Development of an Empirical Model to Explain Atypical Nonlinear Plasma Protein Binding of Eravacycline

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Background

Drugs bind almost instantaneously to the plasma proteins to form protein-drug complexes and a fraction of the total drug remains in free form, which is responsible for the pharmacodynamic response of the drug. This binding to the plasma proteins is reversible (Figure 1)

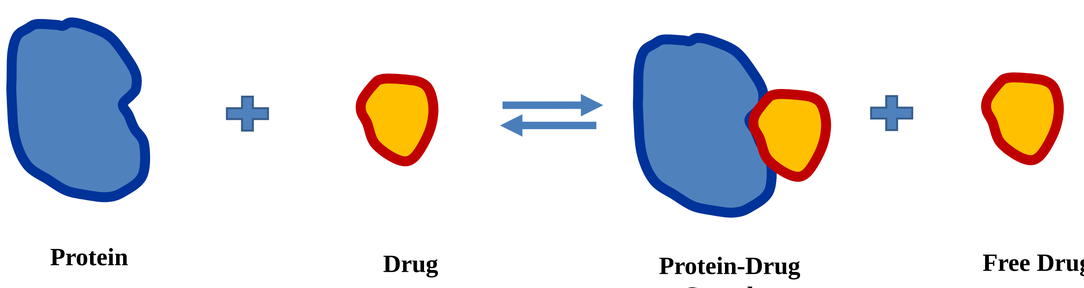

Figure 1: Schematic representation of plasma protein binding of drugs

Linear plasma protein binding: The fraction unbound of the drug remains constant with change in the total concentration

Nonlinear plasma protein binding: The fraction unbound of the drug changes with change in the total concentration (Figure 2)

Typical nonlinear plasma protein binding: The fraction unbound of the drug increases with the increase in the total concentration e.g. Disopyramide

Atypical nonlinear plasma protein binding: The fraction unbound of the drug decreases with increase in the total concentration e.g. Tigecycline, Eravacycline

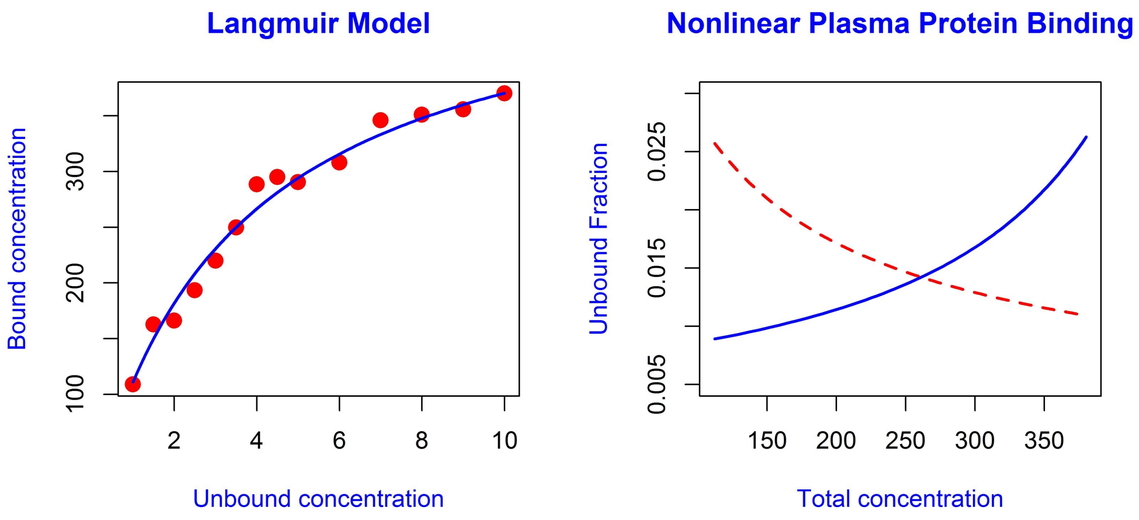

Figure 2: Left panel shows an example of the relationship between bound and unbound concentrations of drugs described by Langmuir model, which can explain linear and typical plasma protein binding. Right panel shows the change in fraction unbound with the change in total concentration in case of typical (blue solid line) and atypical (red dashed line) plasma protein binding

The typical nonlinear plasma protein binding can be described by Langmuir model (equation 1) [1,2]. At lower concentrations, the changes in the bound concentrations of drugs are linear with respect to unbound concentrations while the relation becomes nonlinear at higher unbound concentrations. The proteins are saturated at higher concentrations and therefore, the bound concentrations of drugs do not increase linearly with increase in the unbound concentrations.

$$C_b = \frac{B_{max} \bullet C_u}{K_d + C_u} \qquad \textbf{Equation 1}$$

The Langmuir model is unable to explain the atypical nonlinear protein binding of new antibiotics, tigecycline [3] and eravacycline [4]. The mechanism of such inverse characteristic is currently unknown. No model has been proposed so far to explain atypical nonlinear plasma protein binding.

Objective

To develop an empirical model to explain atypical nonlinear human plasma protein binding of Eravacycline

References

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Methods

Plasma protein binding: The protein binding of eravacycline in human plasma was determined using ultrafiltration technique at 12 different concentrations between 0.1 and 200 µg/mL. Eravacycline was added to pooled heparinized human plasma, equilibrated at 37° C and filtered through Millipore Centrifree® UF device by centrifuging at 1000 × g for 30 minutes to collect the filtrate. The filtrate was analyzed by LC-MS/MS method to determine the unbound concentrations (C_u) in the sample. The bound concentrations (C_b) were obtained by subtracting unbound concentrations from the total concentrations and fraction unbound (f_u) was calculated by dividing unbound concentrations with total concentrations (C_t) of eravacycline.

Model fitting: Model-1 and model-2 described by the equations 2 and 3 were modeled using nonlinear regression in RStudio Version 0.97.312 [5]. The parameters of model-1, A_{max}, K_d and K represents the capacity, affinity and C_b at C_u=1 ng/mL, respectively.

$$\mathrm{Log}(C_b) = \frac{A_{max} \times \mathrm{Log}(C_u)}{K_d + \mathrm{Log}(C_u)} + \mathrm{Log}(K) \qquad \textbf{Equation 2}$$

$$f_u = \beta \bullet C_t^{(-\alpha)} \qquad \textbf{Equation 3}$$

Results

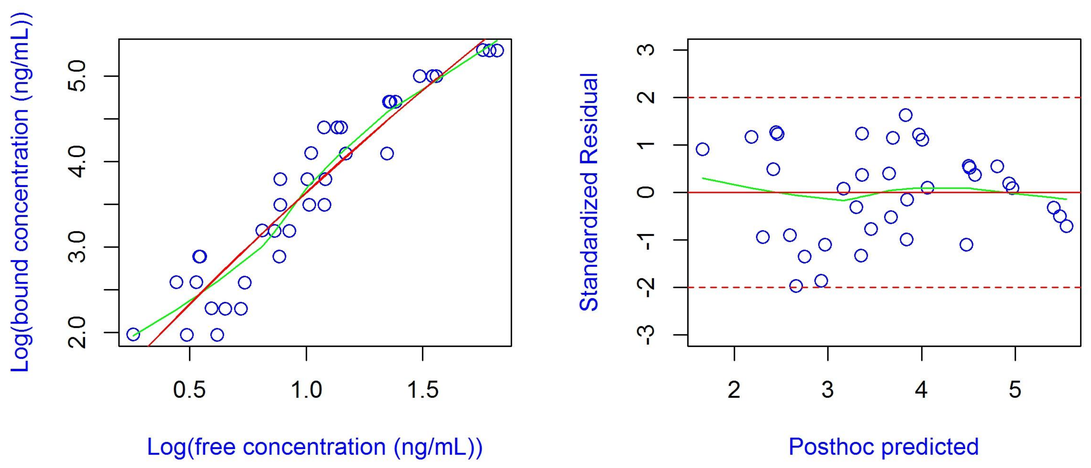

Figure 3: The goodness of fit plots of model-1. Left panel shows the observed (open circle), loess (green solid line) and predicted (red solid line) concentrations. Right panel shows the standardized residual with loess (green).

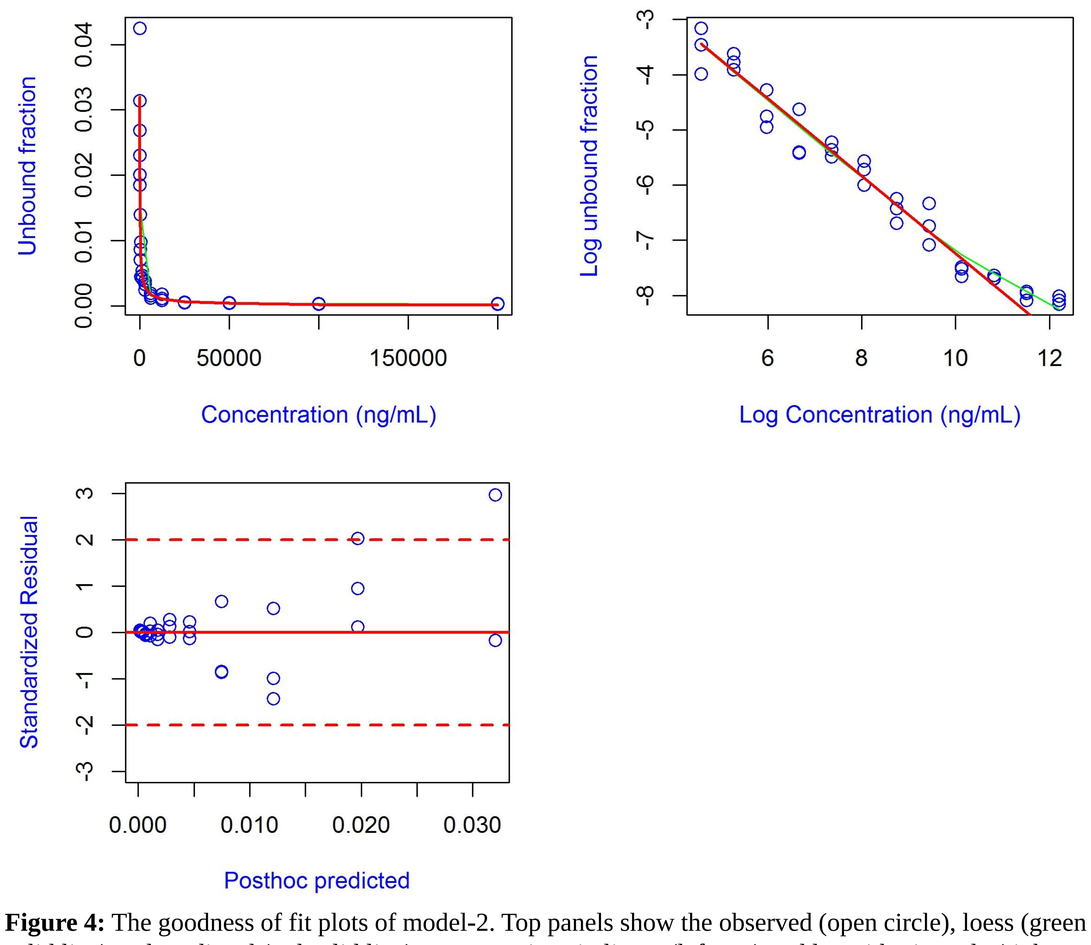

Figure 4: The goodness of fit plots of model-2. Top panels show the observed (open circle), loess (green solid line) and predicted (red solid line) concentrations in linear (left-top) and logarithmic scale (right-top). Bottom panel shows the standardized residual with loess (green).

Model 1 and model 2 adequately described the observed data as shown in figure 3 and 4. The estimates of model-1 parameters A_{max}, K_d and K were 30.8, 10.3 log units and 8.1 ng/mL, respectively. The estimates of model-2 parameters α and β were 0.7 and 0.8, respectively. The AIC values of model-2 (-300.56) was significantly lower than model-1 (31.26). Based on AIC value, goodness of fit plots and parsimony approach, model -2 was selected as the final model. Assuming asymptotic normality the 95% confidence intervals of α and β were calculated as 0.57-0.86 and 0.40-1.78, respectively.

Conclusion

Model-2 explains the relationship between unbound fraction and total concentration of eravacycline in human plasma. However, this model is empirical and does not provide insight into the involved mechanism.

Acknowledgements

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