



Nanoscale pharmacokinetics and pharmacodynamics of isoniazid treatment of tuberculosis in zebrafish larvae

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Introduction

Zebrafish are increasingly popular in tuberculosis (TB) research¹. *Mycobacterium marinum*, the aquatic pathogen, shows pathology similar to *M. tuberculosis* upon infection in zebrafish larvae².

Anti-TB drug isoniazid has been shown to treat *M. marinum* infections in zebrafish larvae upon waterborne treatment. To interpret this drug effect and translate between species, internal drug exposure must be quantified³.

Subsequently to assess scaling of disease dynamics to higher vertebrates, a multistate tuberculosis pharmacometric (MTP) model that has been developed for *in vitro* as well as murine and human data⁴, will be tested.

Here, we quantify the pharmacokinetics of isoniazid in zebrafish larvae, and the natural growth of *M. marinum* using the MTP model to compare it to *M. tuberculosis*; to assess between-species scaling in TB.

Methods

- Zebrafish larvae were treated from 3 days post fertilization (dpf) onwards with waterborne isoniazid concentrations of 0.5, 1, 2, 5, and 10x MIC (15 mg/L). At least n=3 samples consisting of 5 larvae, were washed and homogenized, isoniazid was extracted, followed by a derivatization step and LCMS quantification.
- Blood samples (5dpf, 10x MIC) were taken from the posterior cardinal vein with volumes up to 1.76 nL, of which 19-32 were pooled for quantification.
- *M. marinum* (strain E11 and M-USA) natural growth was obtained *in vitro* over 221 days, by plating colony forming units (CFU). The MTP model was used to fit these data.

Results

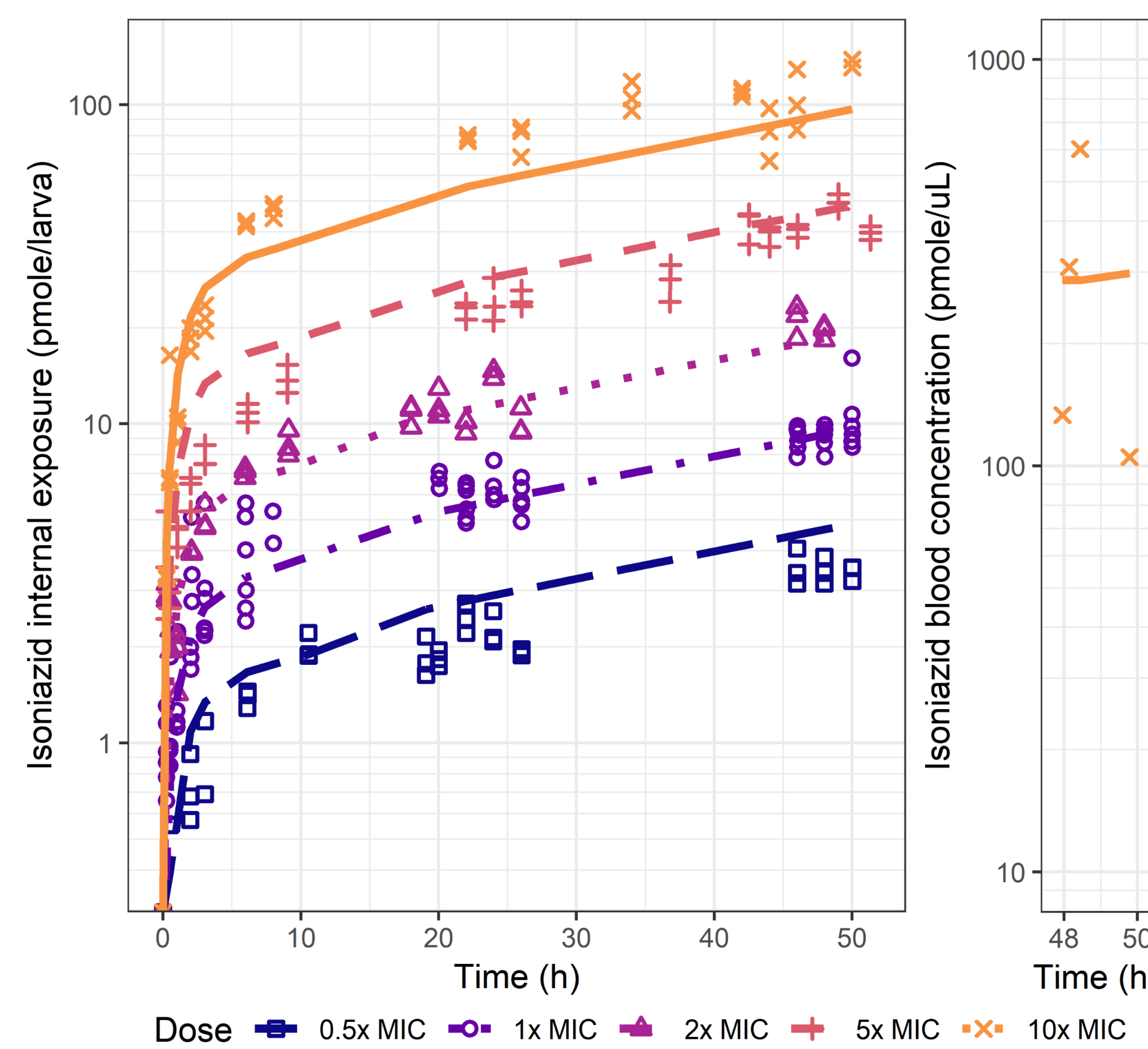


Table 1. Pharmacokinetic model parameters. k_a increases with f_{GI} between 3 and 4 dpf.

Parameter	Estimate	RSE
$k_a = k_{a,base} \cdot c_{age}^{\frac{age}{4.2dpf} \cdot (1 + f_{GI}^*)}$		
$k_{a,base}$ (h ⁻¹)	0.0036	25 %
c_{age} (-)	7.46	17 %
f_{GI} (-)	0.178	51 %
k_e (h ⁻¹)	0.579	32 %
V_D (nL)	325	35 %

< **Figure 1.** Isoniazid internal exposure (left) or blood concentration (right) for 3-5 dpf larvae after constant waterborne treatment at 0.5-10x MIC (symbols; type and colour per dose), including model fit (lines; type and colour per dose).

- Internal isoniazid exposure was best described by a one compartment model with first order age-dependent rate of absorption which increased 17.8 % between 3 and 4 dpf because of opening of the gastro-intestinal (GI) tract⁵, and first order elimination (figure 1, table 1).
- The MTP fit of the two *M. marinum* strains (figure 2) shows different natural growth behaviour, with M-USA in the fast-multiplying state for the majority of the 221 day period. E11 in contrast shifts to slow- and non-multiplying states, more similar to the reported *M. tuberculosis* profile⁴.

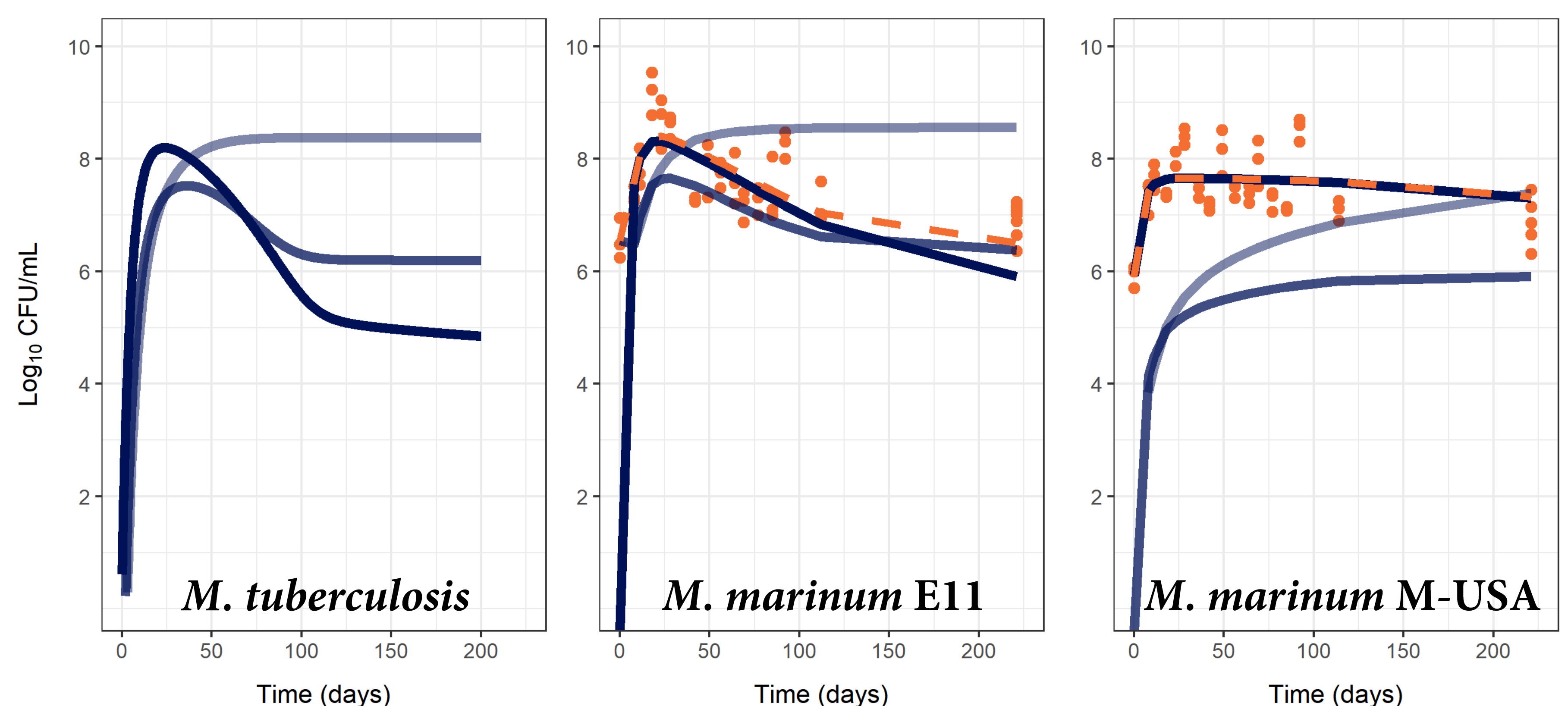


Figure 2. MTP fit of the natural growth of *M. tuberculosis* (published⁴, left) in comparison to that of *M. marinum* strains E11 (middle) and M-USA (right), showing mycobacteria in fast-, slow- or non-multiplying states (resp. dark, medium, light blue). Sum of fast- and slow-multiplying states (dashed orange line) constitutes observations in CFU (orange symbols).

Conclusion and perspectives

For the first time, the pharmacokinetics of isoniazid in zebrafish larvae has been quantified, using homogenates and nanoscale blood samples. The disease dynamics of *M. marinum* were quantified using the MTP model, from which E11 is concluded to mimic TB infections most.

The here quantified pharmacokinetics and disease dynamics will be used to establish the pharmacokinetic-pharmacodynamic relationship essential for translation of pharmacological findings from zebrafish to higher vertebrates.

References

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