DISEASE MODELLING OF REPEATED ACUTE ATTACKS IN AN ACUTE INTERMITTENT PORPHYRIA MOUSE MODEL

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INTRODUCTION AND OBJECTIVES

Acute intermittent porphyria (AIP) is an autosomal dominant hepatic rare disease. It is caused by a genetic mutation in porphobilinogen (PBG) deaminase (PBGD), the third enzyme in the heme biosynthesis pathway. In the presence of precipitating factors that increase hepatic heme demand, patients experience acute attacks associated with the accumulation of neurotoxic heme precursors δ-aminolevulinic acid (ALA) and PBG [1] (Figure 1). Murine models have been developed to study AIP symptoms and heme precursor levels [2]. The aim of this work was to develop a **disease model** to characterize urinary excreted levels of heme precursors (ALA, PBG and porphyrins) during acute attacks in porphyric mice. This computational model may be used as a tool to study the **effect of new therapies** for the treatment of AIP, as well as to guide and design **dose selection** for future studies.

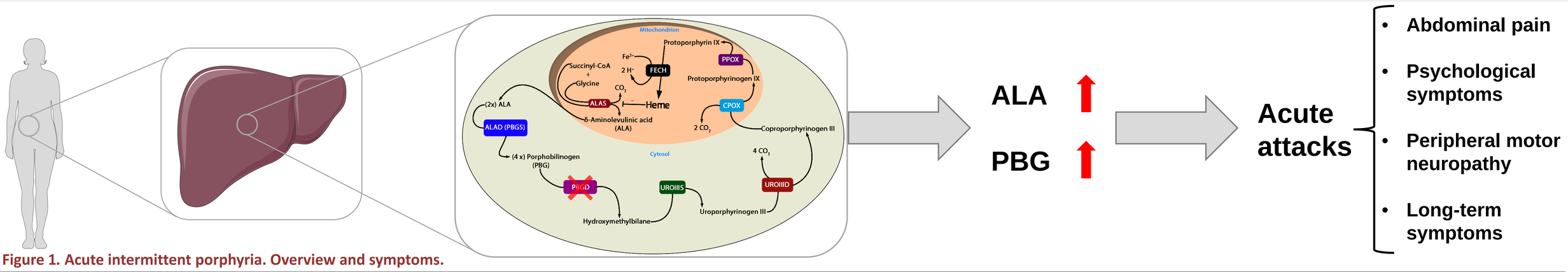


Figure 1. Acute intermittent porphyria. Overview and symptoms.

METHODS

Data was obtained from two experiments where acute attacks were induced by the administration of 4 to 5 escalating **phenobarbital** (PB) doses in multiple challenges to male **AIP mice** (33% PBGD activity) (Figure 2). Mice were housed in metabolic cages in order to collect 24-hour urine in study 1 (**training study**, n=12) and in study 2 (**validation study**, n=11) to quantify heme precursors ALA, PBG and uroporphyrinogen III (URO, representing porphyrins) (Figure 3). Observations were logarithmically transformed. As PB concentrations were not measured, a pharmacokinetic model was adapted from the literature [3]. Data was analyzed by nonlinear mixed effects modelling using NONMEM 7.3. Model selection was carried out by the difference in the minimum value of the objective function (OFV), parameter values and their precision, and by the study of goodness-of-fit plots. Model evaluation was performed through prediction-corrected visual predictive checks using data from training and validation studies.

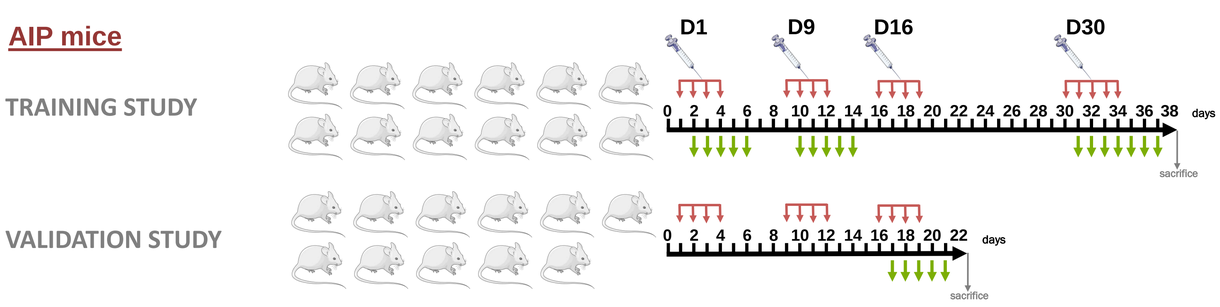


Figure 2. Experimental design for the trainings and validation studies. Two studies were performed: a training study (n=12) and a validation study (n=11). AIP mice were challenged with phenobarbital 4 times (Challenges D1, D9, D16 and D30). 4-5 increasing phenobarbital doses (75, 80, 85 and 90 mg/kg) were administered daily in each challenge. 24-hour urine samples were collected from challenges D1, D9 and D30 in study 1; and 24-hour urine samples from challenge D16 in study 2. Red arrows show PB doses, whereas the green ones are urine collection times.

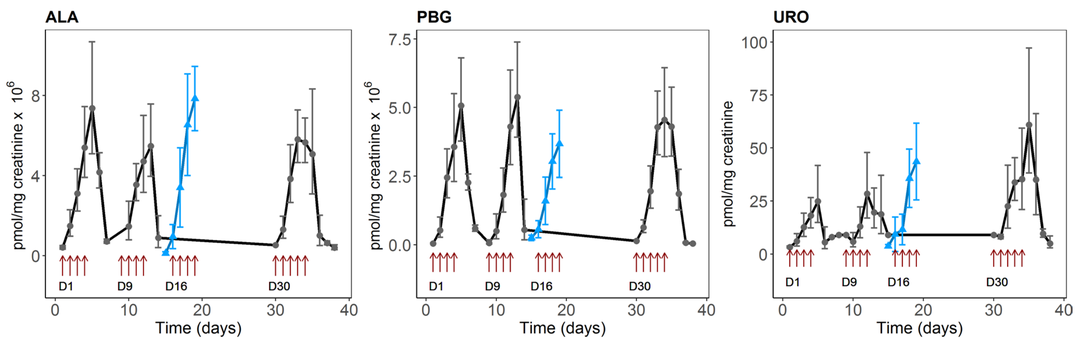
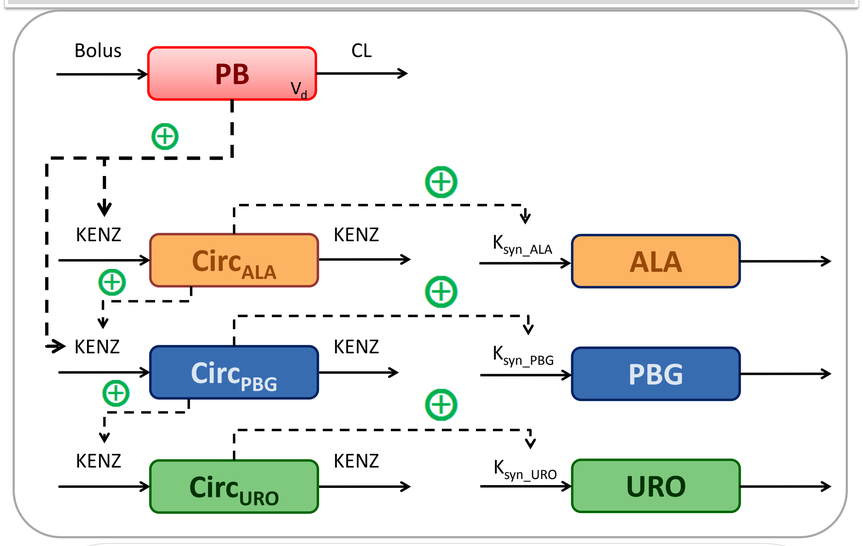


Figure 3. Raw data. 24-hour urinary amounts of ALA, PBG and URO for study 1 (black and grey) and study 2 (blue). Red arrows show PB administration divided into four challenges. Last challenge presented 5 doses, the final one being another dose of 90 mg/kg.

RESULTS

PB effects final model is shown in Figure 4. It was assumed (i) unmeasured circulating biomarker levels (Circ) controlled urinary precursor amounts, (ii) circulating amounts of ALA and PBG were the precursors of circulating amounts of PBG and porphyrins, respectively, and (iii) a phenobarbital effect linearly increasing the synthesis of circulating levels of ALA and PBG was found. PB accumulation in serum was not predicted, thus the increase in maximum concentrations in serum was caused only by increments in dose amounts (Figure 5). This model was capable of predicting the tendency of heme precursors for individual animals (Figure 6) as well as the typical dispersion in both training and validation studies (Figure 7). Model parameter estimates were precise and inter-animal variability was found relevant on urinary ALA and URO synthesis rate constants (Table 1).

I. Model building



$$\frac{dPB}{dt} = -\frac{CL}{V_d}\ x\ PB$$

$$\frac{dCirc_{ALA}}{dt} = KENZ\ x\ (1 + \theta_{Pheno}\ x\ C_{Pheno}) - KENZ\ x\ Circ_{ALA}$$

$$\frac{dCirc_{PBG}}{dt} = KENZ\ x\ (Circ_{ALA} + \theta_{Pheno}\ x\ C_{Pheno}) - KENZ\ x\ Circ_{PBG}$$

$$\frac{dCirc_{URO}}{dt} = KENZ\ x\ Circ_{PBG} - KENZ\ x\ Circ_{URO}$$

$$\frac{dALA}{dt} = K_{syn\_ALA}\ x\ (1 + Circ_{ALA}^{\gamma_{ALA}})$$

$$\frac{dPBG}{dt} = K_{syn\_PBG}\ x\ (1 + Circ_{PBG}^{\gamma_{PBG}})$$

$$\frac{dURO}{dt} = K_{syn\_URO}\ x\ (1 + Circ_{URO}^{\gamma_{URO}})$$

Figure 4. Phenobarbital effects model: Graphical and mathematical representations. PB, Phenobarbital compartment. V_d, PB apparent volume of distribution. CL, PB total clearance. Θ_{PB}, PB lineal effect. $Circ_{ALA, PBG, URO}$, unobserved circulating levels of ALA, PBG, and porphyrins (URO), respectively. KENZ, rate constant governing the turn-over process of the circulating biomarkers. K_{syn_ALA}, K_{syn_PBG} and K_{syn_URO} are the urinary ALA, PBG and URO synthesis rate constants, respectively. ALA, urinary ALA compartment. PBG, urinary PBG compartment. URO, urinary URO compartment. . γ_{ALA}, γ_{PBG} and γ_{URO} are the shape parameters of the modulator compartments.

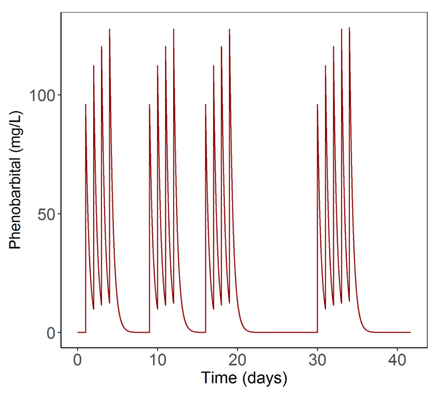


Figure 5. Phenobarbital predicted concentrations. Estimates for drug apparent volume of distribution (V_d) and total drug clearance (CL) were 0.78 L x kg^{-1} and 0.074 L x h^{-1} x kg^{-1}, respectively.

Table 1. Model typical parameter estimates

Parameters	Typical estimate	RSE (%)	IAV (%)	RSE (%)	Shrinkage (%)
Θ_{PB} (L/mg)	0.0178	31.9	-	-	-
KMOD (h^{-1})	0.0747	4.8	-	-	-
K_{syn_ALA} (pmol x mg creatinine^{-1} x h^{-1})	8720	5.1	15	25	17.5
K_{syn_PBG} (pmol x mg creatinine^{-1} x h^{-1})	1090	13.9	-	-	-
K_{syn_URO} (pmol x mg creatinine^{-1} x h^{-1})	0.0766	7.7	21.7	20	18.7
γ_{ALA}	5.14	23.3	-	-	-
γ_{PBG}	5.27	20.3	-	-	-
γ_{URO}	2.61	20.2	-	-	-
Residual error ALA (additive for log data)	0.407	8.6	-	-	-
Residual error PBG (additive for log data)	0.558	6.5	-	-	-
Residual error URO (additive for log data)	0.551	8.2	-	-	-

RSE, relative standard error. IAV, inter-animal variability.

II. Model selection

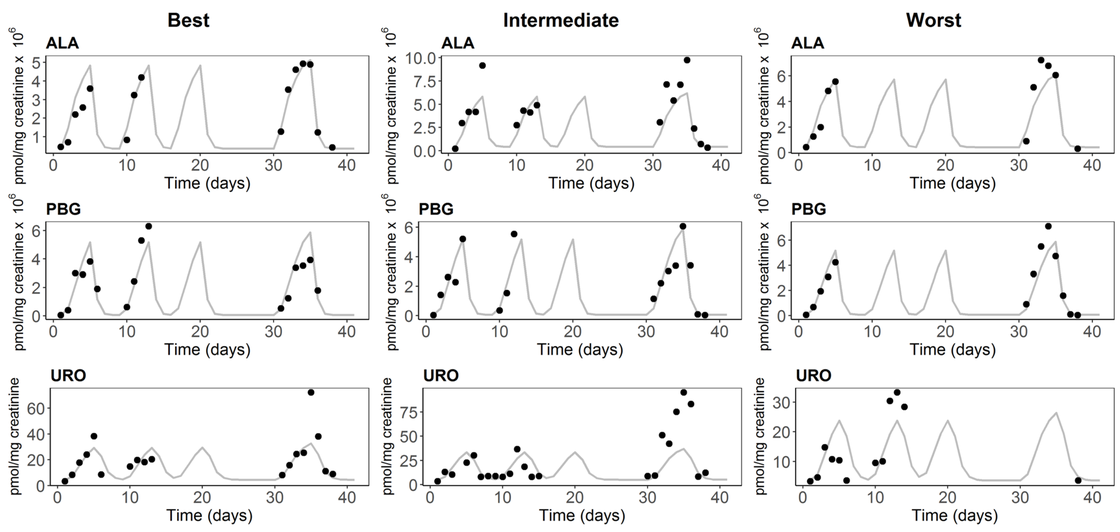


Figure 6. Individual predictions vs time. Heme precursor predictions for the best, intermediate and worst mice model fittings. Lines show individual model predictions, points are observed measurements.

III. Model evaluation

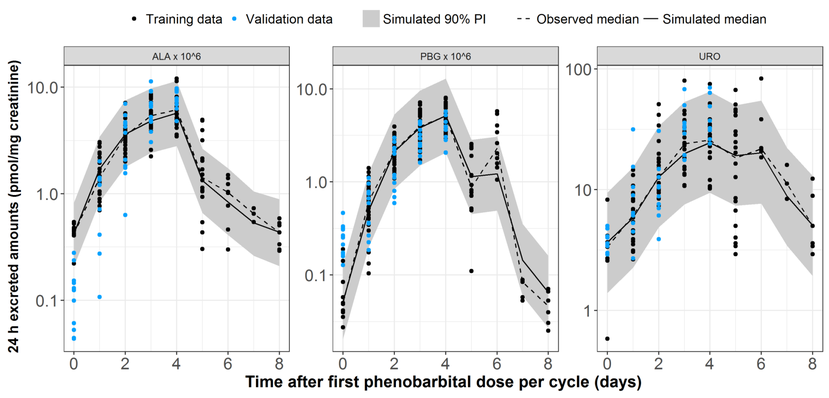


Figure 7. Prediction-corrected Visual Predictive Checks for each heme precursor. Dashed line, median for observed data. Solid line, median for predicted data. Shaded area, 90% prediction interval. Black points: data from training study. Blue points: data from validation study.

CONCLUSIONS & FUTURE PERSPECTIVES

A semi-mechanistic disease model successfully characterizing the temporal course of heme precursors in urine after several phenobarbital challenges has been developed. To the extent of our knowledge, this is the first time that a computational approach is performed to describe heme precursor accumulation during an induced acute attack in AIP mice.

Furthermore, it represents a framework which can be used to explore the impact of new therapies or approaches for this disease and predict their effects on urinary heme precursor levels to support drug development and **therapy optimization**.

References

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[2] Lindberg RLP et al. Nat Genet - 12. 1996;(2):195.
[3] Iven H, Feldbusch E. Naunyn Schmiedebergs Arch Pharmacol. 1983;324(2):153–9.