A parent-metabolite pharmacokinetic model of paracetamol in zebrafish

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Introduction

The zebrafish larva is a promising vertebrate model organism in drug discovery and development. The pharmacokinetics (PK) of the drugs or their metabolites are however often disregarded[1]. Additionally, quantification of elimination rates for different pathways increases our understanding of the PK translation potential of the zebrafish larvae towards higher vertebrates[2].

We developed a non-linear mixed effects (NLME) model to quantify glucuronidation, sulphation and unchanged excretion of paracetamol (acetaminophen, APAP) in zebrafish larvae, extending our concept for paracetamol PK in zebrafish larvae[3,4].

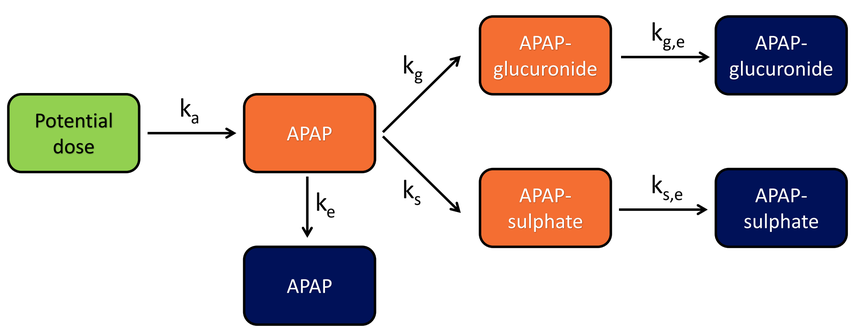
Methods

Zebrafish larvae of 5 days post fertilization (dpf) were exposed to 1 mM paracetamol either for 10-180 minutes (constant exposure) or for 60 minutes followed by a wash-out until 300 minutes. Paracetamol, paracetamol-sulphate, and paracetamol-glucuronide were quantified by UPLC-QTRAP (ABSciex) in 3 replicates of 5 lysed larvae or in their incubation medium.

A NLME model was developed in NONMEM 7.3, simultaneously modelling internal and excreted amounts of parent and metabolites. Destructive sampling imposed fixing the total volume of distribution to total larval volume of 300 nL, which was determined by 3D volume modelling[5], and prevented estimation of inter-individual variability. Paracetamol absorption was estimated as zero order process. Metabolism was tested using first order and Michaelis-Menten kinetics. Excretion of parent and metabolite was estimated as first order process.

Results

A one compartment model described the time course of internal paracetamol and metabolite amounts best (figure 1). Parameter estimates are given in table 1. Model predictions fit the data reasonably (figure 2), using a recovery fraction between constant exposure (solid line) and wash-out (dashed line) experiments.

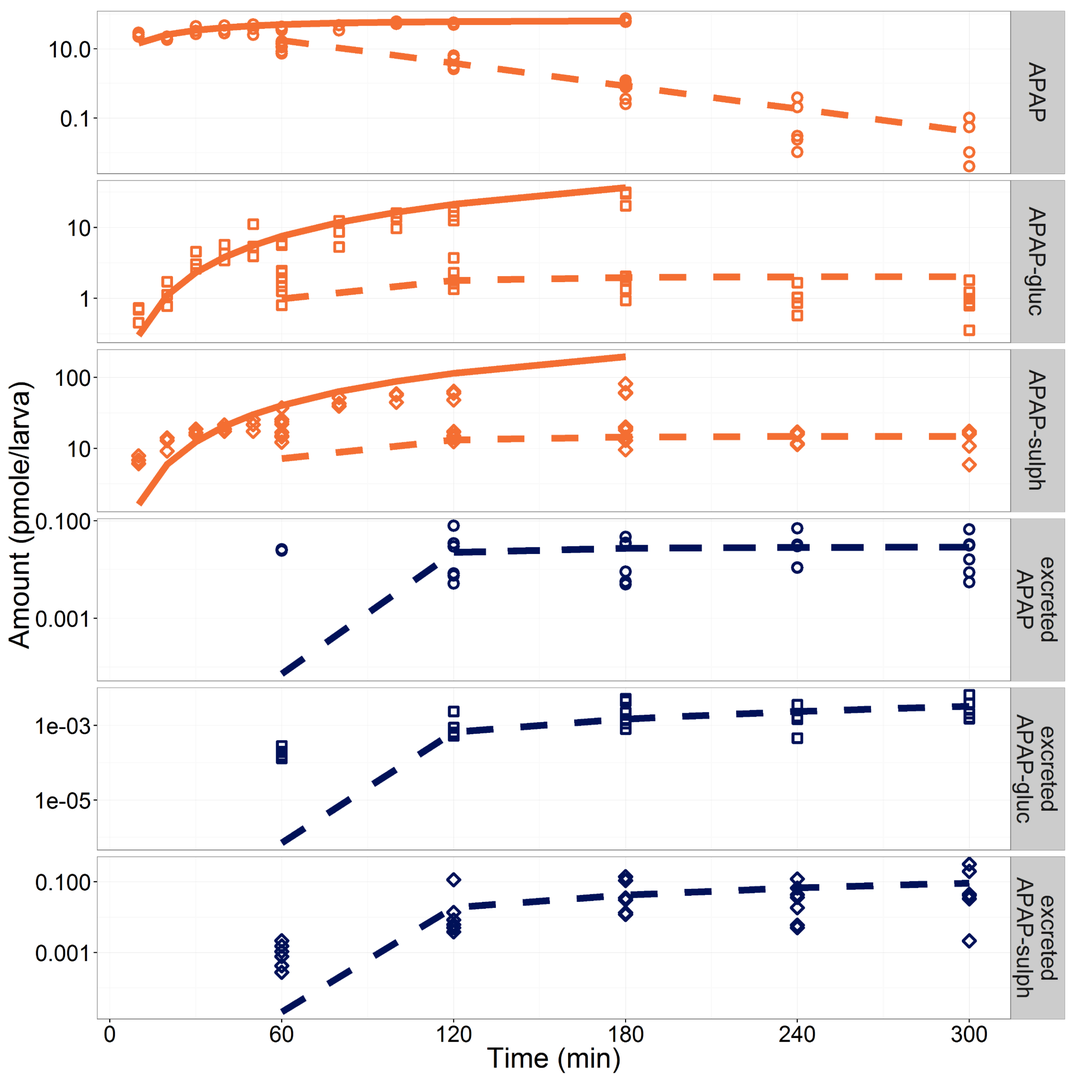


^ Figure 1. Structural model of paracetamol parent and metabolites, with amount in zebrafish in orange and amount excreted to the medium in blue.

> Table 1. Model parameter estimates

Parameter	Estimate	RSE (%)
k_a (pmol/min)	1.64	8
k_e (min^{-1})	1.44 x 10^{-5}	17
k_g (min^{-1})	0.004	11
k_s (min^{-1})	0.0213	6
$k_{g,e}$ (min^{-1})	9.55 x 10^{-7}	18
$k_{s,e}$ (min^{-1})	5.13 x 10^{-6}	31
Experimental recovery fractions		
APAP	0.349	14
APAP-glucuronide	0.131	15
APAP-sulphate	0.178	19
Proportional error zebrafish data		
APAP	0.236	21
APAP-glucuronide	0.314	19
APAP-sulphate	0.984	36
Additive error medium data		
APAP	0.00048	27
APAP-glucuronide	0.00189	34
APAP-sulphate	0.0039	43

v Figure 2. Model predicted amounts (solid line for constant exposure, dashed line for wash-out) and the observations (points).



Conclusion and perspectives

Immature metabolic capacity was observed with more prominent sulphation than glucuronidation, a distinguishing trait of human neonates[6].

This metabolite model may be used to interpret pharmaco- or toxicodynamic effects caused by active metabolites, like that of paracetamol. It also improves mechanistic understanding of drug elimination.

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