

Modelling of protozoa dynamics and drug effects in a murine model of malaria infection

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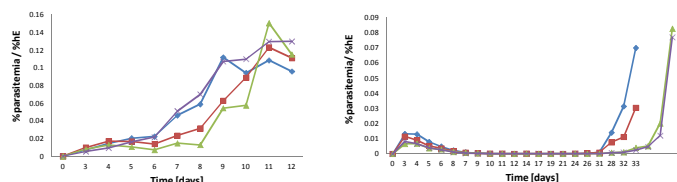


MOTIVATION In humans, severe malaria is responsible of over a million of deaths every year. The development of new antimalarial compounds and vaccines is a critical for malaria eradication. Of particular interest is the development of compounds that are effective against *Plasmodium falciparum*. A new murine model has been developed to assess the therapeutic efficacy of antimalarial drugs using human erythrocytes, which better reflects disease processes (i.e., protozoa clearance) in humans [1]. The objective of this investigation was to develop a PKPD model describing protozoa dynamics and evaluate treatment response to chronic administration of atovaquone (ATV) as compared to a derivative compound (B), for which no efficacy data is available yet.

MATERIALS AND METHODS

Experimental protocol

A cohort of 77 NODscidIL2r^{-/-} mice was injected with human erythrocytes (i.p., qd). As soon as peripheral blood levels reached ~50 % of human erythrocytes (hE), the mice were infected with *Plasmodium falciparum* by i.v. injection of an inoculum of 20x10⁶ Pf3D7^{0087/N9} *P. falciparum*-infected erythrocytes (ihE) /mouse[1]. The mice were randomly assigned an experimental group (control vs. active treatment). The treatment started at day 3 after infection. Blood samples were collected to assess the pharmacokinetics of atovaquone and derivative B. Protozoa load and fraction of infected human erythrocytes were used as pharmacodynamic endpoints.



Individual response in control and treated animals. ATV was dosed at 100mg/kg for 2 days every 24h.

The experimental protocol consisted of 4 – 10 animals per dose level. Drugs were administered orally every 24 h or 48 h for 2 to 10 days with dosage range between 0.1, 1, 8, 20, 50, 100 and 200 mg/kg for ATQ and 0.5, 1, 5, 10, 30 and 50 mg/kg for the derivative compound.

PK and PK/PD analysis

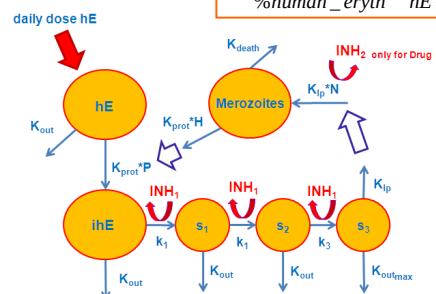
The PK and PKPD analysis were performed in NONMEM v7.1. PK modelling was performed using the ADVAN 2 TRANS 2 subroutine. Clearance, volume and absorption rate constants were defined in conjunction with inter-individual variability and a proportional error model. Model fitting was based on the FO method.

The PKPD model was implemented using ADVAN 13. Model fitting was based on the Stochastic Approximation Expectation Maximization (SAEM) method, combined with the MC Importance Sampling (IMP) method. PD parameters were assumed to show log-normal distribution and residual error was described by a combined error model.

Protozoa dynamics model

The proposed model reflects the dynamics of hE, ihE and merozoites. Drug effect was parameterised in terms of an I_{max} model to capture the inhibition of protozoa replication (INH₁) and of the number of merozoites produced after haemolysis (INH₂).

$$Y = \frac{\% \text{parasitemia}}{\% \text{human_eryth}} = \frac{ihE}{hE + ihE + s_1 + s_2 + s_3}$$



The reproduction time for parasites inside ihE is taken into account through the inclusion of three transit compartments (s₁, s₂ and s₃).

K_{prot} is the infection rate defining the transition from hE to ihE

K₁ is the maturation rate in the transit compartment chain, describing the protozoa replication inside ihE. The latest and faster phase has rate constant K₃ = K₁ / 3.

K_{ip} is the parameter describing the elimination rate due to haemolysis

N is the no. of merozoites released into blood after haemolysis, ready to infect new hE

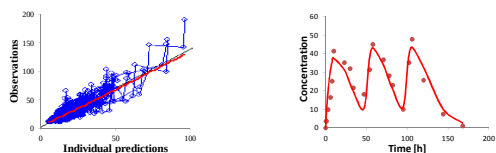
K_{death} represents the clearance of merozoites

K_{out} is the turnover rate of both hE and ihE, described by the following sigmoid function:

$$K_{out} = K_{out_{base}} + \frac{K_{out_{max}} \cdot ihE}{A_m + ihE}$$

RESULTS

PK analysis

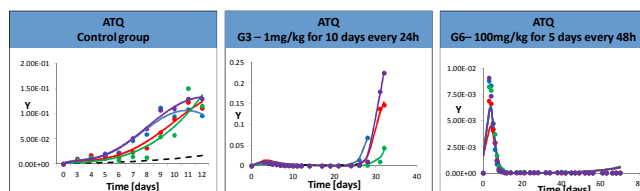


Results obtained from the ATQ PK analysis: individual predictions versus observation and an individual concentration profile fitting. ATV was dosed at 100mg/kg for 3 days every 48h.

Protozoa dynamic model

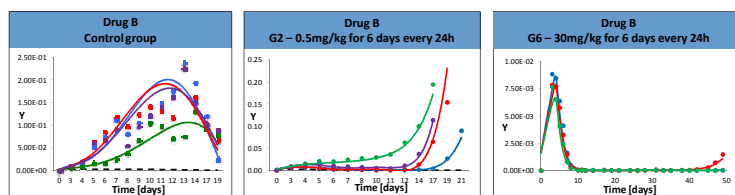
Atovaquone

Parameter	K _{prot}	K _{ip}	K _{death}	N	K ₁	K _{mat}	K _{out}	A _m	IC50 ₁
Parameter Estimates	0.0169 (20%)	23.7 (25%)	2.2 (56%)	24 fix	6.5 (10%)	0.12 fix	0.295 (75%)	0.23 (32%)	0.01 (54%)
ETAs	0.1 fix	0.1 fix	0.309 (35%)	9.05E-3 (16%)	8.87 (47%)	1E-5 (35%)	2.85 (>100%)	31E+1 (>100%)	8E+1 (>100%)
SIGMAs	0.01 (35%)	1E-5 (20%)							



Drug B

Parameter	K _{prot}	K _{ip}	K _{death}	N	K ₁	K _{mat}	K _{out}	A _m	IC50 ₁	IC50 ₂
Parameter Estimates	0.0028 (20%)	36.9 (15%)	0.714 (5.5%)	24 fix	5.8 (7%)	0.12 fix	0.229 (58%)	14.2 (7%)	1.51 (38%)	2.71E-5 (78.5%)
ETAs	0.1 fix	0.1 fix	0.172 (26%)	0.824 (51%)	0.15 (67%)	0.54 (11%)	0.0426 (>100%)	0.0047 (>100%)	0.231 (>100%)	32.4 (17%)
SIGMAs	0.145 (8%)	4.3 E-10 (27%)								



CONCLUSIONS

Our exercise suggests that comparable advantages exist for the evaluation of treatment effect and recrudescence in parasitic infections. In particular, two pharmacological actions point have been considered into the model.

The availability of more data, not only plasma data can improve the performance of the model, characterized by parameter identification problems.

REFERENCES [1] M.B Jiménez-Díaz, T. Mulet, V. Gómez, S. Viera, A. Alvarez, H. Garuti, Y. Vázquez, A. Fernández, J. Ibáñez, M. Jiménez, D. Gargallo-Viola, I. Angulo-Barturen. Quantitative Measurement of Plasmodium-Infected Erythrocytes in Murine Models of Malaria by Flow Cytometry Using Bidimensional Assessment of SYTO-16 Fluorescence, Cytometry Part A, p. 225-235, DOI: 10.1002/cyto.a.20647.