High-dose isoniazid early bactericidal activity against drug-resistant tuberculosis (*katG*-mutated *Mycobacterium tuberculosis*)

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Background and Objectives

Multidrug-resistant (MDR) TB (isoniazid (INH) and rifampicin resistance) threatens global TB control.

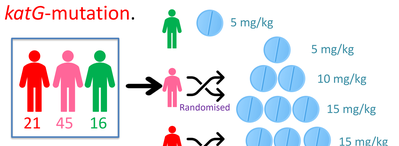
High-dose INH reported to improve MDR-TB treatment outcome.[2,3] *katG*-mutation (activate INH, MICs of 2 to 10 mg/L[1]) is the frequently observed cause of INH-resistance.[4]

Aim: characterize association between INH pharmacokinetics (PK) (at high or standard doses) and its early bactericidal activity (EBA) against *Mycobacterium tuberculosis* (*M.tb*) (drug-sensitive and *katG*-mutated).

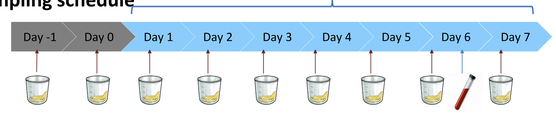
Methods

INHindsight 7-day early bactericidal activity (EBA) of INH dose escalation.[5] Pooled data from the initial analysis (drug-sensitive and *inhA*-mutated isolate) [6], current data has *katG*-mutation.

Study procedure



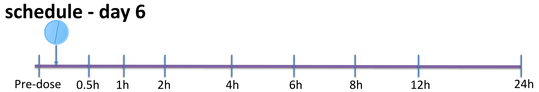
Sampling schedule



Sputum samples were cultured on solid media to capture colony forming unit (CFU) in drug-susceptible and *inhA*-mutated and in liquid media for Time to positivity (TTP) in all drug-susceptibility groups.

Isoniazid minimum inhibitory concentration (MIC) was measured. Median value of drug-sensitive or *inhA*-mutation group imputed when MIC was missing.

PK schedule - day 6



PK-Pharmacodynamic(PD) modelling in NONMEM[7] was used to interpret the data.

- Pre-existing PK-PD model was built on.[7]
- N-acetyltransferase 2 (NAT2) genotype captured (rapid, intermediate or slow). If missing, mixture model was used to assign phenotype.[8]

Results - Study population

Table 1: Participants characteristics

	Drug-sensitive	InhA	KATG
Participants (N)	**16**	**43**	**21**
Sex (M/F)	12/4	31/12	**15**/6
Race (Black/White/Mixed)	6/1/9	15/0/28	**14**/1/6
Age (years)	30(19-58)	34(18-58)	37(19-60)
Weight (kg)	54(40.5 – 80.9)	52(40.6 - 79)	53(41-71)
HIV status (Negative/Positive)	13/3	34/9	**17**/4
Cavities (Yes/No)	12/4	40/3	**13**/8
MIC	0.2(0.2-1)	1(0.2-4)	4(0.03-4)
NAT2 genotype(R/I/S/NA)	4/10/2/0	4/22/13/4	Missing

Results – PK model

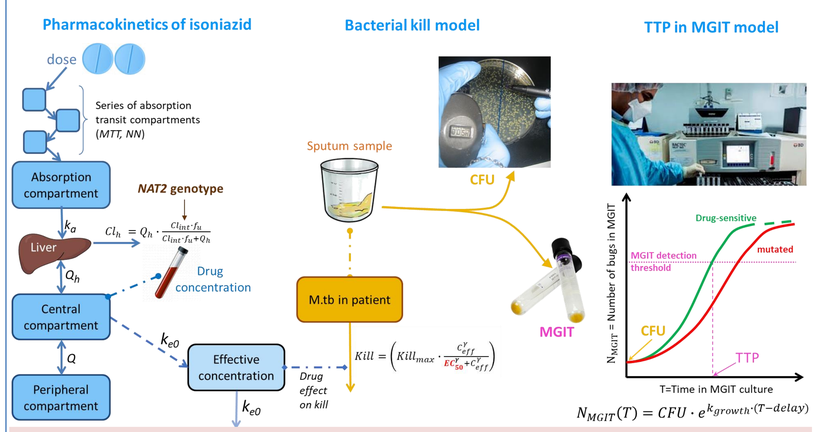


Figure 1. Schematic representation of the PK-PD model used to describe the PK of isoniazid, the drug-induced decline in bacteria load, and the growth of M.tb in MGIT. cfu=colony-forming units; MGIT=Mycobacterial Growth Indicator Tubes; M.tb=Mycobacterium tuberculosis; MTT = absorption mean transit time; NN = number of absorption transit compartment; PD=pharmacodynamics; PK=pharmacokinetics; TTP=time to positivity. C_{eff} concentration in effect compartment, $Kill_{max}$ maximum kill achieved by isoniazid EC_{50} concentration associated with 50% of maximal kill

Table 2: Final parameter estimates

Parameter	Typical value (95% CI[a])	Variability (95% CI[a])
Baseline CFU for drug-sensitive (log_{10}CFU)	5.04 (4.40 – 5.63)	0.942[b] (0.591 – 1.29)
Baseline CFU for mutated (log_{10}CFU)	5.82 (5.22 – 6.25)	
Effect of Cavity on baseline (log_{10}CFU)	**+0.645(0.281 – 0.879)**	
Additive error in CFU (log_{10} CFU)	0.494 (0.435 – 0.579)	
Growth Rate in MGIT drug-sensitive – k_{growth} (1/day)	1.62 (1.40 – 1.82)	
Growth Rate in MGIT for mutated - k_{growth} (1/day)	1.17 (0.967 – 1.32)	
Delay of growth in MGIT after INH treatment (h)	3.32 (2.33 – 6.70)	15.6[d] (11.1 – 20.0)
MGIT detection threshold (log_{10} CFU)	9.26 (8.81 – 9.64)	
Proportional error in TTP (%)	13.6 (11.9 – 15.1)	
EC_{50} for drug-sensitive (mg/L)	0.01 Fixed	
EC_{50} for inhA (mg/L)	0.529 (0.22 – 2.20)	
EC_{50} for katG (mg/L)	5.18 (2.34 – 10.7)	
Gamma – γ ()	2.88 Fixed	
$Kill_{max}$ (1/day)	0.42 (0.367 – 0.856)	65.3[c] (40.3 – 83.5)
$T_{1/2E0}$ – half-life of effect compartment delay (h)	11.0 (5.81 – 22.5)	
Correlation of the CFU-TTP errors (%)	-38.5 (-53.7 – -24.7)	

The ε-shrinkage for CFU = 8% and TTP = 17%.
[a] Estimated from non-parametric bootstrap of the final model (n=200, stratified on arm).
[b] is the between-subject variability of the parameter estimate expressed as standard deviation (in the log10 scale)
[c] is the between-subject variability of the parameter expressed as approximate coefficient of variation (CV%)
[d] is the between-occasion variability of the parameter expressed as approximate coefficient of variation (CV%)

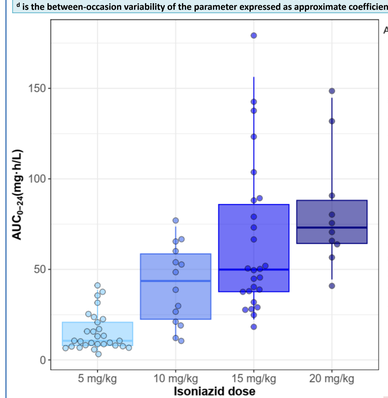


Figure 2. Boxplot of isoniazid AUC_{0-24} across the 4 observed doses

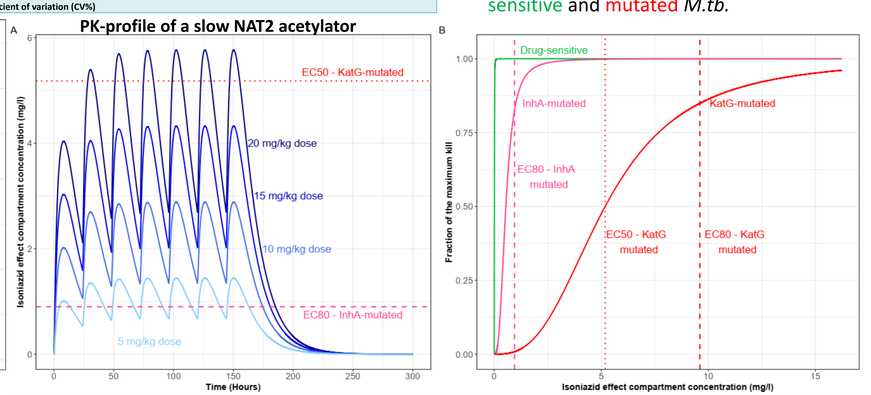


Figure 3. (A)Isoniazid concentration profiles in the effect compartment of a typical individual with slow N-acetyltransferase 2 acetylator status dosed with 5, 10, 15 and 20 mg/kg doses. The pink and red lines represent concentration associated with 80% of maximal kill (EC80) of *inhA*-mutated and concentration associated with 50% of maximal kill (EC50) of *katG*-mutated *M.tb*, respectively. (B)The pharmacokinetics-pharmacodynamics relationship of isoniazid against Mycobacterium tuberculosis (*M.tb*). The green, pink and red profiles represent the pharmacokinetics-pharmacodynamics relationship of the drug-sensitive, *inhA*- and *katG*-mutated *M.tb*, respectively. The pink and green dashed lines represent the concentration EC80 of the *inhA* and *katG*-mutated *M.tb*, respectively, and the dotted line is the EC50.

PK model

- 2-compartment model, transit compartment absorption, with first-pass metabolism hepatic extraction and effect of **NAT2 genotype** on clearance.
- Isoniazid AUC across the 4 doses heavily overlapped, likely due to **NAT2 genotype**.

PK-PD model

- Bacterial kill was modulated by isoniazid concentration using an effect compartment.
- The equilibration between plasma and the effect compartment has a half-life of 11 hours.
- PK-PD relationship was through a sigmoidal Emax.
- Mutated *M.tb* had higher baseline CFU.
- Baseline CFU was high in the presence of cavity.
- Mutated *M.tb* grew with a doubling time 4 hours slower than drug-sensitive in MGIT.
- *M.tb* exposed to drug had a delay of growth in the MGIT of 3.32 hours compared to non-exposed.
- Isoniazid potency against *katG*-mutated *M.tb* was 10 folds lower than *inhA*.
- A slow *NAT2* acetylator, dosed 20mg/kg dose barely achieved the EC50 of *katG*-mutated *M.tb*.
- Maximum kill was similar between drug-sensitive and mutated *M.tb*.

Discussion

Limited to no INH EBA over 7 days against M.tb strains with katG-mutations among participants with MDR-TB.

Drug effect was apparent in participants with high INH AUC, which did not correlate well with dose and was highly variable, likely due to **NAT2 genotype**.

INH is primarily activated by KatG; the EBA of INH against katG-mutated *M.tb* supports the hypothesis that small quantities of INH are activated either by incomplete inactivation of katG[9] or alternative pathways[10-11].

INH monotherapy may not be useful for katG-mutated M.tb as even 20mg/kg did not reach measurable EBA except in select participants.

The role of high-dose isoniazid in MDR-TB regimens needs to be investigated.

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Acknowledgements and Support

The study team is very grateful to the study participates. We appreciate the contributions of the following individuals: Professor Cedric Werely for genotyping participants blood specimens to determine NAT2 acetylator status and Sachiko Miyahara for her input in this project.

Supported by the Division of AIDS, NIH. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases, NIH, under award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701. This work was supported in part by TASK Clinical Research Site grant UM1AI069521. The University of Cape Town Clinical PK Laboratory is supported in part via the AIDS Clinical Trial Group; by the National Institute of Allergy and Infectious Diseases of the NIH under award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701; and the International Maternal Pediatric Adolescent AIDS Clinical Trials Group.

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