Nanoscale blood sampling from zebrafish larvae for the estimation of distribution volume and absolute clearance

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Introduction

Recently, the first pharmacokinetic (PK) model in zebrafish larvae was developed using paradigm compound paracetamol (acetaminophen)[1,2]. This model was based on total paracetamol amounts in lysed larvae, with relative clearance and no estimate of distribution volume. For extrapolation to higher vertebrates, these parameters are however essential.

Aim:

- **Develop an experimental method to sample blood from zebrafish larvae of 5 days old**
- **Estimate PK parameters including distribution volume and absolute clearance.**

Methods

- Blood was sampled from different anatomical locations of the larval circulation[3] using a pulled needle, by 20x microscopic magnification.
- Sample volume within the needle was determined based on sample image (fig 1).
- Zebrafish larvae of 5 days old were exposed to 1 mM paracetamol for 10-170 min, washed, and sampled
- Paracetamol blood concentrations, quantified by UPLC-MS/MS, were combined with data of paracetamol amounts from lysed larvae, and fitted simultaneously (NONMEM 7.3).
- Absorption from medium was parameterized as zero order process, elimination as linear or non-linear, and both one and two compartment models were tested for distribution.
- Because of destructive sampling, only residual variability could be estimated.

Results

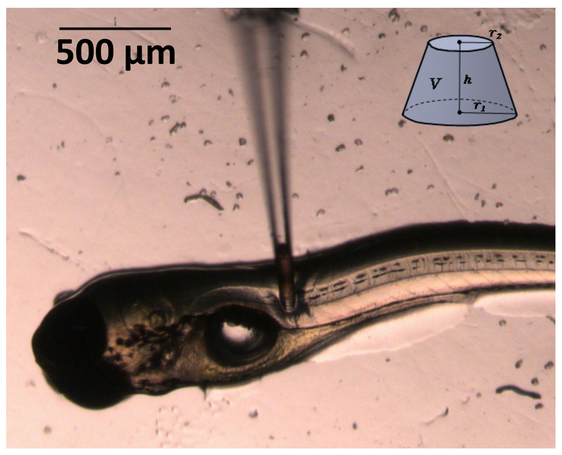
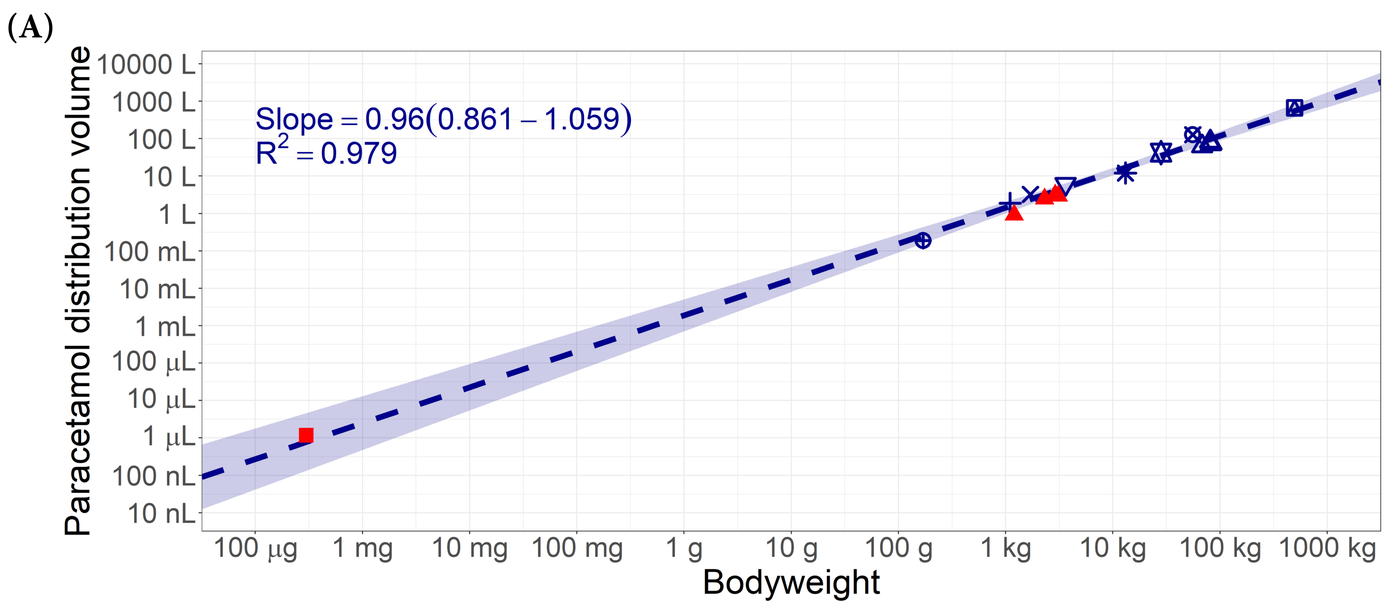


Figure 1. Blood sampling from 5 days old larva. Insert shows calculation of blood volume based on truncated cone formula.

- Blood sampling from posterior cardinal vein was most efficient with highest yields (fig. 1).
- By pooling 15-35 blood samples, detectable blood concentrations could be obtained.
- Paracetamol volume of distribution of 1170 nL and clearance of 1.8 µL/h were both well within the 95% confidence interval of the allometric relationship of those parameters against bodyweight[4] (fig 2-3).



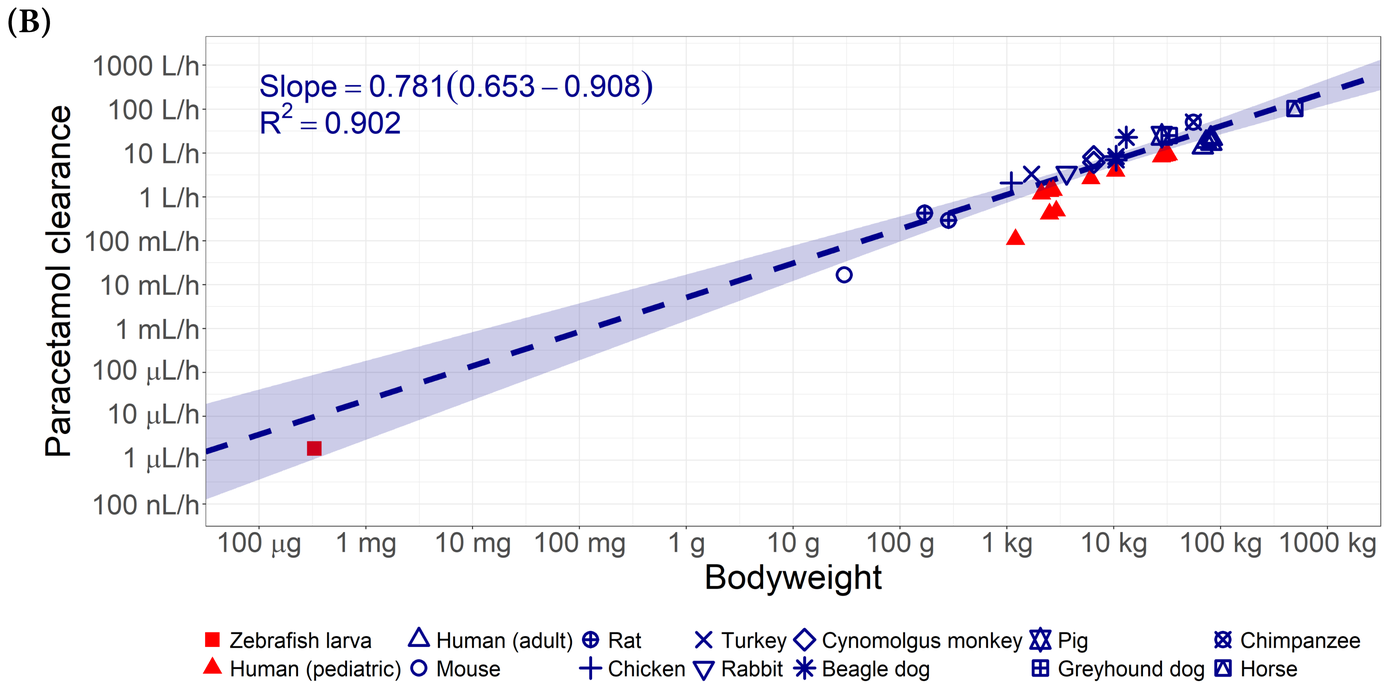


Figure 2. Paracetamol distribution volume (A) and clearance (B) over bodyweight for zebrafish larvae and higher vertebrates. Allometric relationship based on adult vertebrates only (dashed line, slope) with 95% confidence interval (shaded area, brackets).

Conclusion and perspectives

- For the first time, blood samples were taken from zebrafish larvae of only millimetres in size.
- This enabled quantification of blood concentration in this new vertebrate model organism, which is critical for estimating distribution and absolute clearance.

It shows the necessity for quantitative pharmacology to integrate both experimental and computational innovation[5].

Acknowledgements, affiliations and contact details

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