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A PKPD MODEL OF RIBAVIRIN IN LASSA VIRUS INFECTED MICE

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Introduction

Ribavirin is a broad-spectrum antiviral, which is used in the therapy of the Lassa fever. Ribavirin showed inhibitory effects against the replication of the Lassa virus *in vitro*, but *in vivo* this effect was minor. Nevertheless, ribavirin improved the outcome in animals, especially in combination with the strong antiviral favipiravir [1]. Carrillo-Bustamante et al. investigated different mechanisms of action of ribavirin against the Lassa virus and identified no direct antiviral effect, but a relation between dose and aspartate aminotransferase (AST) release from damaged cells suggesting some cell-protective effects of ribavirin [2]. Yet, no PK was considered in [2] and solely an ED50 was estimated. The aim of this project was to link a literature-based PK model to the PD data from [1] in order to develop a PKPD model for ribavirin in mice and to determine an *in vivo* EC50 of ribavirin effects in Lassa infected mice.

Methods

We combined the PD model with the most likely mechanism of action (model C) of Carrillo-Bustamante et al. [2] with the PK model for ribavirin in mice of Endres et al. [3]. Model C describes the cell protective effect of ribavirin by reducing the death rate of the infected cells (Figure 1). The estimates of the PK model were fixed to the pharmacokinetic parameters for Ent1 (+/+) (wildtype for equilibrative nucleoside transporter 1) mice referring to the values described in the literature [3], as no ribavirin plasma concentrations in mice were available to us from the infected mice [1].

The values of the initial target cell inoculum, the initial virus inoculum, the clearance of the free virus and of the AST molecules were fixed to the literature values as in the original PD model [2]. The other PD parameters (basic reproductive number R_0, death rate of infected cells δ_I, viral production rate p, factor α describing AST release from dying infected cells, constant source for AST s_x) were re-evaluated in the PKPD model. Model selection was guided by graphical criteria, i.e. goodness of fit plots and visual predictive checks and the difference in the objective function value.

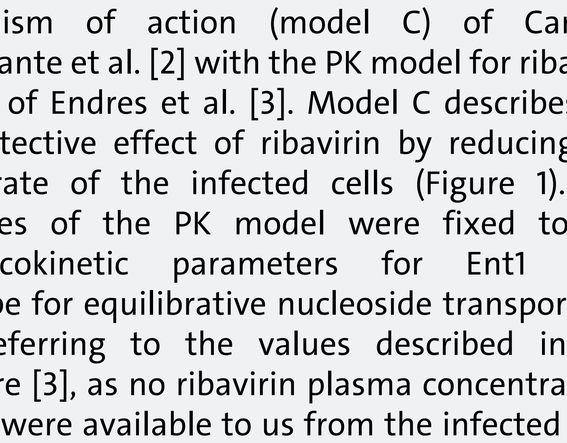

Figure 1: Model sketch of Model C. Abbreviations are explained in Table 1.

The re-evaluated PD part of the model was combined with a human PK model, which was developed with the results of the PAIRR study [4]. Viral and AST kinetics were simulated with the Irrua standard dosing regimen or placebo with 1000 simulated patients each. Therefore, sex-specific body weight was sampled from the mean and variance of the included patient population assuming a log-normal distribution [4].

Results

First, the EC50 was estimated with all other estimates fixed to the literature values. Goodness of fit plots and VPC showed a good model fit for the viral load, but not for the AST concentrations. In the next step all AST related parameters were estimated, while parameters related to viral load remained fixed, which improved the model (dOFV: -38.162). Figure 1 and 2 show the individual model fit plots for the final model. Final model parameters are shown in Table 1.

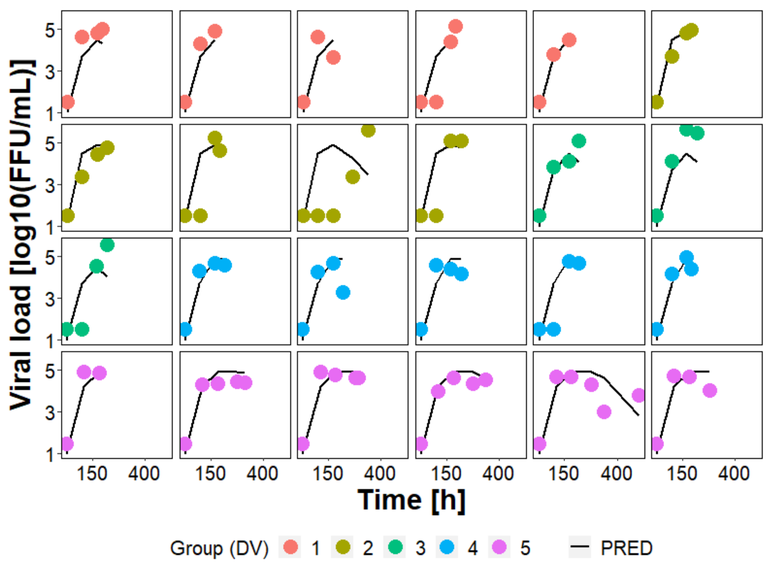

Figure 2: Measured (DV) and predicted (PRED) viral load vs. time in mice. Group 1: placebo, day 0-7 post infection (p.i.); 2: 80 mg/kg ribavirin per day, day 0-7 p.i.; 3: placebo, day 4 p.i. to death; 4: 80 mg/kg ribavirin per day, day 4 post p.i. to death; 5: 2 x 80 mg/kg ribavirin per day, day 4-11 p.i.

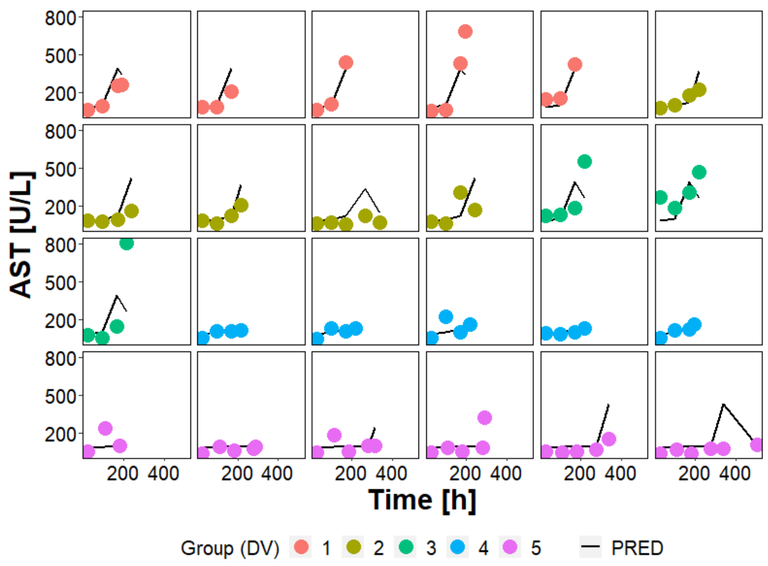

Figure 3: Measured (DV) and predicted (PRED) AST concentration vs. time in mice. Group 1: placebo, day 0-7 post infection (p.i.); 2: 80 mg/kg ribavirin per day, day 0-7 p.i.; 3: placebo, day 4 p.i. to death; 4: 80 mg/kg ribavirin per day, day 4 post p.i. to death; 5: 2 x 80 mg/kg ribavirin per day, day 4-11 p.i.

Table 1: Final parameter estimates for ribavirin mice PKPD model

Parameter	Estimate (RSE)
V1 [mL]*	19.5
k12 [h^{-1}]*	2.34
k21 [h^{-1}]*	0.72
k10 [h^{-1}]*	0.9
initial viral inoculum (VIR_0) [FFU/mL]*	10
available target cells (T_0) [cells/mL]*	10^6
clearance rate free virions (c) [h^{-1}]*	0.833
clearance rate AST (c_A) [h^{-1}]*	0.0417
basic reproduction number (R_0)*	4.96
death rate of infected cells (δ_I) [h^{-1}]*	0.025
production rate new infectious virions (p) [FFU/mL]*	0.0758
AST release from dying infected cells (α)	0.00141 (16%)
constant source for AST (S_x)	79.8 (9%)
EC50 [ng/mL]	40.9 (61%)

* Fixed to literature values

Using the linked human PK-PD model, no antiviral effects were predicted, on the contrary viral load is slightly increasing. AST was predicted to increase only moderately indicating that the effect of ribavirin on AST might be achievable in the clinical setting.

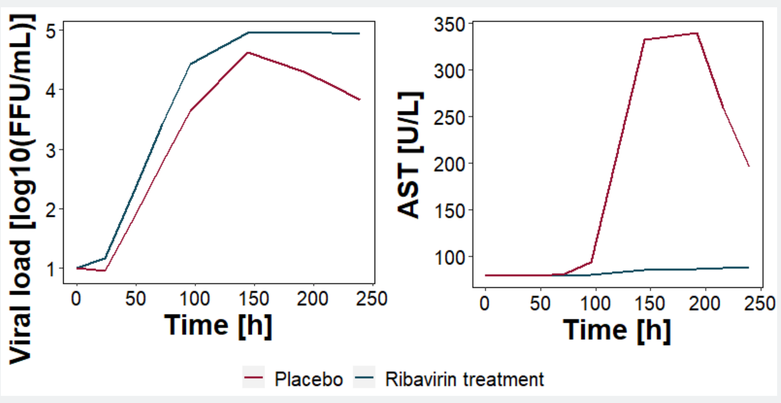

Figure 3: Left: Simulated viral load in human. Right: Simulated AST concentration in human.

Conclusion

Using the developed PKPD model, we could successfully estimate an *in vivo* EC50 for the cell protective effects of ribavirin against Lassa fever.

Simulation with the human PKPD model showed the cell protective effect of the Irrua regimen, resulting in lower AST concentrations compared to placebo. On the other hand viral load is predicted to be higher under ribavirin therapy, due to the increased amount of infected cells protected from dying. If this reflects the true clinical outcome, has to be evaluated in the next step by fitting the model to clinical data.

Literature

[1] L. Oestereich et al., "Efficacy of Favipiravir Alone and in Combination With Ribavirin in a Lethal, Immunocompetent Mouse Model of Lassa Fever," J. Infect. Dis., vol. 213, no. 6, pp. 934–938, 2016, doi: 10.1093/infdis/jiv522.

[2] P. Carrillo-Bustamante, T. H. T. Nguyen, L. Oestereich, S. Günther, J. Guedj, and F. Graw, "Determining Ribavirin's mechanism of action against Lassa virus infection," Sci. Rep., vol. 7, no. 1, p. 11693, Dec. 2017, doi: 10.1038/s41598-017-10198-0.

[3] C. J. Endres, A. M. Moss, R. Govindarajan, D. S. Choi, and J. D. Unadkat, "The role of nucleoside transporters in the erythrocyte disposition and oral absorption of ribavirin in the wild-type and equilibrative nucleoside transporter 1(-/-) mice," J. Pharmacol. Exp. Ther., vol. 331, no. 1, pp. 287–296, 2009, doi: 10.1124/jpet.109.153130.

[4] C. Erameh et al., "Prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in patients with Lassa fever in Nigeria," BMJ Open, vol. 10, no. 4, pp. 1–11, 2020, doi: 10.1136/bmjopen-2020-036936.

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