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## Objective

To develop a PK/PD model of adverse, drug-mediated increase in heart rate in the anaesthetized mouse in order to determine a dose with acceptable heart rate alterations (defined as < 10% increase relative to baseline).

## Methods

- Heart rate, arterial blood pressure, and body temperature were continuously measured in anaesthetized, male Swiss mice for up to 95 min.
- Drug or vehicle was injected i.v. and plasma samples for exposure measurements were obtained at 1-3 time points.
- PK and heart rate data were fitted to an appropriate model using non-linear modelling with the naïve pooled approach implemented in Phoenix WinNonlin 6.3.
- The following models were used to describe the time-dependent change in heart rate effects (E) as a function of drug concentration (C):

Turnover model with stimulation of  $k_{in}$ : 
$$\frac{dE}{dt} = k_{in} \cdot \frac{E_{max} \cdot C}{EC_{50} + C} - k_{out} \cdot E$$

Receptor binding model [1]: 
$$\frac{dE}{dt} = k_{off}/K_D \cdot [C] \cdot (E_{max} - E) - k_{off} \cdot E$$

with  $k_{in}$  and  $k_{out}$  the 0 order and 1<sup>st</sup> order rate constants for formation and degradation of the effect,  $E_{max}$  = maximum effect,  $EC_{50}$  = concentration for half-maximal effect,  $K_D = k_{off}/k_{on}$  the equilibrium dissociation constant of the drug-receptor complex (equated with E here) and  $k_{on}$  and  $k_{off}$  the 2<sup>nd</sup> and 1<sup>st</sup> order rate constants for association and dissociation of the drug-receptor complex.

## Results

- For assessment of the concentration dependence of cardiovascular effects, sequential, escalating i.v. bolus doses of drugs A-C were used to achieve a step-wise increase in plasma concentrations (Figure 1A). Simulations of a PK model previously fitted to PK profiles (data not shown, see Table 1 for fitted parameter estimates) were within the assay error of exposure data.
- Drugs A, B and C caused a dose-dependent increase in heart rate with a time delay between plasma concentration and effect (Figure 1B), suggesting a slow emergence of heart rate effects.
- Heart rate data could be described by a turnover model (Figure 2 and Table 1).
- The turnover model predicted that the maximum heart rate effect occurs only 15-30 min after administration of drug C (blue lines in Figure 3).
- A follow-up study with longer heart rate monitoring following single i.v. doses of drug C showed that effects emerge slower than predicted by the turnover model, with maximal effects being reached at  $\geq 60$  min for most animals (black lines in Figure 3).
- The time-dependence of heart rate was dose-dependent, with a slower heart rate increase at lower doses. This behaviour was best captured by a receptor binding model (red lines in Figure 3, Table 2).
- The receptor binding model predicted that doses  $\leq 40 \mu\text{g}/\text{kg}$  have an acceptable heart rate increase.

Figure 1: Exposure (A) and heart rate effects (B) of drugs A, B and C in anaesthetized mice.

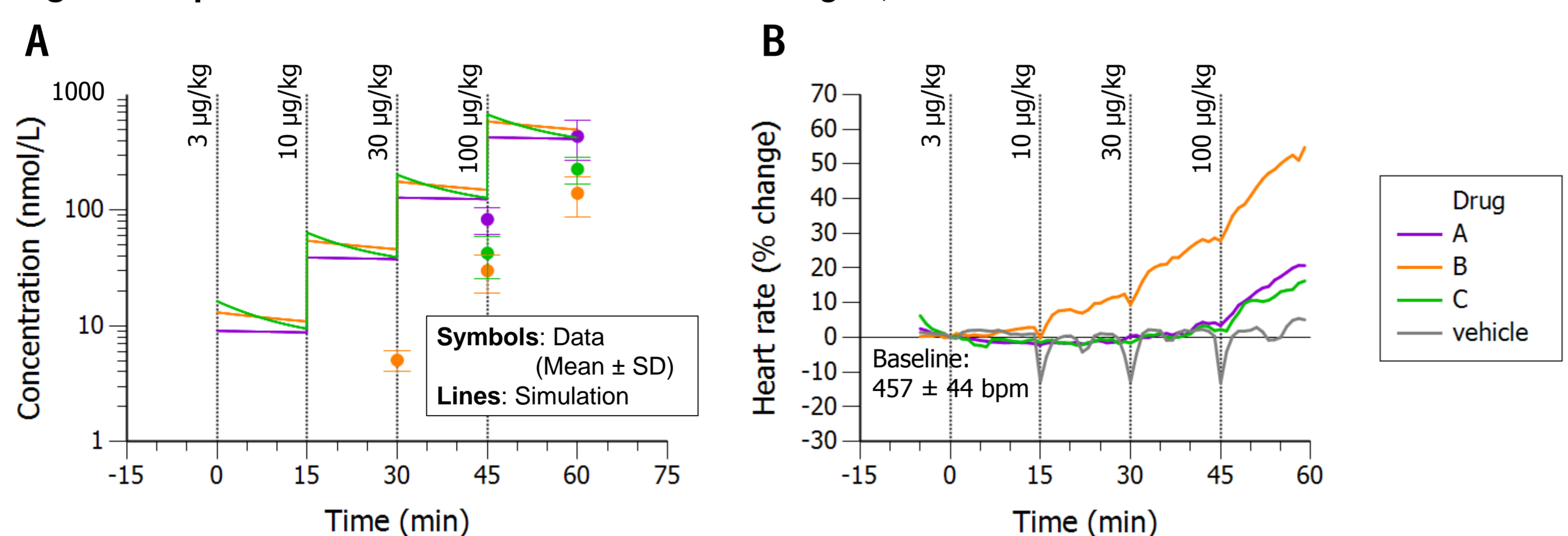


Figure 2: Turnover model fit for drug B

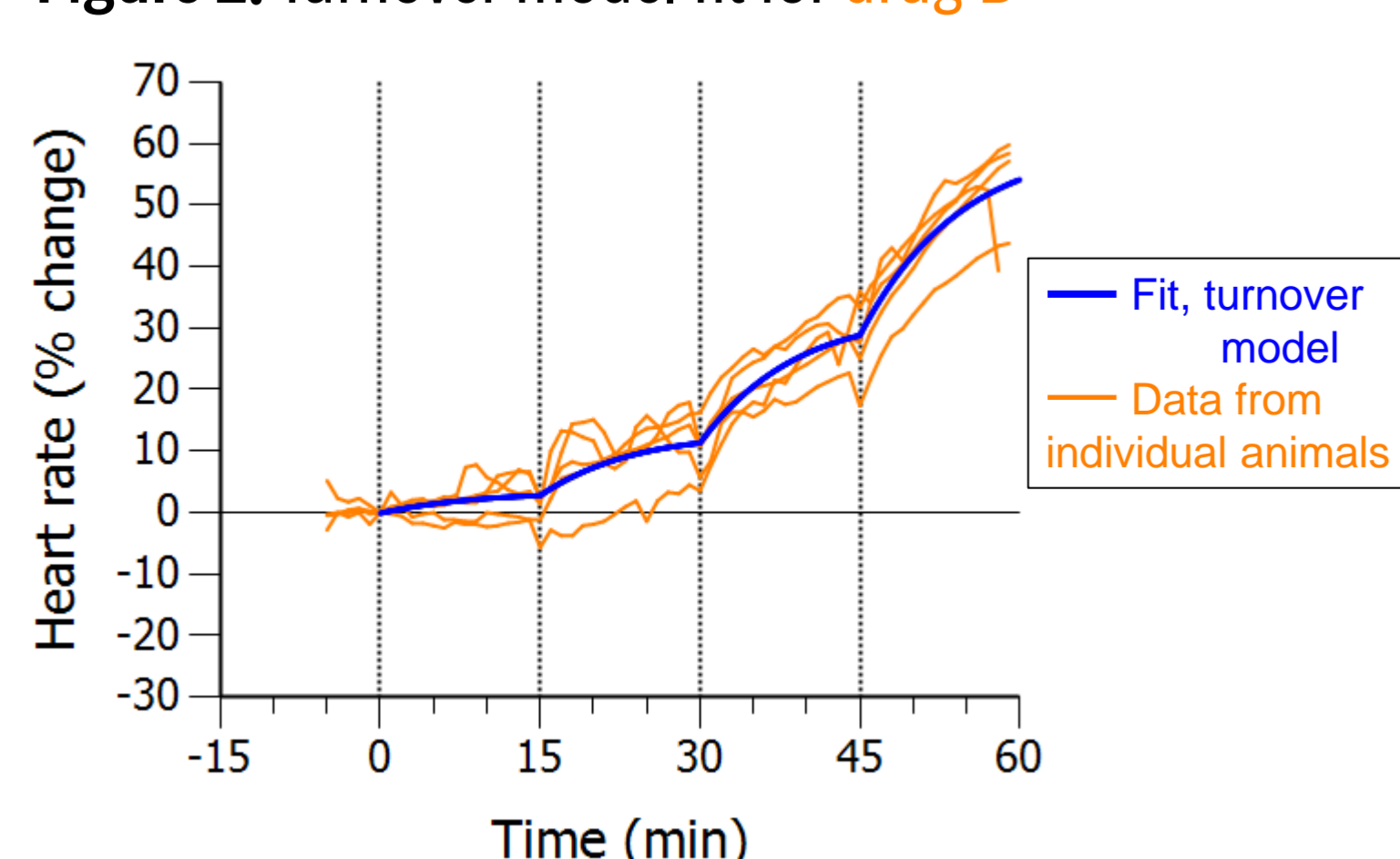


Table 1: Parameters of the turnover model

Parameter	Drug A	Drug B	Drug C
V [L/kg]	0.0888 (3.78)	0.0615 (9.70)	0.0497 (22.9)
Cl [L/hr/kg]	0.0111 (3.21)	0.0107 (6.96)	0.0123 (6.00)
V <sub>2</sub> [L/kg]	-	0.0291 (26.7)	0.0504 (24.1)
Cl <sub>2</sub> [L/hr/kg]	-	0.0400 (69.2)	0.148 (48.5)
E <sub>max</sub> [rfb*]	as drug B	0.962 (6.64)	as drug B
k <sub>in</sub> [rfb*/hr]	as drug B	5.50 (27.1)	as drug B
k <sub>out</sub> [1/hr]	as drug B	5.50 (27.2)	as drug B
EC <sub>50</sub> [nM]	1470 (4.58)	280 (12.7)	2629 (8.18)

Numbers in parentheses are CV%  
\*rfb = ratio from baseline  
(The turnover model was fitted to ratio-normalised data)

Figure 3: Heart rate effects after single i.v. administration of vehicle (A) or drug C (B-G) to anaesthetized mice, and associated exposure at the end of the study (H).

— Simulation, turnover model (table 1) — Fit, receptor binding model (table 2)  
— Data from individual animals

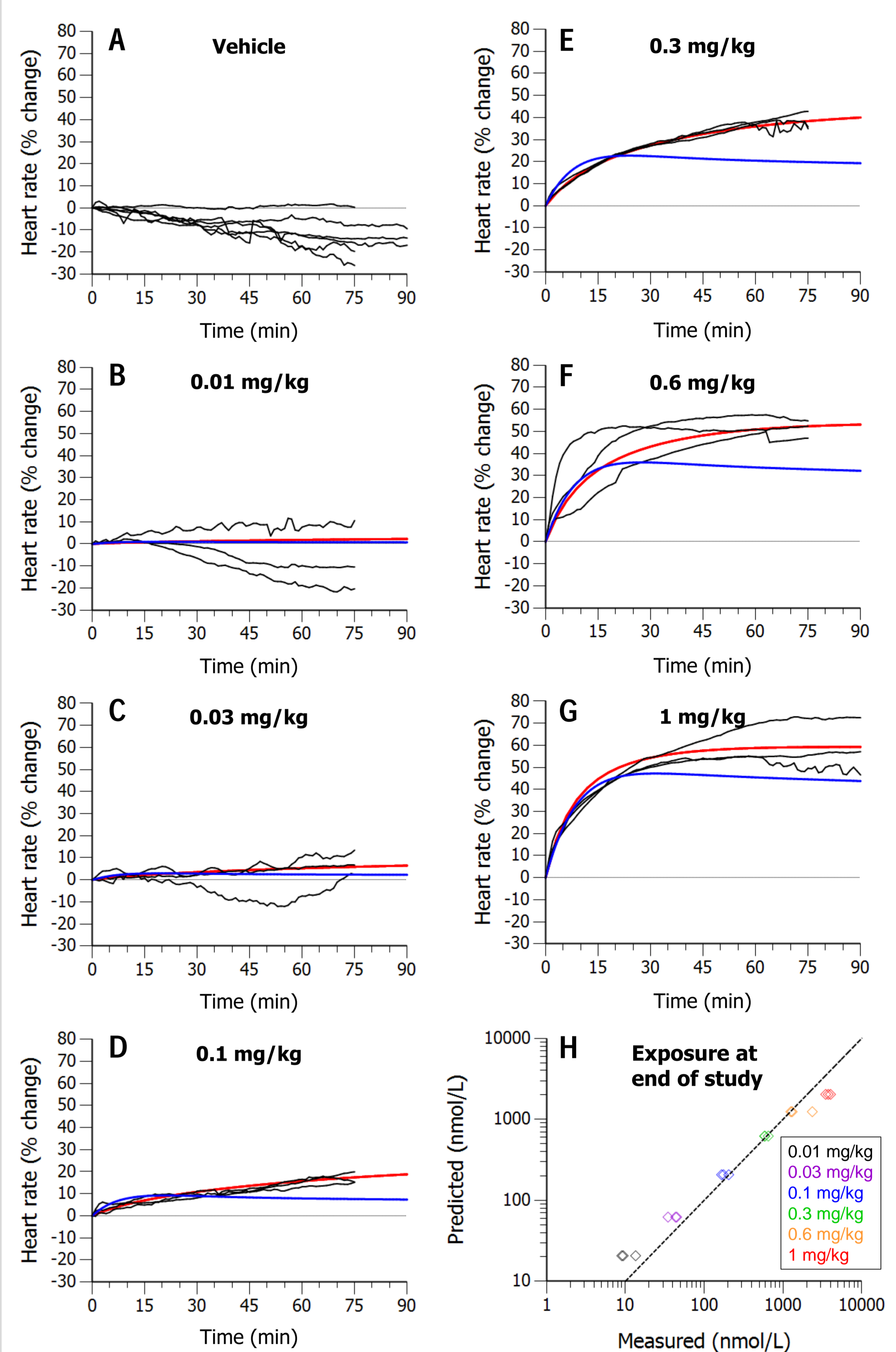


Table 2: Parameters of the receptor binding model for drug C

E <sub>max</sub> [% change]	K <sub>D</sub> [nM]	k <sub>off</sub> [1/hr]	k <sub>on</sub> = k <sub>off</sub> /K <sub>D</sub> [nM <sup>-1</sup> hr <sup>-1</sup> ]
68.8 (1.95)	350 (13.7)	0.400 (13.3)	0.00114

Numbers in parentheses are CV%

## Conclusions

- Modelling and simulation of heart rate data assisted the design of follow-up studies (info about time + magnitude of maximum effect, suitable doses).
- The observed time delay between plasma concentration and heart rate increase was best described by a receptor binding model, suggesting that slow receptor binding kinetics may be responsible for the slow emergence of heart rate effects.
- The model could be used to determine a dose having an acceptable heart rate increase (40  $\mu\text{g}/\text{kg}$ ).
- Consideration of the time delay between concentration and effect was crucial to prevent under-estimation of adverse effects and an over-estimation of the acceptable dose.

## References

[1] Shimada S, Nakajima Y, Yamamoto K, Sawada Y, Iga T. Comparative pharmacodynamics of eight calcium channel blocking agents in Japanese essential hypertensive patients. Biol Pharm Bull (1996) 19(3): 430-437.