PREDICTION OF THE ORAL CLEARANCE OF ISONIAZID IN VIRTUAL SUBJECTS WITH DIFFERENT NAT-2 ACETYLATOR STATUS

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Objectives

The aim of this study was to develop a scaling strategy for cytosolic enzymes that incorporates *in vitro* metabolism data with information on relative abundance in conjunction with the appropriate phenotype frequencies and demographics to simulate the range of isoniazid clearances in a virtual South African Population. The use of 2 *vs*. 3 phenotypes to describe the population variability was also compared.

Methods

- Information relating to demographics and bodyweights in South African populations were collected from publically available databases.
- A meta-analysis was carried out for NAT-2 phenotype frequencies in South African subjects where mean values were weighted for the size of study populations.
- Clinical data were collected to provide oral and renal (CL_R = 2.76 L/h) clearance values for isoniazid [1-5].
- The *in vitro* intrinsic clearance for the cytosolic metabolism of isoniazid (3.13 µL/min/mg) was back calculated from the average oral clearance (CLpo = 26.3 L/h) in fast acetylator subjects reported by Peloquin et al.[3], using a retrograde approach.
- Relative abundances of the enzyme in fast and slow acetylators were calculated based on data reported by Weber and Hein [6] and Werely et al. [7].
- The pharmacokinetics of isoniazid were simulated in a virtual population with demographics matching those represented in available clinical studies, using the Simcyp Simulator (V14R1).
- Simulated plasma concentration-time profiles and distribution of CLpo values were compared to observed data.

Results

Four studies with sufficient genetic information to classify subjects into slow, Intermediate and fast phenotypes were identified (Table 1). The data from these studies were used to calculate weighted mean frequencies for each of the phenotypes.

The relative activity of N-acetyltransferase in PM was set at 0.25 of the activity in extensive metabolisers [6]. When used the activity in the IM phenotype was set to 0.61 of the activity in the EM subjects.

Table 1 Phenotypic frequencies for N-acetyltransferase

Ref.	n	Genotype/ Phenotype	Subjects	% Phenotype		
				PM	IM	EM
Donald 2004	87	Gene	TB infected, Western Cape.	32.2	41.4	26.4
Zhu 2012	151	Gene	Infants, Capetown/ Durban	31.8	44.4	23.8
Parkin 1997	47	Gene	TB infected, Stellenbosch	31.9	44.7	23.4
Lotkionov 2002	101	Gene	Tswana speaking	39.6	39.6	20.8
Sum	386		Weighted Mean	33.9	42.5	23.6

Two different scenarios were investigated using simulation approaches. In scenario 1 the frequency of EM. PM and IM subjects were set to 0.236, 0.339 and 0.425, respectively based on the results of the meta-analysis.

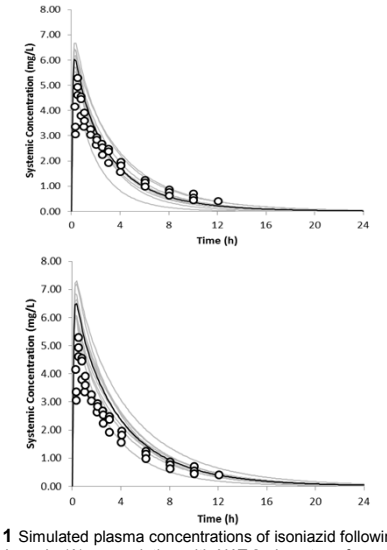

Figure 1 Simulated plasma concentrations of isoniazid following a 300 mg oral dose in (A) a population with NAT-2 phenotype frequencies of 0.236, 0.339 and 0.425 in EM, PM and IM subjects and (B) a population with NAT-2 phenotype frequencies of 0.236 and 0.764 in EM and PM subjects. A virtual population of 10 trials of 14 subjects was simulated (age 20-50; 0.429 female) (grey lines) and the dots represent the observed data from the clinical study by Peloquin et al.[6]

Table 2 Compound specific information used to develop the PBPK model for isoniazid

Parameter	Value	Units
	Isoniazid	
Mol Wt	137.1	(g/mol)
Log P	-0.7	
Compound Type	Monoprotic Base	
pKa	1.82	
B/P	0.825	
fu_p	0.95	
Main plasma binding protein	Albumin	
fa	1	
ka	3.55	1/h
fu_{gut}	1	
Q_{gut}	2.39	L/h
P_{eff}man	0.318	10^{-4} cm/s
V_{ss}	0.5	L/kg
CLu_{int} user cytosol	3.125	µl/min/mg cytosolic protein
CL_R	2.76	L/h

In the second scenario the frequency of EM and PM subjects was set to 0.236 and 0.764 (sum of IM & EM) respectively.

The pharmacokinetics of isoniazid were simulated using a compound file constructed using the data presented in Table 2. The simulated concentrations in the two scenarios were compared to clinical data (Figure 1).

The simulations were extended to a larger virtual population (n = 1000; Age 18-65; 0.5 female) and the individual oral clearance values of isoniazid were compared with those observed in the study of Werely et al ([7] Figure 2)

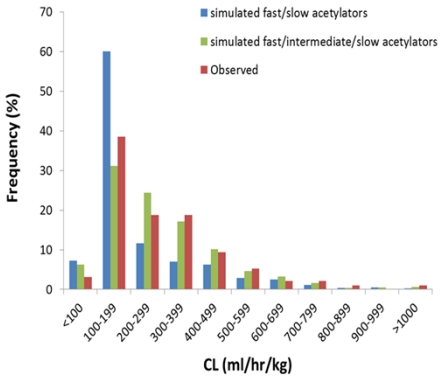

Figure 2 Histogram showing the frequency of Clearance (ml/hr/kg) values of isoniazid in a population of subjects. Simulations were performed using two scenarios. In the first individuals were considered to be either EM (freq 0.236) or PM (0.764), in the second scenario individuals were assigned to be EM (0.236), IM (0.425) or PM (0.339).

Conclusions

Using information on NAT-2 metabolism in fast acetylators, together with information on the frequency of intermediate and slow acetylator phenotypes, and relative activity of NAT-2 across phenotypes it was possible to simulate the plasma concentration-time profile and distribution of clearances of isoniazid within a South African population.

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