltstraat 106, 3572 BJ Utrecht, the Netherlands

Phone: + 31 30 230 7140 Fax: +31 30 230 7148

Email: info@virology-education.com / www.virology-education.com

Program - Friday 16 September 2011

08.30h	Opening of the Workshop	Workshop chair	
Session 1 Pharmacokinetics & Pharmacodynamics of approved TB drugs			
Chairs: Rob Aarnoutse & Kelly Dooley			
08.45h	<i>Invited lecture:</i> Optimizing existing drugs for the treatment of MDR-TB	K. Dooley	
09.15h	Evaluation of protein-unbound, active concentrations of rifampicin in Indonesian tuberculosis patients	R. Aarnoutse	O_01
09.30h	Pharmacokinetics and safety of high dose rifampicin and moxifloxacin for tuberculosis meningitis: preliminary data from a RCT in Indonesia	R. Ruslami	O_02
09.45h	The safety and pharmacokinetics of adjusted dose lopinavir /ritonavir when given with tuberculosis treatment in South African HIV-infected patients.	H. McIlleron	O_03
10.00h	CYP3A induction by rifampin and rifapentine: Which drug and dose does it best?	K. Dooley	O_04
10.15h	Moxifloxacin exposure is significantly lower during early treatment of tuberculosis compared to a later stage of treatment	J. Alffenaar	O_05
10.30h	Discussion		
10.45h	Coffee Break		
Session 2 Pharmacokinetics & Pharmacodynamics of new TB drugs			
Chairs: Eric Nuermberger & Khisi Mdluli			
11.15h	<i>Invited lecture:</i> Combination therapy with novel TB drugs	K. Mdluli	
11.45h	Exploratory pharmacokinetics-pharmacodynamics (PK-PD) target attainment analysis of PA-824	A. Barth	O_06
12.00h	Novel TMC207-containing regimens have sterilizing activity in murine tuberculosis	E. Nuermberger	O_07
12.15h	Safety, tolerability, and pharmacokinetic interactions between single-dose TMC207 and steady-state efavirenz in healthy volunteers: ACTG study A5267	K. Dooley	O_08
12.30h	Rapid evaluation of candidate regimens for XDR-TB including PNU-100480, TMC207, and PA-824 using whole blood culture	R. Wallis	LB_01
12.45h	Discussion		
13.00h	Lunch		

Session 3 Pharmacokinetic- & Pharmacodynamic modeling**Chairs: Charles Peloquin & David Haas**

14.00h	<i>Invited lecture:</i> Pharmacogenomics of Antituberculous Drugs	D. Haas	
14.30h	Population PK of Isoniazid in South African Adults	P. Denti	O_09
14.45h	Population pharmacokinetics of rifapentine and its active metabolite in healthy volunteers: nonlinearities in clearance and bioavailability	K. Dooley	O_10
15.00h	Modeling the Pharmacokinetics of Rifapentine in TB patients receiving 10 mg/kg daily	C. Peloquin	O_11
15.15h	Population Pharmacokinetics and Pharmacodynamics of Ofloxacin in South African patients with drug-resistant Tuberculosis	H. McIlleron	O_12
15.30h	Discussion		
15.45h	Coffee Break		

Session 4 New Drug Development Methods**Chairs: Helen McIlleron & Andreas Diacon**

16.15h	<i>Invited lecture:</i> EBA: Theoretical aspects and practical experience	A. Diacon	
16.45h	A novel Pharmacodynamic model for treatment of Tuberculosis using days to positivity in automated liquid mycobacterial culture	H. McIlleron	O_13
17.00h	Rifampicin concentration-effect relationships for resistance development differ between Mycobacterium tuberculosis genotypes	J. de Steenwinkel	O_14
17.15h	Dose-ranging activity of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis	E. Nuermberger	O_15
17.30h	New methods for therapeutic drug monitoring of moxifloxacin in patients with tuberculosis in rural areas and resource limited settings	J. Alffenaar	O_16
17.45h	Discussion		
18.00h	Closure of the Workshop		

Invited Speakers

Optimizing existing drugs for the treatment of MDR-TB



Kelly Dooley, MD, PhD, is an Assistant Professor of Medicine, Pharmacology & Molecular Sciences at Johns Hopkins University. She completed fellowships in Infectious Diseases and Clinical Pharmacology and a doctoral degree at the Johns Hopkins Bloomberg School of Public Health with a thesis focused on the methodological challenges in the evaluation of new tuberculosis treatment regimens.

Dr. Dooley is an investigator with the Tuberculosis Trials Consortium and the AIDS Clinical Trials Group.

Her research interests include the clinical pharmacology of new anti-tuberculosis regimens with an emphasis on Phase I clinical trials of new or existing anti-TB drugs including maximal tolerated dose studies and studies of drug-drug interactions between anti-TB agents and antiretrovirals used to treat HIV; strategies for treating HIV in patients who cannot take NNRTIs but require (rifampin-based) TB treatment; optimization of existing drugs for the treatment of MDR-TB; and the evaluation of TB and HIV drug concentrations in pregnant co-infected women and the relationship between drug exposure and maternal and perinatal HIV outcomes.

Combination therapy with novel TB drugs



Khisi Mdluli, PhD, joined the TB Alliance R&D team in May as the Project Leader of Research, working closely with Dr. Zhenkun Ma, Head of Research. Dr. Mdluli brings over 10 years of drug discovery expertise, particularly in assay development and high throughput screening, to help build and advance the TB Alliance's portfolio.

A native of Swaziland, Dr. Mdluli is a highly regarded expert in the microbiology, molecular biology and biochemistry of *Mycobacterium tuberculosis*. A three-year visiting fellowship at the U.S. National Institutes of Health resulted in several important publications describing the mechanism of action of two currently used drugs, including isoniazid. Dr. Mdluli also spent two years at Cumbre Inc., a Texas based biotech, and six years at Chiron Corporation as a Senior Scientist where he helped establish and manage a major antimicrobial discovery program.

Pharmacogenomics of Antituberculous Drugs



David W. Haas, MD is Professor of Medicine, Pharmacology, Pathology, Microbiology & Immunology at Vanderbilt University School of Medicine, and a member of the Vanderbilt Center for Human Genetics Research.

He devotes considerable effort into HIV pharmacogenomics research. He directs the Vanderbilt HIV Therapeutics Clinical Research Site, and previously chaired the Pharmacology Committee for the NIH funded AIDS Clinical Trials Group (ACTG). He has led the ACTG's pharmacogenomics initiative since 2000. He oversaw the establishment of the ACTG Human DNA Repository (housed at Vanderbilt, with extracted DNA from nearly 13,000 HIV-infected individuals), and is now working to extend DNA banking to non-US ACTG sites in resource-limited countries in worldwide. His work led to the seminal observations that CYP2B6 516G>T predicts delayed clearance of efavirenz, which largely explains increased plasma exposure among individuals of African descent, and may help predict CNS side effects and virologic response. He collaborates on numerous HIV pharmacogenomic projects in the US and abroad, and is principal investigator on an R01 from NIAID, "Pharmacogenomics of HIV Therapy". He has led studies of pharmacokinetic interactions between antiretroviral and antituberculous drugs.

EBA: Theoretical aspects and practical experience



Andreas Henri Diacon MD. is a specialist physician holding qualifications for Internal Medicine and Pulmonology (Lung Diseases) in Switzerland and South Africa. He emigrated to South Africa in 2000 to become a research fellow with the Lung Unit of the Department of Internal Medicine and completed his PhD thesis at the University of Stellenbosch in 2007.

He joined the Department of Biomedical Sciences in 2008 as Associate Professor with the Division of Medical Physiology and consultant for Internal Medicine, Pulmonology and Intensive Care at Tygerberg Hospital, Cape Town.

His clinical research interest is the evaluation of antituberculous agents and treatment regimens. He is the director of the Centre of Clinical Tuberculosis Research (CCTR) at the DST/NRF Centre of Excellence in Biomedical Tuberculosis Research (Director: Prof Paul van Helden). This activity encompasses the supervision and coordination of clinical trials in the departmental MRC laboratory and the development of techniques for the evaluation of antituberculosis agents. Prof Diacon is also involved in research programs in exercise physiology, interventional pulmonology and pleural disease.

4th International Workshop on Clinical Pharmacology of Tuberculosis Drugs

Abstracts

Abstract: O_01

PK/PD of approved TB drugs

Evaluation of protein-unbound, active concentrations of rifampicin in Indonesian tuberculosis patients

R.E. Aarnoutse¹, F.W. Mooren¹, H. Nijland¹, L. Apriyani², F. Wieringa², R. van Crevel³, R. Ruslam²

¹*Radboud University Nijmegen Medical Centre, Nijmegen, Dept of Clinical Pharmacy, Nijmegen, The Netherlands;*

²*Health Research Unit, Faculty of Medicine, Universitas Padjadjaran /Hasan Sadikin Hospital, Bandung, Indonesia;*

³*Radboud University Nijmegen Medical Centre, Nijmegen, Dept of Internal Medicine, Nijmegen, The Netherlands*

Background: Concentrations of tuberculosis (TB) drugs in plasma are supposed to predict efficacy and adverse effects to these drugs. Pharmacokinetic (PK) studies of approved and new TB drugs focus on total (i.e. protein-bound plus unbound or free) plasma concentrations, whereas only free drug is active and able to diffuse to the site of action. Malnutrition (BMI • 18.5 as defined by the WHO) often occurs with TB and has been associated with a decrease in protein binding and an increase in free fraction (protein-unbound / total concentration) for many drugs, but very few data are available for TB drugs. The aim of this study was to evaluate free plasma concentrations of the pivotal TB drug rifampicin.

Materials & Methods : A PK study was performed in adult Indonesian pulmonary TB patients in the intensive phase of treatment. In agreement with the Indonesian national TB program, patients received the same fixed oral dose of rifampicin (450 mg, circa 10 mg/kg daily) together with other first-line TB drugs. Intensive PK sampling was performed after 2 weeks of treatment after witnessed intake of drugs on an empty stomach. Total and free plasma concentrations of rifampicin were analyzed with validated HPLC methods. Measurement of free rifampicin was based on ultrafiltration. PK parameters were assessed with non-compartmental methods, and were compared between subgroups using the independent-samples T-test.

Results: Thirty-six patients were included (mean age 34 years, 39% male, mean BMI 18.6 kg/m²). Geometric mean AUC_{0-24h} and C_{max} values for rifampicin were 54.7 h•mg/L and 10.9 mg/L, respectively. The geometric mean ratio of free and total AUC_{0-24h} of rifampicin (AUC_{0-24h}) was 10.3%, with a two-fold range in this free fraction (range 7.6-15%). The height of total plasma concentrations only slightly affected the free fraction of rifampicin, e.g. the geometric mean free fraction was 10.2% at 2h post dose and 12.2% at 6h post dose. 11 malnourished (BMI • 18.5, with mean 15.4 kg/m²) and 25 well-nourished patients (BMI >18.5, with mean 20.0 kg/m²) showed no differences at all in AUC_{0-24h} or C_{max} based on total or free plasma concentrations of rifampicin, despite a somewhat higher dose/kg in malnourished versus well-nourished patients (11.6 vs 9.7 mg/kg). Female participants showed higher mean total and free rifampicin AUC_{0-24h} values than men (+23% and +29% respectively) without displaying a different free fraction of rifampicin, an effect that remained significant in a multivariate analysis.

Conclusions: The mean free fraction of rifampicin in Indonesian TB patients is 10-12% based on AUC_{0-24h} or single concentrations, which is higher than reported before in other populations (Bennet et al: 60-90%, Holdiness: 80%). The free, active fraction in plasma varies two-fold amongst patients with the same total exposure. These findings are relevant for interpretation of rifampicin PK parameters across populations and for Therapeutic Drug Monitoring of rifampicin based on total concentrations. Based on active rifampicin concentrations, malnourished Indonesian patients are not at an increased risk of treatment failure. Further study on the effect of gender on the PK of rifampicin is warranted.

No conflict of interest

Abstract: O_02*PK/PD of approved TB drugs***Pharmacokinetics and safety of high dose rifampicin and moxifloxacin for tuberculosis meningitis: preliminary data from a RCT in Indonesia***R. Ruslami¹, A.R. Ganiem², S. Dian², L. Apriani³, T.H. Achmad⁴, R. van Crevel⁵, R.E. Aarmoutse⁶*

¹Padjadjaran University Faculty of Medicine, Pharmacology & Therapy, Bandung, Indonesia; ²Padjadjaran University Faculty of Medicine/Hasan Sadikin Hospital, Neurology, Bandung, Indonesia; ³Padjadjaran University Faculty of Medicine/Hasan Sadikin Hospital, Health Research Unit, Bandung, Indonesia; ⁴Padjadjaran University Faculty of Medicine, Biochemistry, Bandung, Indonesia; ⁵Radboud University Nijmegen Medical Center, Internal Medicine, Nijmegen, The Netherlands; ⁶Radboud University Nijmegen Medical Center, Pharmacy, Nijmegen, The Netherlands

Background: Tuberculosis meningitis (TBM) is the most lethal form of tuberculosis. Up to 30% of patients are dying, mostly in the first month after diagnosis. The optimal treatment for TBM has not been established and follows the model of pulmonary TB treatment. Fluoroquinolones are promising drugs for TBM, especially moxifloxacin that shows good penetration into the cerebrospinal fluid (CSF). A higher dose of rifampicine as well as moxifloxacin may increase the efficacy of these drugs and result in increased exposure in CSF. The aim of this study was to evaluate the pharmacokinetics and safety/tolerability of an intensified regimen for TBM using higher doses of rifampicin and moxifloxacin.

Materials & Methods: An open-label, randomized, phase II clinical trial was conducted in a referral hospital in Indonesia. Adult TB meningitis patients were randomized to 14-day treatment with either standard dose (450 mg or 10 mg/kg, administered orally) or high dose (600 mg, 90 min infusion) of rifampicin, and (in a second randomization) to moxifloxacin 400 mg once daily, 800 mg once daily, or ethambutol 750 mg once daily (no moxifloxacin). All patients received 300 mg isoniazid, 1500 mg pyrazinamide and dexamethason 0.4 mg/kg, and after 14 days all patients continued with a standard regimen. Intensive pharmacokinetic

(PK) sampling took place within the first 3 days of TBM treatment, together with CSF samples taken between 3-6 h after administration of drugs. Plasma and CSF concentrations of rifampicin and moxifloxacin were analyzed with validated HPLC methods, PK parameters were assessed with non-compartmental methods and were compared between subgroups using the independent-samples t-test after logarithmic transformation. Safety/tolerability in this patient group with severe signs of disease was evaluated by assessment of grade 3 and 4 adverse events attributed to TB treatment.

Results: Twenty-three patients have been included so far (mean age 32 years, 44% male, HIV-positive 13%, mean BMI 19.5 kg/m²). Increasing the dose of rifampicin by 33% given intravenously led to a 1.8-fold increase in the geometric mean of AUC₀₋₂₄ (130.4 vs. 72.5 h*mg/L, p=0.002), a 2.5-fold increase in AUC₀₋₆, and a 2.8 fold increase in rifampicin C_{max} (21.1 vs 7.5 vs mg/L, p<0.001). Doubling the dose of moxifloxacin resulted in an increase in C_{max} by almost 3 times (9.3 vs. 3.4 mg/L, p<0.001) and a three-fold increase in AUC₀₋₆. Geometric mean CSF concentrations for rifampicin (n=19) were 0.50 vs. 0.37 mg/L for high respectively standard dose of rifampicin (p=0.31), and 3.9 vs. 1.7 mg/L (p<0.05) for high versus standard moxifloxacin (n=12). Six patients (26%, 3 in both rifampicin-arms) developed grade 3-4 drug-induced hepatitis, three (rifampicin 600 mg) experienced anemia and thrombocytopenia, and one (moxifloxacin 800mg) had prolonged QTc. Seven patients (30%) patients, well distributed over the regimens, died within one month, and none because of drug-toxicity.

Conclusions: Increasing the dose of rifampicin and moxifloxacin is associated with a significant increase in exposure to these drugs both in plasma and CSF, and with an increase in the incidence of non-fatal adverse events.

No conflict of interest

Abstract: O_03

PK/PD of approved TB drugs

The safety and pharmacokinetics of adjusted dose lopinavir /ritonavir when given with tuberculosis treatment in South African HIV-infected patients

E.H. Decloedt¹, P. Smith¹, M. Concepta², F. Bango³, G. Maartens¹, H. McIlleron¹

¹University of Cape Town, Division of Clinical Pharmacology, Cape Town, South Africa; ²Trinity College Dublin, Department of Pharmacology, Dublin, Ireland; ³Site B Khayelitsha Day Hospital, Ubuntu Clinic, Cape Town, South Africa

Objective: Rifampicin co-administration dramatically reduces plasma lopinavir concentrations. Studies in healthy volunteers and HIV-infected patients showed that doubling the dose of lopinavir/ritonavir (LPV/r) or adding additional ritonavir overcame this interaction. However, high rates of hepatotoxicity, nausea and vomiting were observed in healthy volunteers. We prospectively evaluated the safety and steady-state pharmacokinetics of LPV in HIV infected adults established on a LPV/r regimen treated with rifampicin-based TB treatment.

Methods: Adult patients treated with a LPV/r-based antiretroviral regimen and rifampicin-based TB treatment were enrolled. Doubled doses of LPV/r or an additional 300 mg of ritonavir were used to overcome the inducing effect of rifampicin. Steady-state LPV trough concentrations were evaluated bi-monthly.

Results: 18 patients, of whom 11 were female, were enrolled. The median (IQR) age was 38.5 (33-48) yrs and the median (IQR) CD4-count was 111 (41 – 181) cells/mm³. The median (IQR) month on TB treatment when enrolled was 4 (2-5) with a total of 79 patient months of observation. 11/18 patients were followed up until TB treatment completion. During TB treatment, the median (IQR) lopinavir trough concentration was 6.8 (1.2 - 9.15) mg/L and 36/47 (77%) of lopinavir trough concentrations

were above the recommended trough concentration of 1 mg/L. Treatment was generally well tolerated with no grade 3 or 4 toxicity: 8 patients developed grade 1 or 2 transaminase elevation, 1 patient defaulted additional ritonavir due to nausea and 1 patient developed diarrhea requiring dose reduction. Viral loads after TB treatment were available for 12 patients and 10 were undetectable.

Conclusion: Once established on treatment, adjusted doses of LPV/r co-administered with rifampicin-based TB treatment was relatively well tolerated and LPV trough concentrations were adequate.

No conflict of interest

Abstract: O_04*PK/PD of approved TB drugs***CYP3A induction by rifampin and rifapentine: Which drug and dose does it best?**

E. Bliven-Sizemore¹, A.D.M. Kashuba², S. Malone³, M. Weiner⁴, E. Nuernberger⁵, W. Burman⁶, S. Dorman⁵, K.E. Dooley⁷

¹Centers for Disease Control and Prevention, Division of Tuberculosis Elimination, Atlanta, USA; ²University of North Carolina at Chapel Hill, UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core, Chapel Hill, USA; ³University of North Carolina at Chapel Hill, School of Pharmacy, Chapel Hill, USA; ⁴University of South Texas Health Science Center, Audie L. Murphy VA Hospital, San Antonio, USA; ⁵Johns Hopkins University School of Medicine, Center for Tuberculosis Research, Baltimore, USA; ⁶Denver Public Health, Infectious Diseases Clinic, Denver, USA; ⁷Johns Hopkins University School of Medicine, Clinical Pharmacology & Infectious Diseases and Center for Tuberculosis Research, Baltimore, USA

Background: Rifamycins are potent inducers of cytochrome P450 (CYP) enzymes, including CYP3A, the isoform most commonly involved in P450-mediated drug metabolism. Previous human hepatocyte experiments have suggested that rifampin is a more potent inducer of CYP3A than rifapentine or rifabutin. Midazolam, metabolized almost exclusively by CYP3A, is a commonly used probe drug to determine if a candidate drug affects CYP3A enzyme activity. In this study, we evaluated the effect of rifapentine dose on CYP3A activity, using oral midazolam as a probe drug and rifampin at standard doses as a comparator. Understanding the relationship between increasing rifapentine dose and magnitude of CYP3A induction can provide important information regarding potential drug interactions involving rifapentine as it is optimized for the treatment of tuberculosis.

Materials & Methods: Healthy volunteers enrolled in Tuberculosis Trials Consortium Study 29B received oral rifapentine 5, 10, 15, or 20 mg/kg or rifampin 10 mg/kg daily for 14 days (n=6 per cohort). Subjects received two single 15 mg oral doses of midazolam, one alone and the other co-administered with steady-state rifapentine or rifampin. Samples for midazolam pharmacokinetic analysis were collected after each midazolam dose. Plasma concentrations of

midazolam and its metabolite (1-OH-MDZ) were determined by a validated HPLC/MS procedure. Pharmacokinetic parameters of midazolam and 1-OH-MDZ were calculated using standard noncompartmental methods using WinNonlin version 6.1 (Pharsight, Cary, NC).

Results: Mean 12-hour area under the time-concentration curve (AUC₀₋₁₂) of midazolam was reduced 74% by co-administration with rifampin and 92, 92, 94, and 94% by co-administration with rifapentine 5, 10, 15, or 20 mg/kg, respectively. Mean maximum plasma concentration (C_{max}) of midazolam was likewise diminished -- 73% by co-administration with rifampin and 83, 84, 84, and 88% by co-administration with rifapentine. 1-OH-MDZ concentrations were similarly reduced when midazolam was co-administered with rifampin or rifapentine. Mean midazolam oral clearance was increased 925, 1658, 1392, 1923 and 1952% by steady-state rifampin or rifapentine 5, 10, 15 or 20 mg/kg, respectively (p=0.05, comparing the change in oral clearance by rifampin vs. rifapentine). At a dose of 10 mg/kg, median steady-state C_{max} and AUC of rifapentine and rifampin were 22 mg/mL and 330 mg*h/mL and 7.5 mg/mL/45 mg*h/mL, respectively. Average mM concentrations of rifampin and rifapentine at this dose were 2.3 μM and 15.7 μM, respectively.

Conclusions: Similar to results from isolated human hepatocytes, we saw that rifampin was a more potent CYP3A inducer than rifapentine on a μM scale. However, at clinically-relevant doses in healthy volunteers, we observed that rifapentine was a stronger inducer of CYP3A than rifampin. This may be due to the greater exposures observed with daily mg/kg doses of rifapentine compared to rifampin, not doses in μM. There was no evidence of a dose-response relationship between rifapentine dose and CYP3A induction at the studied doses. Recommendations for dose adjustments that result from drug-drug interaction studies involving rifampin and companion drugs may not be readily extrapolated to rifapentine. Careful testing of drug interactions involving rifapentine at clinically-relevant doses and drugs metabolized by key cytochrome P450 enzymes will help guide dosing considerations for the future.

No conflict of interest

Abstract: O_05*PK/PD of approved TB drugs***Moxifloxacin exposure is significantly lower during early treatment of tuberculosis compared to a later stage of treatment***J.W.C. Alffenaar¹, A.D. Pranger¹, R. van Altena², W.C.M. de Lange², D. van Soolingen³, D.R.A. Uges¹, J.G.W. Kosterink¹, T.S. van der Werf⁴*

¹University Medical Center Groningen, Hospital and Clinical Pharmacy, Groningen, The Netherlands; ²University Medical Center Groningen, Tuberculosis Center Beatrixoord, Groningen, The Netherlands; ³National Institute for Public Health and the Environment and Radboud University Medical Center, National Mycobacteria Reference Laboratory, Groningen, The Netherlands; ⁴University Medical Center Groningen, Tuberculosis and Lung Diseases, Groningen, The Netherlands

Introduction: Moxifloxacin (MFX) is a powerful second-line agent with high *in vitro* and *in vivo* activity against *Mycobacterium tuberculosis*. The AUC_{0-24h} to MIC ratio is the most predictive parameter for *in vivo* efficacy of MFX and should be over 100 for optimal killing. Studies in healthy volunteers showed that MFX exposure was proportional to the dose after repeated administration. The non-clinically relevant accumulation beyond steady-state concentrations showed the absence of non linear pharmacokinetics. Age, gender, renal or hepatic dysfunction did not alter the pharmacokinetics in a clinically relevant manner to advocate dose adjustments. To optimize TB treatment therapeutic drug monitoring (TDM) can be recommended in patients infected with *M. tuberculosis* with higher MIC values for MFX or patients concomitantly treated with Rifampicin (RIF) that lowers the exposure by approximately 30%. However, data on dose escalation in TB patients is scarce. As variability in the pharmacokinetics of MFX between TB patients has been observed we studied the intra patient pharmacokinetics of MFX in TB patients that were selected for TDM.

Materials & Methods: TB patients (aged > 18 yrs) who received MFX 400 mg once daily as part of their treatment were eligible for evaluation if they were subjected to TDM after initiation of

MFX and later on during treatment (e.g. after dose adjustment). Intensive pharmacokinetic sampling (t=0, 1, 2, 3, 4, 8h) was performed after at least 5 days of treatment (steady-state). Plasma concentrations of MFX were determined using a validated LC-MS/MS method. Pharmacokinetic parameters were assessed using the KINFIT module of MWPharm (Mediware, the Netherlands). The Middlebrook 7H10 agar dilution method was applied for drug susceptibility testing of the patients' isolates.

Results: Evaluation was performed in 5 TB patients. At 400 mg once daily a mean AUC_{0-24h} of 20.1 (range: 12.1-25.7) was observed after 11 (range: 6-20) days of treatment which is considerably lower compared to the observed exposure in healthy volunteers (i.e. 33.9 ± 1.22 mg*h/L). None of the patients was concomitantly treated with RIF. Although the MFX dose (i.e. 400 mg qd) in two patients remained unchanged as AUC/MIC ratio was over 100, the AUC had increased during treatment with 52% (day 40) and 57% (day 54), respectively. In another patient the dose was increased to 600mg once daily and to 800 mg once daily in two other patients. The AUC had increased disproportional from 18.7 to 66.8mg*h/L at 600mg (day 46), from 12.1 to 46.2 mg*h/L at 800mg (day 41) and from 20.7 to 58.5 mg*h/L at 800mg (day 112), respectively. All patients showed a clinically relevant accumulation in time but in only 2 cases AUC values were reached similar to healthy volunteers. This accumulation may be explained by a reduction in disease severity/critically illness.

Conclusion: The pharmacokinetics of MFX may be influenced during the stage of active and high burden of disease. Later on pharmacokinetics may normalize but do not always reach similar AUC values as determined in healthy volunteers. This may result in development of resistance or treatment failure. More prospective pharmacokinetic studies are needed in TB patients to characterize this phenomenon.

No conflict of interest

Abstract: O_06*PK/PD of new TB drugs***Exploratory pharmacokinetics-pharmacodynamics (PK-PD) target attainment analysis of PA-824**

A.B. Barth¹, R.P. Singh¹, E.F. Egelund¹, Z. Ahmad², E.L. Nuermberger^{2,3}, C.A. Peloquin¹, H. Derendorf¹

¹College of Pharmacy, University of Florida, Gainesville, Florida; ²Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; ³Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

Background: PA-824 is a new nitroimidazole chemotherapeutic agent in clinical trials for improving the treatment of tuberculosis, including multidrug resistance TB. In mice, it has demonstrated dose-dependent bactericidal and sterilizing activity. The efficacy of the drug was mainly associated with the PK/PD index %T>MIC. According to a recent clinical study, the maximum efficacy was unexpectedly achieved at the lowest dosage tested, indicating activity of lower doses should be explored. Therefore, the present study had the aim of performing an exploratory study that could aid dose optimization.

Methods: The population pharmacokinetic parameter estimates associated with the pharmacokinetic variability of PA-824 were used in Monte Carlo simulations (MCS). MIC and PK data derived from a pre-clinical study were used, where PA-824 PK was described as a one compartment body model. The magnitude of the %T>MIC was used as the index that best predicted microbial kill based on the inhibitory sigmoid Emax model. Different levels of free drug (5, 7.5 and 10 %) and inter-individual variability as a whole (10, 20 and 30%) were simulated at different doses. The target attainment (TA) rates were calculated for each regimen by conducting 10,000 MCS. The software Model Risk® (Vose Software, Belgium) was used for the simulations.

Results: The fraction of simulated subjects who achieved a decrease in 1.5 log CFU was

calculated for the range of MICs from 0.031 to 0.125 mg/liter and range of percent free drug (5-10%). The results showed that an average T>MIC of 50% is required for at least 80% of simulated subjects to attain target. The change in free fraction from 5 to 7.5% with 10-30 percent inter individual variability did not show much change in %T>MIC required for TA. The change in free fraction from 5 to 10%, though, increased the probability of attaining target by 100%. The average %T>MIC varied largely by dose. However, the obtained percentage of animals with the decrease in CFU values (in relation to non-treated animals) higher than 1.5 log CFU count did not change extensively at doses higher than 75 mg.

Conclusion: There was a variation of %T>MIC according to the dose. At higher doses there was not a substantial difference in the percentage of animals with a decrease higher than 1.5 log CFU count.

No conflict of interest

Abstract: O_07

PK/PD of new TB drugs

Novel TMC207-containing regimens have sterilizing activity in murine tuberculosis

E. Nuermberger¹, R. Tasneen¹, S. Li¹, C. Peloquin², K. Andries³, K. Mdluli⁴, J. Grosse¹

¹Johns Hopkins University School of Medicine, Center for TB Research, Baltimore, USA; ²University of Florida, College of Pharmacy, Gainesville, USA; ³Johnson & Johnson, Tibotec BVBA, Beerse, Belgium; ⁴Global Alliance for TB Drug Development, Research & Development, New York, USA

Background: Novel combinations containing at least 2 new drugs and having sterilizing activity greater than the first-line regimen of rifampin, isoniazid and Z (RHZ) could transform the treatment of both drug-susceptible and multidrug-resistant (MDR) TB. TMC207 (J) is a new diarylquinoline which improves sputum culture conversion rates when added to a background regimen for MDR-TB. Previous studies in mice identified combinations containing J and pyrazinamide (Z) with sterilizing activity superior to RHZ. Because as many as 50% of MDR-TB isolates are also Z-resistant in some areas, we evaluated the sterilizing activity of novel J-containing regimens with and without Z.

Materials & Methods: BALB/c mice were aerosol-infected with $\sim 4 \log_{10}$ CFU of *M. tuberculosis* H37Rv. Daily treatment began 2 weeks later. Controls received 2 months of RHZ, then 2 months of RH. Test regimens included 3-drug combinations of J, Z, rifapentine (P), moxifloxacin (M), and PA-824 (Pa) in human-equivalent doses. Outcomes were lung CFU counts during treatment and culture-positive relapse 3 months after treatment.

Results: The mean lung CFU count at treatment initiation was $8.21 \log_{10}$. After 2 months, JZP, JZM and PZM reduced the mean lung CFU count to < 0.50 vs. 1.68, 2.70, 3.45, 3.74, 4.44, and 4.58 in the PZPa, JPM, PaMZ, PMPa, RHZ and JPaM groups. At 2 months, 0% and 33% relapse was noted in mice receiving JZP and JZM, and 100% in other groups. At 4 months, 50%, 50%, 67%, 87% and 100% relapsed in the

JPM, JPaM, PaMZ, PMPa and 2RHZ/2RH groups.

Conclusions: While JZP may be superior in its sterilizing activity, JZM may represent an ultrashort regimen for MDR-TB. Regimens without Z were not as sterilizing but, despite evidence of modest antagonism between J and Pa in preceding experiments, 4JPaM was still more effective than 2RHZ/2RH. Therefore, the combined regimen of JZM+Pa may represent a novel regimen for all forms of TB which is significantly shorter than RHZ against Z-susceptible strains and at least as effective as RHZ against strains resistant to R, H and Z.

No conflict of interest

Abstract: O_08*PK/PD of new TB drugs***Safety, tolerability, and pharmacokinetic interactions between single-dose TMC207 and steady-state efavirenz in healthy volunteers: ACTG study A5267**

K. Dooley¹, J.G. Park², S. Swindells³, R. Allen⁴, D.W. Haas⁵, F. Aweeka⁶, A. Gupta⁷, P. Lizak⁸, S. Qasba⁸, Y. Cramer², R. van Heeswijk⁹, C. Flexner¹⁰

¹Johns Hopkins University School of Medicine, Clinical Pharmacology and Infectious Diseases, Baltimore, USA; ²Harvard School of Public Health, Statistical & Data Analysis Center, Boston, USA; ³University of Nebraska Medical Center, Internal Medicine/Infectious Disease, Omaha, USA; ⁴Social & Scientific Systems Inc., Clinical Research and Bioscience, Silver Spring, USA; ⁵Vanderbilt University School of Medicine, Medicine/Infectious Disease, Nashville, USA; ⁶University of California San Francisco, Clinical Pharmacology, San Francisco, USA; ⁷Johns Hopkins Center for Global Health, Clinical Global Health Education, Baltimore, USA; ⁸Montgomery County Department of Health and Human Services, TB Control Program, Silver Spring, USA; ⁹Johnson & Johnson, Clinical Pharmacology, Antwerp, Belgium; ¹⁰Johns Hopkins University School of Medicine, Pharmacology and Molecular Sciences, Baltimore, USA

Introduction: TMC207 is an investigational agent under development for the treatment of drug-resistant and drug-sensitive tuberculosis (TB). TB is the leading cause of death in HIV-infected patients, and TB and HIV must often be treated concomitantly. TMC207 is metabolized by cytochrome P450 (CYP) 3A to a less-active M2 metabolite, and the antiretroviral drug efavirenz (EFV), may induce or inhibit CYP isoforms, including CYP3A.

Materials & Methods: This was a phase I open-label, two-period, sequential-design, multicenter pharmacokinetic (PK) drug interaction trial. Healthy, HIV-seronegative volunteers received a first 400 mg dose of TMC207 and, 14 days later, initiated EFV 600 mg once daily for 28 days. A second 400 mg dose of TMC207 was given after the 14th dose of EFV. Plasma PK sampling for TMC207 and its M2 metabolite was performed over 14 days after each TMC207 dose. Plasma sampling for EFV was performed over 24 hours after the 14th dose of EFV. Concentrations of TMC207 and M2 were determined using LC-

MS/MS, EFV using HPLC. Noncompartmental PK analyses were performed in SAS and confirmed using WinNonLin 6.1. EFV metabolizer status was based on CYP2B6 composite 516/983 genotype.

Results: We enrolled 37 subjects, of whom 36 completed ≥ 1 PK sampling and 33 completed both. Among the 37, median age was 44 years, median weight was 82.9 kg, 22% were African-American, and 8% were women. The geometric mean ratio (GMR) was 0.820 (90% CI 0.753 to 0.893) for the 14-day area under the concentration-time curve (AUC_{0-14d}) and 0.996 (90% CI 0.878 to 1.131) for the maximum concentration of the drug in plasma (C_{max}) for TMC207 with steady-state EFV versus TMC207 alone. For the M2 metabolite, the GMR was 1.073 (90% CI 0.968 to 1.190) for the AUC_{0-14d} and 1.886 (90% CI 1.656 to 2.148) for the C_{max}. There were no Grade 3 or 4 clinical adverse events. One subject developed a Grade 3 transaminase elevation while taking EFV, and as a result of this laboratory abnormality, study drug was discontinued. Mean increase in QTcF from baseline at 4-6 hours after the first and second TMC207 doses was 12.3 msec (95% CI 6.92-17.62) and 12.8 msec (95% CI 7.49-18.14), respectively. There were no statistically significant associations between TMC207 or M2 C_{max} and changes in QTcF for either TMC207 dose administration period. EFV concentrations were similar to historical control data when stratified by CYP2B6 genotype.

Conclusions: Single-dose TMC207 was well-tolerated alone and with steady state EFV. Changes in TMC207 concentrations when given with EFV are unlikely to be clinically significant. Implications of increased M2 C_{max} are unclear. PK modeling of these single-dose data is underway and may help predict PK for TMC207 and M2 during prolonged co-administration with EFV.

No conflict of interest

Abstract: O_09*PK/PD modelling***Population PK of Isoniazid in South African Adults**

P. Denti¹, R. Rustomjee², T. Mthiyane², P. Onyebujoh³, P. Smith¹, H. McIlleron¹

¹University of Cape Town, Division of Clinical Pharmacology, Cape Town, South Africa; ²South African Medical Research Council, TB Research Unit, Durban, South Africa; ³World Health Organization, Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland

Introduction: Isoniazid is a key drug used for the prevention and treatment of tuberculosis. Its clearance is highly dependent on genetic polymorphisms of N-acetyltransferase 2. A large range of values has been reported for the pharmacokinetic parameters of isoniazid amongst different populations.

Material and Methods: 62 South African HIV infected subjects with pulmonary tuberculosis were treated with a fixed dose combination of rifampicin, isoniazid, pyrazinamide, and ethambutol, following the WHO weight-based dosing recommendations. Blood samples were drawn on 4 occasions, on the 1st, 8th, 15th and 29th day of antituberculosis treatment and isoniazid plasma concentrations measured. The data was interpreted with nonlinear mixed-effects modelling using NONMEM VII. Different absorption and disposition models were tested, and between-subject (BSV) and –occasion variability (BOV) in the pharmacokinetic parameters were quantified. Samples with concentrations below the limit of quantification were handled with the M6 method and an additive error structure. Allometric scaling based on fat-free mass was used to adjust clearance and volume of distribution parameters for body size. As no direct information about the genotype of the patients was available, a mixture model was employed to evaluate the presence of subpopulations with different values of pharmacokinetic parameters due to acetylator phenotype.

Results: Isoniazid disposition was best described by a two-compartment model, as previously reported, and absorption was modelled through a series of transit

compartments. A significant amount of BOV was observed in absorption and bioavailability. Conversely, clearance was strongly subject-specific, with a BSV of >50%. The introduction of a mixture model describing 2 subpopulations with estimated clearance values of 28 L/h (in about 45% of subjects) and 60 L/h (for the remaining subjects) reduced unexplained BSV in clearance to 20%. Moreover, in the cluster of subjects with fast clearance, bioavailability was about 70% lower than patients with in the subpopulation with slow clearance. Higher doses of isoniazid (375 mg) were found to have a higher bioavailability.

Conclusions: Even though no direct information on the acetylator status of the subjects was available in this study, the improvement in fit obtained with the introduction of the mixture modelling indicated the presence of at least 2 subpopulations, likely corresponding to slow and fast acetylators. The introduction of a 3rd population was not supported by the data. Fast metabolizers had reduced bioavailability, which might be due to more extensive first-pass metabolism. The higher bioavailability for the largest dose in the study (375 mg) may indicate saturation of this process. Our estimates of clearance are significantly larger than previously reported in an ethnically distinct population of South African patients. This difference should be investigated further.

No conflict of interest

Abstract: O_10*PK/PD modelling***Population pharmacokinetics of rifapentine and its active metabolite in healthy volunteers: nonlinearities in clearance and bioavailability**R. Savic¹, E. Bliven-Sizemore², M. Weiner³, E. Nuermberger⁴, W. Burman⁵, S. Dorman⁶, K. Dooley⁷

¹University of California San Francisco, Department of Bioengineering & Therapeutic Sciences, San Francisco, USA; ²Centers for Disease Control and Prevention, Division of Tuberculosis Elimination, Atlanta, USA; ³University of Texas Health Science Center, Department of Medicine, San Antonio, USA; ⁴Johns Hopkins University School of Medicine, Center for Tuberculosis Research, Baltimore, USA; ⁵Denver Public Health, Infectious Disease Clinic, Denver, USA; ⁶Johns Hopkins University School of Medicine, Center for Tuberculosis Research, Baltimore, USA; ⁷Johns Hopkins University School of Medicine, Division of Pharmacology & Infectious Diseases and Center for Tuberculosis Research, Baltimore, USA

Background: Rifapentine (RPT), a rifamycin antibiotic, is under active investigation as a potent TB drug that may help shorten treatment duration. Available studies suggest several plausible nonlinearities in RPT pharmacokinetics (PK) related to bioavailability and time-dependent changes in clearance (CL) for RPT and its metabolite.

Materials & Methods: Cohorts of 6 healthy volunteers each received RPT for 14 days at a dose of 5, 10, 15, or 20 mg/kg daily in Tuberculosis Trials Consortium Study 29B. PK sampling was performed after the 1st and 14th doses. Trough samples were also collected on days 5 and 10. Population PK analysis was performed using non-linear mixed effects (NLME) modelling using NONMEM (Version 7) software. Model building involved sequential development of absorption, time-varying clearance and bioavailability models for RPT and its metabolite, followed by the establishment of the joint model where all data were simultaneously analyzed. In order to accurately describe highly variable absorption, several models were tested, including first order, zero order with and without lag time, transit compartment model, etc. To account for changes

in CL over time, range of empirical and semi-mechanistic auto-induction models were investigated, for both, parent and metabolite. Additionally, relative changes in bioavailability (F) and fraction metabolized in liver (F_m) with respect to dose and time were explored. Model building was guided by the likelihood ratio test, visual model diagnostics, and model evaluation techniques, including predictive checks.

Results: A one-compartment model described the data well for both parent and metabolite. RPT relative bioavailability decreased linearly by 2.5% for each 100 mg increase in dose. The transit compartment model substantially improved the model fit compared to other absorption models: absorption rate constant of 2 hr⁻¹, mean transit time of 1.1 hr and 10 transit compartments were required. The single dose model under-predicted the multiple-dose data, suggesting CL increase over time for both parent and metabolite. All of the auto-induction models investigated suggested a similar auto-induction pattern of linear increase of CL/F and CL_m/F_m over time independent of the dose administered, with baseline values of 1.2 and 3.1 L/h at day 1 increasing to 2.15 and 5.01 L/h at day 14 for the parent drug and metabolite, respectively. Relative fraction metabolized in the liver (F_m) appeared to increase with dose, suggesting possible saturation of other elimination routes (e.g. renal).

Conclusions: The proposed transit compartment model described the PK of RPT and its metabolite well. The modelling exercise revealed the following in RPT pharmacokinetics: (1) decrease in bioavailability with increased dose; (2) increase of F_m with increased dose; and (3) an auto-induction process increasing the elimination over time for both the parent drug and metabolite. The proposed transit compartment model incorporating RPT dose and time related bioavailability and clearance changes provides a useful tool for correct quantification of drug exposure which is essential information for optimization of drug dosing for TB treatment.

No conflict of interest

Abstract: O_11*PK/PD modelling***Modelling the Pharmacokinetics of Rifapentine in TB patients receiving 10 mg/kg daily**

C. Peloquin¹, M. Weiner², E. Bliven-Sizemore³, E. Egelund¹, M. Engle⁴, J. Johnson⁵, P. Nsubuga⁶, W. Mac Kenzie³

¹University of Florida College of Pharmacy, Infectious Disease Pharmacokinetics Lab, Gainesville, USA; ²University of Texas Health Science Center, Department of Medicine, San Antonio, USA; ³Centers for Disease Control and Prevention, Division of Tuberculosis Elimination, Atlanta, USA; ⁴University of Texas Health Science Center, Department of Medicine, San Antonio, USA; ⁵Case Western Reserve University School of Medicine, Department of Medicine, Cleveland, USA; ⁶Case Western Reserve University School Research Collaboration, Department of Pharmacy, Kampala, Uganda

Background: Rifapentine (RPNT) is the cyclopentyl derivative of rifampin, and is used for the treatment of drug-susceptible tuberculosis (TB). RPNT was approved by the FDA for use in HIV-uninfected patients with TB in the initial phase of treatment at 600 mg twice-weekly, and once-weekly in the continuation phase. A murine model of TB showed that higher dosages and more frequent administration of RPTN result in more rapid bactericidal activity and stable cure. TBTC Study 29 evaluated 10 mg/kg by directly observed therapy five times per week in the intensive phase of treatment. A PK substudy included intensively and singly sampled patients.

Materials & Methods: The participants were administered a reference PK dose of RPNT either fed or fasted, and food descriptions were recorded. During the initial 2 months of therapy, following at least 3 consecutive daily doses such that drug would be at steady state, participants had blood collected at 0,1,2,6,9,12 and 24 hours post dose (intensive, n = 43) or within one hour of the 24 hour trough (single, n = 157). Data were analyzed using non-compartmental techniques (WinNonlin 5.2.1), followed by population methods (Monolix 3.1S). Covariates tested to date include age, sex, height, weight, serum creatinine, and location (African vs. non-African site). A series of models were tested, including 1 and 2 compartment models, zero and first order absorption, and constant, proportional,

or exponential residual error models, beginning with intensively sampled participants.

Results: The final base model included a lag time (t_{lag}) (0.748 hr, inter-individual variation [IIV] 44%), absorption rate constant (k_a) (0.450 1/hr, 4%), volume of distribution (V/F) (51.4 L, 18%), and clearance (CL/F) (2.22 L/hr, 16%). Calculated elimination rate constant (k_e) was 0.043 1/hr and half-life (t_{1/2}) was 16.0 h. African location was the only significant co-factor after extensive testing of single and combination covariates, and was applied to both volume and clearance. The exponential residual error was 19.4%. The most highly correlated parameters were t_{lag} and k_a (0.45). Visual predictive checks (VPC) (N=1000) showed excellent capture of the observed data. eta-Shrinkage values were 1% CL/F, 39% V/F, 78% t_{lag}, and 97% k_a.

Participants with single sampling were added to this base model. Covariates and residual error models were not altered. The t_{lag} changed to 0.773 hr, IIV 46%, k_a to 0.622 1/hr, 14%, V/F to 53.1 L, 23%, and CL/F to 2.04 L/hr, 13%. Calculated k_e was 0.038 1/hr and t_{1/2} was 18.0 h. The exponential residual error was 19.0%. The most highly correlated parameters were V/F and CL/F (0.58). VPC showed excellent capture of the observed data. eta-Shrinkage values increased to 22% CL/F, 73% V/F, 96% t_{lag}, and 99% k_a.

Conclusion: Monolix provided sound initial models for RPNT. Participants with single sample data did not provide information about t_{lag}, k_a or V/F, with resulting increases in eta-shrinkage and higher correlation between V/F and CL/F. Further modelling will include evaluations of food effect and 25-desacetyl-rifapentine (metabolite) concentrations.

No conflict of interest

Abstract: O_12*PK/PD modelling***Population Pharmacokinetics and Pharmacodynamics of Ofloxacin in South African patients with Drug-Resistant Tuberculosis**

E. Chigutsa¹, S. Meredith¹, L. Weisner¹, N. Padayatchi², J. Harding³, W. Mac Kenzie⁴, M. Weiner⁵, H. McIlleron¹, C.M.J. Kirkpatrick⁶

¹University Of Cape Town, Medicine, Cape Town, South Africa; ²University of KwaZulu Natal, TBTC Study 30, Durban, South Africa; ³DP Marais Hospital, Tuberculosis, Cape Town, South Africa; ⁴Centers for Disease Control and Prevention, TBTC Study 30, Atlanta, USA; ⁵Centers for Disease Control and Prevention, TBTC Study 30, San Antonio, USA; ⁶Monash University, Centre for Medicine Use and Safety Faculty of Pharmacy and Pharmaceutical Sciences, Melbourne, Australia

Background: Ofloxacin is a renally eliminated fluoroquinolone drug used in the treatment of multi-drug resistant tuberculosis (MDR-TB). There is scant data on the pharmacokinetics and pharmacodynamics of ofloxacin in patients with MDR-TB, and even less data in South African patients. The best known predictor of in-vivo efficacy of ofloxacin has been shown to be the ratio of the drug's area-under-the-curve (AUC) to the minimum inhibitory concentration (MIC). We used a population model to describe ofloxacin pharmacokinetics and the expected probability of obtaining target exposures (free ofloxacin AUC/MIC > 100) in the study population.

Materials and Methods: Thirty-eight patients were recruited from Cape Town and 27 patients were recruited from Durban, South Africa. The patients were on a multi-drug regimen for the treatment of MDR-TB including anamycin, pyrazinamide, terizidone, ethionamide and a daily 800 mg dose of ofloxacin. Pharmacokinetic sampling involved drawing 5-7 blood samples from patients during a single dosing interval. Liquid chromatography with tandem mass spectrometry was used for quantifying the ofloxacin plasma concentration. Sputum was collected from the patients at baseline and used for determination of the ofloxacin MIC using the agar dilution method. The software NONMEM® was used for data analysis.

Results: A two compartment model with a transit absorption compartment model best described the pharmacokinetics of ofloxacin. Drug clearance was estimated to be a combination of glomerular filtration, which was affected by creatinine clearance, and active tubular secretion, which was higher in heavier patients. The rate of drug absorption was slower in patients who took the drug after a meal compared to the fasting state. Monte Carlo simulations were performed to estimate the probability of target attainment (PTA; free AUC/MIC > 100). A free fraction of plasma ofloxacin of 0.75 was used to obtain the free AUC from the total AUC. MIC data was available from 23 patients in the Durban cohort and ranged from 0.5 mg/L (38% of patients), 1.0 mg/L (52%), 2 mg/L (4%) to 8 mg/L (4%) of patients. The corresponding PTA for each MIC was 0.97, 0.52, 0.11 and 0.00 respectively. Assuming that the distribution of MICs in Durban and Cape Town are similar, this results in a true PTA expectation value of 0.64.

Conclusion: The pharmacokinetics of ofloxacin in South African MDR-TB patients have been elucidated, taking into account glomerular filtration and active tubular secretion as the primary elimination pathways. Thirty-five percent of patients failed to achieve the minimum free AUC/MIC ratio of 100, and higher doses should be considered if more effective fluoroquinolones are not available.

No conflict of interest

Abstract: O_13*New Drug Development Methods***A novel pharmacodynamic model for treatment of Tuberculosis using days to positivity in automated liquid Mycobacterial culture***E. Chigutsa¹, K. Patel², M. Visser³, G. Maartens¹, C.M.J. Kirkpatrick², H. McIlleron¹, M.O. Karlsson⁴*

¹University Of Cape Town, Medicine, Cape Town, South Africa; ²Monash University, Faculty of Pharmacy and Pharmaceutical Sciences, Melbourne, Australia; ³University of the Western Cape, School of Public Health Cape Town, Cape Town, South Africa; ⁴Uppsala University, Department of Pharmaceutical Biosciences, Uppsala, Sweden

Background: Measurements of treatment response in patients receiving treatment for tuberculosis include the decline in Mycobacterium tuberculosis (Mtb) colony forming unit counts (CFUs) in serial sputum samples. Counting of CFUs is expensive, labour intensive, and is fraught with methodological difficulties leading to inaccurate counts. Another measure of bacillary load in patients is time to a positive test result in liquid culture, which is inversely related to the inoculum concentration. We describe the decline in viable Mtb in the sputum during the intensive phase of standard short course chemotherapy in patients with sputum smear-positive pulmonary tuberculosis using the quantitative measure of days to positivity in liquid culture using a nonlinear mixed effects model.

Materials and Methods: One hundred and forty four patients starting a 5 day per week drug regimen containing rifampicin, isoniazid, pyrazinamide and ethambutol were recruited. Sputum was collected from patients before they started treatment, and then once weekly for 8 weeks. Sputum was processed for culture on liquid media (BACTEC MGIT 960; Becton Dickinson, Sparks, MD) and the days to a positive result were recorded. If no growth was observed after 42 days of incubation, the result was recorded as negative. A time to event model implemented in the software NONMEM® was used for data analysis.

Results: A biexponential decay model was used to characterise decline in bacillary burden of Mtb in patients over the 8 weeks of treatment. The relative concentration of Mtb from the patients' weekly sputum obtained from the biexponential model was incorporated as the baseline hazard of a positive MGIT culture result. An exponential model was used to describe growth of Mtb in MGIT culture. The exponential growth was preceded by a fixed lag phase of 3 days for baseline sputum samples, and 5 days for samples collected during treatment. The rate of bacterial growth in the MGIT culture was dependent upon the relative proportions of fast-replicating bacilli and slow-replicating bacilli in the sputum from each patient, estimated from the biexponential decay model. The hazard of a positive culture then increased with incubation time, proportional to the rate of growth of the bacteria, until a positive culture result was recorded. In the case of a negative MGIT result, this meant that the baseline hazard was so low that culturing for 42 days would still result in a low probability of a positive MGIT result. The typical value for the half-life of the kill of fast-replicating bacilli in patients without lung cavitation was 3 days (19% of patients), whilst it was 6 days in patients with lung cavitation (81% of patients) ($p < 0.01$). The ratio of fast-replicating bacilli to slow replicating bacilli was 484:1. The typical value for the half life of kill of slow-replicating bacilli was 54 days.

Conclusion: This is the first model describing the pharmacodynamics of the treatment of tuberculosis using a weekly time to event model based on days to positivity in liquid culture. The model can be used for investigation of covariates such as drug exposure or different Mtb chemotherapy combinations.

No conflict of interest

Abstract: O_14*New Drug Development Methods***Rifampicin concentration-effect relationships for resistance development differ between *Mycobacterium tuberculosis* genotypes**

*J.E.M. de Steenwinkel*¹, *M.T. ten Kate*¹, *G.J. de Knegt*¹, *K. Kremer*², *H.A. Verbrugh*¹, *M.J. Boeree*³, *R.E. Aarnoutse*⁴, *D. van Soolingen*², *I.A.J.M. Bakker-Woudenberg*¹

¹Erasmus MC, Department of Medical Microbiology & Infectious Diseases, Rotterdam, The Netherlands; ²National Institute of Public Health and the Environment Centre for Infectious Disease Control, National Tuberculosis Reference Laboratory, Bilthoven, The Netherlands; ³Radboud University Nijmegen Medical Centre, Department of Pulmonary Diseases, Nijmegen, The Netherlands; ⁴Radboud University Nijmegen Medical Centre, Department of Pharmacy, Nijmegen, The Netherlands

Introduction: Emergence of resistance in *Mycobacterium tuberculosis* (Mtb) against anti-tuberculosis (TB) drugs is one of the largest public health challenges threatening the WHO targets of TB elimination. There are multiple factors contributing to low cure rates, treatment failures, and relapses after curative treatment. However, there is one underestimated factor that presumably fuels the worldwide problem of emerging resistance; the evolution of Mtb. The Beijing genotype of Mtb is significantly associated with drug resistance in various geographic areas and may have a higher intrinsic ability to withstand treatment with anti-TB drugs. Therefore, the *in vitro* activity of anti-TB drugs towards Beijing genotype strains and East-African-Indian (EAI) genotype strains from the same endemic area (Vietnam) was compared. Such knowledge could attribute to the insight in genotype-specific pharmacodynamics (PD) and thus improve study design for future drug-development.

Materials and Methods: We assessed the minimal inhibitory concentration (MIC) of isoniazid (INH), rifampicin (RIF), moxifloxacin (MXF) and amikacin (AMK) and the *in vitro* mutation frequency (MF) for five Beijing and five EAI genotypes strains. Additionally, we determined the time-kill kinetics of these anti-TB drugs towards the different strains, and we assessed the *in vitro* emergence of resistance and the range of concentrations within which solely resistant mycobacterial populations were selected. Also the mutant prevention concentration (MPC) was assessed.

Results: All Beijing and EAI genotype strains appeared susceptible to INH, RIF, MXF and AMK using the BACTEC MGIT liquid culturing system. Also MIC values of were similar. The MF of the Beijing and EAI genotype strains were similar regarding INH, MXF and AMK. However, regarding RIF the MF of the Beijing genotype strains and EAI genotype strains differed significantly. Time-kill kinetics of RIF showed a strong time-dependent as well as concentration-dependent activity. At low mycobacterial density, low RIF concentrations were needed to achieve >99% killing. However, to achieve 100% killing, the RIF concentrations had to be increased substantially, and this effect was much more pronounced for the Beijing genotype strain. Similarly differences between low- and high density cultures were observed, particularly for Beijing genotype strain. A substantial increase in RIF concentrations was needed to kill 100% of the high density Beijing culture. MPC distribution of the Beijing and EAI genotypes showed a very small range.

Discussion: This study shows that conventional anti-TB drug susceptibility assays do not discriminate between the *in vitro* susceptibility of the Beijing and EAI genotype strains and additional determination of the MF may be more informative. As shown for the Beijing genotype strains, the observed high MF for RIF, strong dependency of the killing capacity of RIF on the mycobacterial density and the large RIF concentration window in which RIF-resistant mutants were selected might attribute to the less favourable treatment outcome in the Beijing genotype TB infections.

Conclusion: This proof of genotype specific RIF PD is indicating that future drug-development studies should use a range of (endemic) Mtb genotypes, in order to assess the concentration-effect-relation of new drugs and that genotype-specific MF assessment should be performed in drug-development.

No conflict of interest

Abstract: O_15*New Drug Development Methods***Dose-ranging activity of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis**

R. Tasneen¹, C. Peloquin², E. Nuermberger¹

¹Johns Hopkins University School of Medicine, Center for TB Research, Baltimore, USA; ²University of Florida, College of Pharmacy, Gainesville, USA

Background: Rifamycins are key sterilizing drugs in the treatment of tuberculosis (TB) but the optimal drug and dose remain unresolved. We previously studied the dose-ranging activity of rifampin (R) and rifapentine (P) in infected BALB/c mice and found that (i) P is 4 times more potent than R when administered daily, and (ii) substitution of P for R (each at 10 mg/kg and combined with isoniazid-pyrazinamide (HZ)), shortens the treatment needed to cure mice by 2-3 months. However, BALB/c and other conventional mouse strains are criticized for the intracellular nature of infection and the lack of histopathological changes observed in human TB. Therefore, we sought to compare R and P in C3HeB/FeJ mice, which develop necrotic granulomas with extracellular bacilli more closely resembling human TB lesions.

Materials & Methods: Single dose PK of R and P were compared in each mouse strain. Mice were aerosol-infected with 2.0 log₁₀CFU of *Mycobacterium tuberculosis* H37Rv. At treatment initiation, 6 wk later, mean lung CFU counts were 7.29 ± 0.46 and 6.62 ± 0.16 in C3HeB/FeJ and BALB/c mice, respectively. Mice received escalating doses (in mg/kg) of R (10, 20, 40) or P (5, 10, 20) alone, or either rifamycin at 10 mg/kg in combination with. Treatment was administered 5 days per week for 8 wk (monotherapy) or 12-16 wk (combination therapy). Lung CFU counts were assessed at 4 and 8 wk. Relapse was assessed 12 wk after completing 4, 8, 12 or 16 wk of treatment.

Results: Single dose serum PK was similar in the 2 strains. Infected BALB/c mice developed characteristic lung histopathology, with well-circumscribed aggregates of mononuclear cells

and intracellular acid-fast bacilli, whereas C3HeB/FeJ mice develop necrotic granulomas with a high proportion of extracellular bacilli. Both R and P exhibited dose-dependent bactericidal activity in both models. Cell kill at a given dose was similar between mouse strains; but tender to be greater in C3HeB/FeJ mice. For a given dose of rifapentine, the equipotent dose of rifampin was approximately 4 times higher, irrespective of mouse strain. Two months of PHZE rendered both strains of mice culture negative, whereas RHZE-treated mice averaged 1.10 to 1.25 log₁₀ CFU. Relapse results after 2 months of treatment are pending.

Conclusions: The superior potency of P over R observed in BALB/c mice is also seen in C3HeB/FeJ mice, which develop histopathological changes more closely resembling human TB. These results suggest that the superiority of P over R is not determined by differential activity against intracellular organisms or drug penetration into necrotic lesions.

No conflict of interest

Abstract: O_16*New Drug Development Methods***New methods for therapeutic drug monitoring of moxifloxacin in patients with tuberculosis in rural areas and resource limited settings***J.W.C. Alffenaar¹, D.H. Vu², A.D. Pranger¹, R. van Altena³, T.S. van der Werf⁴, W.C.M. de Lange³, J.G.W. Kosterink¹, D.R.A. Uges¹**¹University Medical Center Groningen, Hospital and Clinical Pharmacy, Groningen, The Netherlands; ²Hanoi University of Pharmacy, Clinical Pharmacy, Hanoi, Vietnam; ³University Medical Center Groningen, Tuberculosis Center Beatrixoord, Haren, The Netherlands; ⁴University Medical Center Groningen, Tuberculosis and Lung Diseases, Groningen, The Netherlands*

Introduction: The AUC_{0-24h} to MIC ratio is the most predictive parameter for efficacy of Moxifloxacin (MFX). Therefore therapeutic drug monitoring (TDM) can be recommended to assure adequate exposure to optimize treatment and prevent development of resistance. Unfortunately, TDM requires advanced laboratory facilities and cooled transportation of blood samples and is therefore often not available. Dried Blood Spot analysis (DBS) and Thin Layer Chromatography (TLC) are two methods that may be helpful to support TDM in rural areas and resource limited settings. MFX was used as model compound for the development and clinical evaluation of these methods of analysis.

Materials & Methods: Intensive pharmacokinetic sampling (t=0, 1, 2, 3, 4 and 8h) was performed at steady-state in 9 TB patients who received MFX 400 mg qd. At the same time saliva samples were obtained in 4 patients using a Salivette®. DBS samples were obtained at t=0, 2, 8h. DBS samples and plasma concentrations of MFX were determined using a validated LC-MS/MS method. Saliva concentrations were determined by applying 10ul of saliva extract on a TLC plate and comparing the intensity, size and shape of the fluorescence spot (366nm) with three reference spots (0.5, 1, 2.5 mg/L). The correlation between MFX concentration in DBS and plasma was evaluated by simple linear

regression and Passing-Bablok regression. The level of agreement between five analysts for two different batches (i.e. sheet 1 and 2) of TLC plates (weighted *k*) was calculated to evaluate the robustness of the TLC method. The overall agreement was expressed as an intraclass correlation coefficient for the latent variable. A Bland-Altman analysis was used to calculate the correlation between predicted (DBS or saliva) and observed AUC_{0-24h}.

Results: The median AUC_{0-24h} after 400 mg MFX in plasma was 28.4 (range: 11.0 – 61.9) mg*h/L, 35.1 (range: 12.7–97.2) mg*h/L in DBS. DBS calibration curves were linear in the range of 0.05–6.00 mg/L with inter-day and intra-day precisions and biases of less than 11.1%. The recovery was 84.5, 85.1 and 92.6% in response to blood concentration of 0.15, 2.50 and 5.00 mg/L, respectively. MFX in DBS was stable for at least 4 weeks at room condition (temperature of 25 °C and humidity of 50%). The simple linear regression showed excellent correlations between the plasma level and the DBS level (R² = 0.996). The ratio saliva: plasma was 0.2; 0.3; 0.3 and 0.8. Regarding the TLC method, the weighted kappa was determined at 0.84 and 0.92 for sheet 1 and sheet 2, respectively. The overall agreement between analysts was estimated at 0.969 (95% CI: 0.722 – 0.997). There were no interfering spots observed by simultaneous determination of commonly used anti-TB drugs in saliva. The Bland-Altman analysis showed a good correlation between predicted and observed AUC_{0-24h} values.

Conclusion: This study showed that MFX AUC_{0-24h} could be predicted accurately using DBS and semi quantitative by TLC using saliva. Both methods can be used to individualize treatment in rural areas and resource limited settings.

No conflict of interest

Abstract: LB_01*New Drug Development Methods***Rapid evaluation of candidate regimens for XDR-TB including PNU-100480, TMC207, and PA-824 using whole blood culture**

R. S. Wallis¹, W. Jakubiec², M. Mitton-Fry¹, L. Ladutko², S. Campbell^{2,3}, D. Paige¹, P. F. Miller¹

¹ Pfizer, Groton, CT; USA ² VA CT Healthcare, West Haven, CT; USA ³ Yale University, New Haven CT, US

There presently is no rapid method to test novel drug combinations for tuberculosis. This study examined the bactericidal activities of PNU-100480, TMC207, PA-824, and pyrazinamide, singly and in various combinations, using whole blood culture. The addition of 1,25 dihydroxy-vitamin D facilitated detection of the activity of TMC207 in the 3-day cultures. Low, mid, and high therapeutic concentrations of each drug pair were tested in a checkerboard fashion. Observed bactericidal activity was compared to that predicted by the sum of the effects of individual drug. Combinations of PNU-100480 and TMC207 were fully additive, whereas those including PA-824 were less than additive or antagonistic. The cumulative whole blood bactericidal activities (WBA) of 2, 3, and 4 drug combinations were predicted based on the observed concentration-activity relationship and published pharmacokinetic data after oral dosing. The most active regimen, which consisted of PNU-100480 600 mg BID, TMC207 400 mg QD, and standard doses of pyrazinamide, was predicted to have cumulative activity comparable to standard TB therapy. Further testing of regimens including these compounds is warranted. Measurement of whole blood bactericidal activity can accelerate the development of novel TB regimens.

Author	Abstract title	Abstract #	Page #
Aarnoutse, R.	Evaluation of protein-unbound, active concentrations of rifampicin in Indonesian tuberculosis patients	O_01	03
Alffenaar, J-W.	Moxifloxacin exposure is significantly lower during early treatment of tuberculosis compared to a later stage of treatment	O_05	07
Alffenaar, J-W.	New methods for therapeutic drug monitoring of moxifloxacin in patients with tuberculosis in rural areas and resource limited settings	O_16	18
Barth, A.	Exploratory pharmacokinetics-pharmacodynamics (PK-PD) target attainment analysis of PA-824	O_06	08
de Steenwinkel, J.	Rifampicin concentration-effect relationships for resistance development differ between Mycobacterium tuberculosis genotypes	O_14	16
Denti, P.	Population PK of Isoniazid in South African Adults	O_09	11
Dooley, K.	CYP3A induction by rifampin and rifapentine: Which drug and dose does it best?	O_04	06
Dooley, K.	Safety, tolerability, and pharmacokinetic interactions between single-dose tmc207 and steady-state efavirenz in healthy volunteers: actg study a5267	O_08	10
Dooley, K.	Population pharmacokinetics of rifapentine and its active metabolite in healthy volunteers: nonlinearities in clearance and bioavailability	O_10	12
McIlleron, H.	The safety and pharmacokinetics of adjusted dose lopinavir /ritonavir when given with tuberculosis treatment in South African HIV-infected patients.	O_03	05
McIlleron, H.	Population pharmacokinetics and pharmacodynamics of ofloxacin in South African patients with drug-resistant tuberculosis	O_12	14
McIlleron, H.	A novel pharmacodynamic model for treatment of tuberculosis using days to positivity in automated liquid mycobacterial culture	O_13	15
Nuermberger, E.	Novel TMC207-containing regimens have sterilizing activity in murine tuberculosis	O_07	09
Nuermberger, E.	Dose-ranging activity of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis	O_15	17
Peloquin, C.	Modeling the Pharmacokinetics of Rifapentine in TB patients receiving 10 mg/kg daily	O_11	13
Ruslami, R.	Pharmacokinetics and safety of high dose rifampicin and moxifloxacin for tuberculosis meningitis: preliminary data from a RCT in Indonesia	O_02	04
Wallis, R	Rapid evaluation of candidate regimens for XDR-TB including PNU-100480, TMC207, and PA-824 using whole blood culture	LB_01	19



virology education

a medical education company

Biltstraat 106
3572 BJ Utrecht
the Netherlands
www.virology-education.com

www.virology-education.com
