

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/58472> holds various files of this Leiden University dissertation.

Author: Witte, W.E.A. de

Title: Mechanistic modelling of drug target binding kinetics as determinant of the time course of drug action in vivo

Issue Date: 2017-12-19

Chapter 5. The influence of drug distribution and drug-target binding on target occupancy: The rate-limiting step approximation

W.E.A. de Witte^a, G.Vauquelin^b, P.H. van der Graaf^{a,c}, E.C.M de Lange^{a*}

^a Division of Pharmacology, Leiden Academic Centre for Drug Research, Leiden University, 2333 CC Leiden, The Netherlands

^b Department of Molecular and Biochemical Pharmacology, Vrije Universiteit Brussel, Brussels, Belgium

^c Certara Quantitative Systems Pharmacology, Canterbury Innovation Centre, Canterbury CT2 7FG, United Kingdom

* Correspondence: ecmdelange@lacdr.leidenuniv.nl

Eur J Pharm Sci 2017; epub ahead of print

Abstract

The influence of drug-target binding kinetics on target occupancy can be influenced by drug distribution and diffusion around the target, often referred to as “rebinding” or “diffusion-limited binding”. This gives rise to a decreased decline of the drug-target complex concentration as a result of a locally higher drug concentration that arises around the target, which leads to prolonged target exposure to the drug. This phenomenon has been approximated by the *steady-state approximation*, assuming a steady-state concentration around the target. Recently, a *rate-limiting step approximation* of drug distribution and drug-target binding has been published. However, a comparison between both approaches has not been made so far.

In this study, the *rate-limiting step approximation* has been rewritten into the same mathematical format as the *steady-state approximation* in order to compare the performance of both approaches for the investigation of the influence of drug-target binding kinetics on target occupancy.

While both approximations clearly indicated the importance of k_{on} and high target concentrations, it was shown that the *rate-limiting step approximation* is more accurate than the *steady-state approximation*, especially when dissociation is fast compared to association and distribution out of the binding compartment.

It is therefore concluded that the new *rate-limiting step approximation* is to be preferred for assessing the influence of binding kinetics on local target site concentrations and target occupancy.

Introduction

Although drug-target binding kinetics (the association and dissociation rate constants) are important determinants of the kinetics of drug action [1–4], their role can be influenced by rebinding or diffusion-limited binding. [5–7] The term rebinding has been introduced to describe the result of a (micro-)environment around the target site which is not in instantaneous equilibrium with the plasma or target tissue, and where a concentration difference between target site and plasma or target tissue concentrations can be enhanced by drug-target binding. This local target site concentration can thus induce a delay in both drug-target association and dissociation and should therefore be considered in the analysis and prediction of the relationship between drug-target binding kinetics and target occupancy. This is especially important when *in vitro* values for drug-target binding are used to explain or predict *in vivo* target occupancy and effect.

The local concentration that drives rebinding has been approached historically from different perspectives. The biophysical approach started by describing diffusion around clustered receptors on a spherical or planar surface, which was subsequently discretized by dividing the space surrounding the receptor into the target vicinity and the bulk solution [8,9]. The pharmacological approach started from *in vitro/in vivo* observations of target binding that could not be explained by drug-target binding from bulk/tissue concentrations, which was solved by assuming the existence of a micro-compartment surrounding the target [10–12]. The effect compartment model is a less mechanistic and more general approach that is often used in PKPD modelling to explain a delay between drug concentrations and drug effect. In this approach, the drug concentrations in a hypothetical compartment drives the drug effect. The effect compartment model is most often combined with the assumption of fast target binding in the effect compartment (resulting in a “Emax model”) [13–16], although binding kinetics have also been incorporated in the effect compartment model [17,18].

The mathematical models that have been proposed from the different approaches as listed above share a similar compartmental structure and give rise to similar equations. In these models, the compartment in which binding takes place (in this paper referred to as the binding compartment) represents the target site, which is the (micro-) environment that surrounds the target.[5,9,11,17,19,20] An approximation of these compartmental models has been derived previously by assuming quasi steady-state in the binding compartment [19] and has been used since for simulation studies.[5,6] We will refer to this approximation as the *steady-state approximation*. A recent comparison between the *steady-state approximation* of rebinding and the effect compartment model (which has the same mathematical structure as the full two-compartment model from which the *steady-state approximation* is derived) indicated that the *steady-state approximation* is not capturing the behavior of the effect compartment model for fast dissociating ligands.[6]

A recently published approximation for describing target binding from a local (tissue) compartment, assumed that the overall decline of target occupancy is most influenced by the slowest process (the rate-limiting step).[7] We will refer to this approximation as the *rate-limiting step approximation*.

The *rate-limiting step approximation* has not yet been compared with the *steady-state approximation* as described by deLisi (deLisi, 1980). In this study, we use the *rate limiting step approximation* and the *steady-state approximation* of drug distribution and drug-target binding and compare their ability to capture the behavior of the original compartmental model, from which both models are derived.

Methods

To allow comparison of the *steady-state approximation* and the *rate-limiting step approximation* of drug-target binding and drug distribution as proposed here, both approximations should be written in the same mathematical format. In appendix A, the steady-state rebinding formula is rewritten in our format of choice, resulting in equation 1.

$$df_1 = \frac{k_{out}}{k_{out} + k_{on} \cdot N}$$

In equation 1, df_1 is the delay factor for Model 3 that is multiplied with k_{off} and k_{on} to account for the influence of the local concentration, k_{out} is the first-order distribution or diffusion rate constant from the micro compartment into plasma, k_{on} is the second-order association rate constant and N is the unbound target concentration. In appendix B, our rate-limiting step formula for rebinding is derived from our

previously published approximation of target binding, tissue distribution and plasma elimination, resulting in equation 2.

$$df_2 = \frac{k_{out} \cdot (1 - BF)}{k_{off} + k_{out} \cdot (1 - BF) + k_{on} \cdot N}$$

In Equation 2, df_2 is the delay factor for Model 4, the additional parameter BF is the fraction of target that is bound to the drug and k_{off} is the first-order dissociation rate constant. It should be noted that k_{out} is used to replace the drug distribution rate constant that has been called k or just k in the biophysical approach, k_{out} in the mechanistic pharmacological approach and k_{eo} in the non-mechanistic PKPD modelling approach.

Our *rate-limiting step approximation* of target binding was intended to approximate the duration of target occupancy after its maximal value and is thus applicable to calculate the delay factor for k_{off} . However, both k_{off} and k_{on} need to be multiplied with the same delay factor to ensure that rebinding does not affect equilibrium target occupancy. Multiplying k_{on} and k_{off} with the same factor is also common practice in previous rebinding studies.[6,9]

To assess the performance of the *rate-limiting step approximation* and to compare this with the recently published evaluation of the *steady-state approximation*, four different mathematical models were compared.

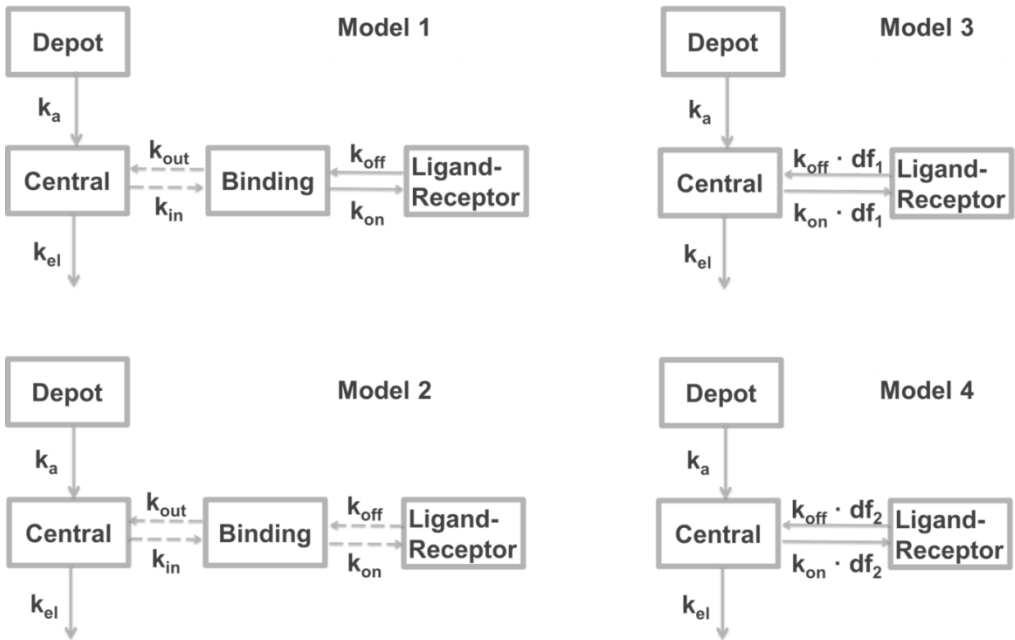


Figure 1. Schematic representation of all model structures that are used for simulations. Model 1 and Model 2 share a similar model structure, as depicted in the left panels. Model 3 and Model 4 share the model structure as depicted in the right panels. Dashed arrows indicate the absence of mass transfer between compartments. The binding compartment in Model 1 and Model 2 is assumed to be very small and the mass transfer between the Central and Binding compartment is ignored. This allows the direct modelling of the concentrations in the Binding compartment and the assumption that k_{out} equals k_{in} .

Model 1 (fig 1) is the full compartmental model that consists of a depot compartment from which absorption into plasma occurs, a central compartment representing the blood, a binding compartment in which binding occurs and a ligand-receptor compartment that represents ligand in the target-bound state. Since rebinding is often hypothesized to occur from a small “micro-compartment”, the mass transfer from plasma to this compartment was assumed to be negligible and was excluded from the model equations. However, since rebinding can result in accumulation or depletion of ligand in the binding compartment, mass transfer was incorporated in the model equations describing the concentration in the binding

compartment.

Model 2 (fig 1) is equal to Model 1, except that it does not take into account mass transfer between the binding compartment and the target-bound compartment. This model was required for comparison with the previous publication on the performance of the steady-state rebinding formula in which Model 2 was compared to Model 3 [6].

Model 3 (fig 1) encompasses an absorption and central compartment in the same way as Model 1, but drug-target binding is now driven by the plasma concentrations. Both k_{on} and k_{off} are multiplied by the delay factor, according to the steady-state approximation (equation 1).

Model 4 (fig 1) is the same as model 3, but k_{on} and k_{off} are now multiplied by the delay factor according to the newly derived rate-limiting step approximation (equation 2).

As Model 1 is the original compartmental model that is approximated by Model 3 and Model 4, we can assess the performance of Model 3 and Model 4 by comparing the simulation outcomes of these models to the outcome of Model 1. More similarity to Model 1 means a more accurate approximation. Model 2 is included for comparison with the recent publication of Vauquelin [6], in which Model 2 was compared to Model 3. Also, the parameter values for k_{on} , k_{off} , k_{out} , k_{el} and k_a were set to the same values as for the simulations in the study of Vauquelin [6]. In contrast to the publication of Vauquelin, the delay factor is calculated from equation 1 and 2, where k_{out} is used both for simulation of distribution to the binding compartment and for calculation of the delay factor. The total target concentration was chosen to yield similar results as in the recent publication of Vauquelin.[6]

The differential equations for all models are given in appendix C. The initial concentration in the absorption compartment was $15 \cdot K_D$ in all simulations. The differential equations were solved by using the Isoda solving method in the deSolve package in R, version 3.3.1[21,22].

Results

The only differences between equation 1 and 2 are the addition of k_{off} in the denominator and the correction for the influence of target saturation in the factor $(1-BF)$. Since $k_{off} + k_{out} \cdot (1-BF) + k_{on} \cdot N$ reduces to $k_{out} \cdot (1-BF) + k_{on} \cdot N$ if $k_{off} \ll k_{out} \cdot (1-BF) + k_{on} \cdot N$, equation 1 and 2 only give similar results for the delay factor df if k_{off} is relatively small compared to $k_{out} \cdot (1-BF)$ or $k_{on} \cdot N$. This corresponds to the steady-state approximation that is used to derive equation 1, which assumes that the concentration in the binding compartment adapts quickly to the surrounding concentrations. This requires that the rate at which the drug is distributed out of the binding compartment (determined by $k_{out} \cdot (1-BF)$ and $k_{on} \cdot N$) is relatively large compared to the rate at which drug is distributed into the binding compartment (determined by k_{off}). It should be noted that the influence of the saturation factor $(1-BF)$ only has a major influence for high values of target occupancy.

The performance of our *rate-limiting step approximation* has been visualized in figure 2 and 3. Figure 2A demonstrates that both approximations (steady-state: Model 3 and rate-limiting step: Model 4) produce similar results as Model 1, although Model 4 seems to approximate Model 1 a bit better than Model 3 does. In figure 2B, the difference between Model 3 and Model 4 is clearly demonstrated for the simulations with the lowest values of k_{out} and Model 4 performs much better for the parameter values that were used for figure 2B (i.e. high k_{off} , low affinity and average receptor concentration). The mismatch between Model 3 and Model 1 in figure 2B is not only the result of the high dissociation rate constant: if the target concentration is set to 1 pM instead of 1 nM, a similar mismatch as in figure 2B is observed, as demonstrated in figure 2C. As can be derived from equation 1 and 2, the same effect is observed when the k_{on} value is lowered instead of the target concentration (data not shown). Similarly, the mismatch between Model 3 and Model 1 in figure 2B can be almost completely reversed by a thousand-fold increase in the target concentration or the k_{on} (figure 2D).

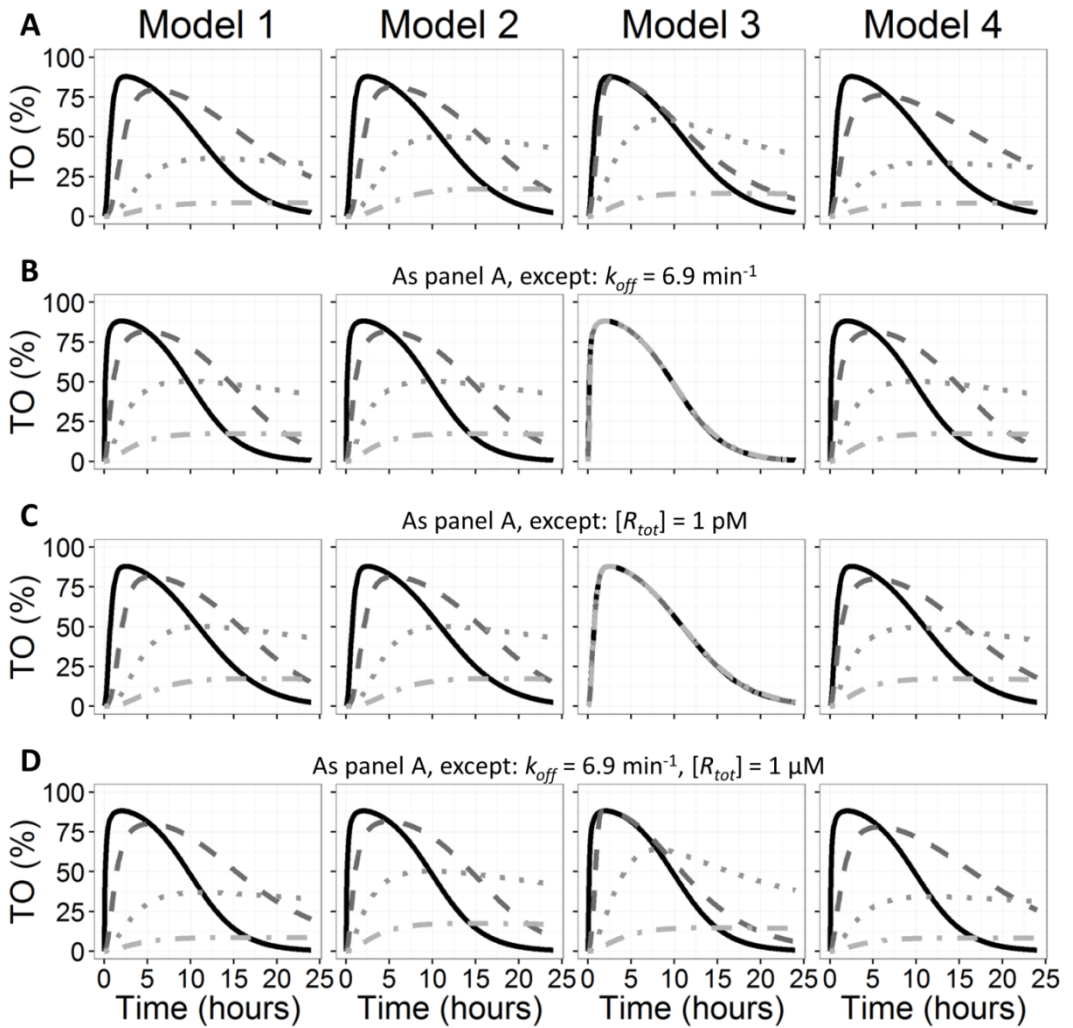


Figure 2. Comparative simulations of target occupancy (TO) over time for the models 1-4. Unless otherwise indicated above the panels, the parameter values remain the same as for panel A: $k_{out} = 10$ (solid line), 0.0047 (dashed line), 0.0005 (dotted line) or 0.000087 (dash-dotted line) min^{-1} . $k_{off} = 0.0069 \text{ min}^{-1}$, $k_{on} = 1 \cdot 10^7 \text{ M}^{-1} \cdot \text{min}^{-1}$, $[R_{tot}] = 1 \text{ nM}$, $k_a = 0.0115 \text{ min}^{-1}$ and $k_{el} = 0.00575 \text{ min}^{-1}$. The different grey tones are used for visual distinction of the lines.

The performance of our *rate-limiting step approximation* of rebinding was also investigated in the context in which rebinding formulas are frequently applied: in simulations of the influence of k_{off} on target occupancy. Figure 3A demonstrates that the influence of k_{off} is much better approximated by Model 4 than by Model 3. Although Model 4 also shows a mismatch in the increase rate of target occupancy for the lowest k_{off} values, the duration of target occupancy and the influence of k_{off} thereon resembles Model 1 closely (note the increase in the simulation duration).

For a more typical drug treatment situation, with nanomolar drug-target affinity and target concentration and a moderate delay in distribution from plasma to the binding site, both Model 3 and Model 4 produce comparable results with Model 1, as illustrated in figure 3B. The result of incorporating a correction for target saturation in our *rate-limiting step approximation* is illustrated in figure 3C and 3D: While for figure 3C Model 3 and Model 4 result in similar simulations, an increased dose results in a mismatch between Model 3 and Model 1, which is not observed for Model 4.

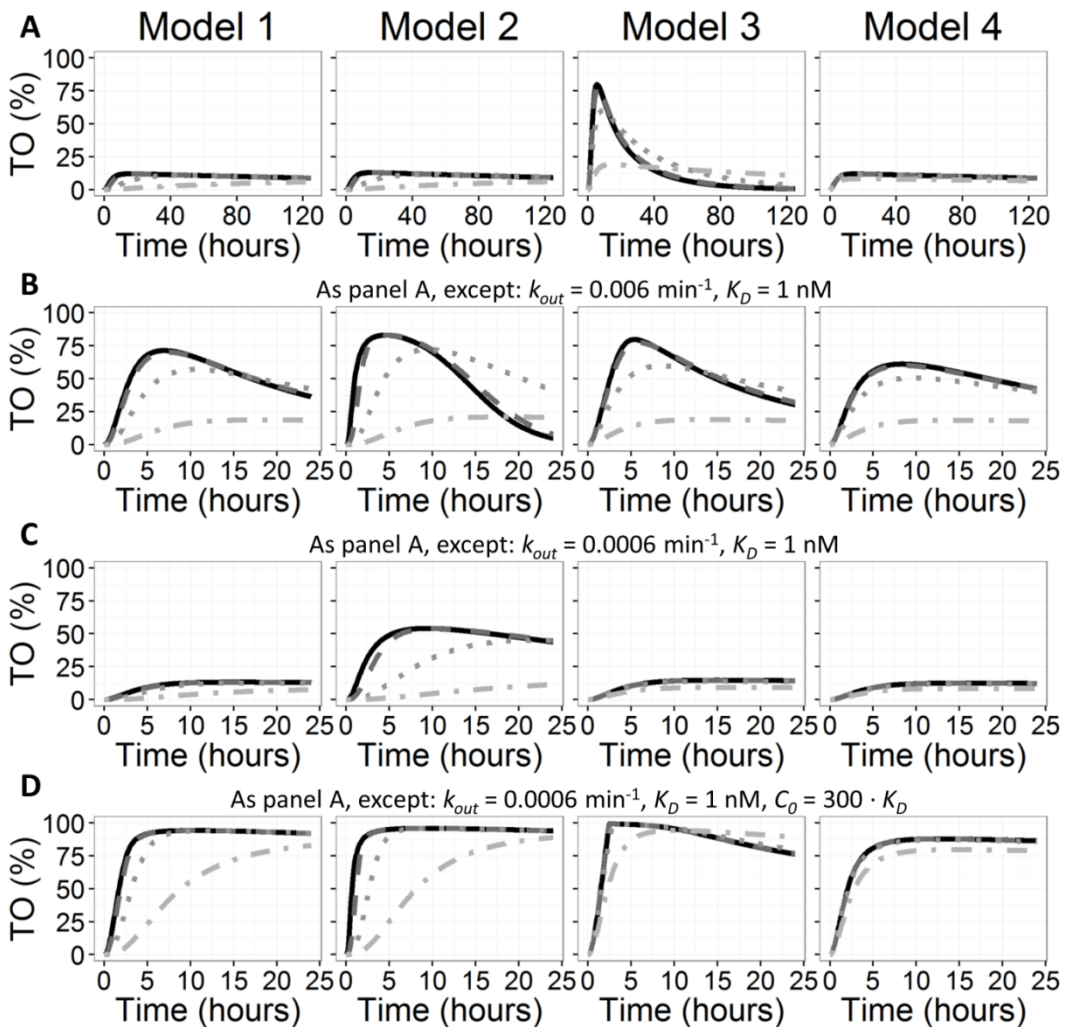


Figure 3. Comparative simulations of target occupancy (TO) over time for the different models. Unless otherwise indicated above the panels, the parameter values remain the same as for panel A: $k_{off} = 0.1$ (solid line), 0.01 (dashed line), 0.001 (dotted line) or 0.0001 (dash-dotted line) min^{-1} . $k_{out} = 0.00006 \text{ min}^{-1}$, $K_D = 1 \cdot 10^{-7} \text{ M}$, $[R_{tot}] = 10 \text{ nM}$, $k_a = 0.0115 \text{ min}^{-1}$, $k_{el} = 0.00575 \text{ min}^{-1}$ and C_0 (the initial concentration) $= 15 \cdot K_D$. The different grey tones are used for visual distinction of the lines.

Discussion

The *rate-limiting step approximation* for drug distribution and drug-target binding that we propose here on basis of our previous publication differs significantly from the *steady-state approximation* when the dissociation rate constant (k_{off}) is high, compared to the distribution rate constant (k_{out}) and the product of the association rate constant and the unbound target concentration ($k_{on} \cdot N$). This difference results in an improved approximation of the original compartmental model (Model 1), from which both approximations are derived.

The improved robustness of the *rate-limiting step approximation* compared to the *steady-state approximation* that we demonstrated here, yields a mathematically reliable simulation of the influence of k_{on} and k_{off} for a wider range of pharmacological situations. Moreover, our new approximation can help to understand the role of the relevant parameters and to interpret the observed influence of rebinding from *in vivo*, *in vitro* or *in silico* data. For example, the *steady-state approximation* results in a decline of target occupancy that is linearly related to k_{off} as long as the drug elimination from plasma is not rate-limiting (equation 1). This is not in line with the intuitive thinking that the distribution/diffusion out of the target vicinity could also be determining the decrease of target occupancy, which would make k_{off} less influential. With our *rate-limiting step approximation*, this intuitive thought is confirmed and the influence of k_{off} on the decline rate of target occupancy decreases when k_{off} becomes relatively large. Also, a correct approximation of binding and distribution is essential if this approximation is used to discriminate between various mechanisms that can explain the duration of drug action or target occupancy.

While the *rate-limiting step approximation* as presented here is a more robust approximation than the *steady-state approximation*, the difference between these two approximations is most significant when the extent of influence of drug distribution is low (since the product of target concentration and k_{on} has to be relatively small). We do not provide a rigorous mathematical proof here that the *rate-limiting step approximation* is better than the *steady-state approximation*. However, the determining equations and the simulations make clear that the *rate-limiting step approximation* can be significantly different and more accurate for conditions with limited rebinding, slow distribution out of the binding compartment and relatively fast dissociation. An approximation of the full rebinding model, such as the two approximations discussed here, is not required for simulations or model fitting of drug distribution and drug-target binding, since the full compartmental model (Model 1) can be used. However, the previous use of the *steady-state approximation* demonstrates the value of the *rate-limiting step approximation* for investigations in the role of drug-target binding kinetics and rebinding.

In conclusion, the rate-limiting step approximation provides an improved approximation of drug-target binding and drug distribution which can be used as an alternative for the existing *steady-state approximation*. Using the *rate-limiting step approximation* as presented here is especially important when dissociation is fast compared to association and distribution out of the binding compartment.

Acknowledgements

This research is part of the K4DD (Kinetics for Drug Discovery) consortium which is supported by the Innovative Medicines Initiative Joint Undertaking (IMI JU) under grant agreement no 115366. The IMI JU is a project supported by the European Union's Seventh Framework Programme (FP7/2007–2013) and the European Federation of Pharmaceutical Industries and Associations (EFPIA).

Appendices

Appendix A: Rewriting the steady-state approximation of rebinding

In the steady-state approximation of rebinding, both k_{on} and k_{off} are multiplied with the delay factor df_1 that is defined in equation A.1, where all parameters are defined as explained in methods[6]:

$$df_1 = \frac{1}{1 + \frac{k_{on} \cdot N}{k_{out}}}$$

Equation A.1 can be rewritten as equation A.2, which equals equation A.3, which provides equation 1 of the main text.

$$df_1 = \frac{1}{\frac{k_{out} + k_{on} \cdot N}{k_{out}}}$$

$$df_1 = \frac{k_{out}}{k_{out} + k_{on} \cdot N}$$

Appendix B: Derivation of the rate-limiting step approximation of target binding and drug distribution.

For the rate-limiting step approximation, our previously published approximation of pharmacokinetics and target binding was used as the starting point [7]. From this publication, equation S35 is the most relevant which is given as equation B.1 here. In equation B.1, $\lambda_{TO}(BF)$ is the fractional decrease in target occupancy, as a function of the target fraction bound (BF). $\lambda_{elTO}(BF)$ is the value that $\lambda_{TO}(BF)$ would have if the elimination would be rate limiting and thus determining the decrease of target occupancy. $\lambda_{outTO}(BF)$ is the value that $\lambda_{TO}(BF)$ would have if drug distribution from the binding compartment to plasma would be rate limiting. $\lambda_{TO}(BF)$ equals the reverse rate constant $k_{off} \cdot df$ from equation 1 and 2 if there would be no drug in plasma, i.e. if the elimination rate would be extremely high. Such a high elimination rate can be assumed for equation 1 and 2, since these equations are meant for models that simulate plasma concentrations separately. Assuming an extremely high elimination rate constant leads to an extremely high value of $\lambda_{elTO}(BF)$, which reduces equation B.1 to equation B.2.

$$\lambda_{TO}(BF) = \frac{1}{\frac{1}{\lambda_{elTO}(BF)} + \frac{1}{\lambda_{outTO}(BF)} + \frac{1}{k_{off}}} \quad (B.1)$$

$$\lambda_{TO}(BF) = \frac{1}{\frac{1}{\lambda_{outTO}(BF)} + \frac{1}{k_{off}}} \quad (B.2)$$

Since $\lambda_{outTO}(BF)$ is given by equation B.3 according to our previous publication (equation S33), equation B.2 equals equation B.4, which can be rewritten as equation B.5.

$$\lambda_{outTO}(BF) = \frac{k_{out} \cdot (1-BF)}{1 + [R_{tot}] \cdot \frac{1-BF}{K_D}} \quad (B.3)$$

$$\lambda_{TO}(BF) = \frac{1}{\frac{1}{\frac{k_{out} \cdot (1-BF)}{1 + [R_{tot}] \cdot \frac{1-BF}{K_D}}} + \frac{1}{k_{off}}} \quad (B.4)$$

$$\lambda_{TO}(BF) = \frac{1}{\frac{1 + [R_{tot}] \cdot \frac{1-BF}{K_D}}{k_{out} \cdot (1-BF)} + \frac{1}{k_{off}}} \quad (B.5)$$

Equation B.5 can be rewritten as equation B.6 by realizing that K_D equals the ratio of k_{off} to k_{on} and by multiplying each fraction by unity in such a way that the denominators become equal and the two fractions can be summed as in equation B.7. Equation B.7 equals equation B.8 and since $[R_{tot}] \cdot (1-BF)$ is equal to the unbound target concentration, equation B.8 provides equation 2.

$$\lambda_{TO}(BF) = \frac{1}{\frac{k_{off} + [R_{tot}] \cdot \frac{1-BF}{\left(\frac{k_{off}}{k_{on}}\right)} - k_{off}}{k_{out} \cdot (1-BF) \cdot k_{off}} + \frac{k_{out} \cdot (1-BF)}{k_{out} \cdot (1-BF) \cdot k_{off}}} \quad (B.6)$$

$$\lambda_{TO}(BF) = \frac{1}{\frac{k_{off} + [R_{tot}] \cdot (1-BF) \cdot k_{on} + k_{out} \cdot (1-BF)}{k_{out} \cdot (1-BF) \cdot k_{off}}} \quad (B.7)$$

$$\lambda_{TO}(BF) = \frac{k_{out} \cdot (1-BF) \cdot k_{off}}{k_{off} + [R_{tot}] \cdot (1-BF) \cdot k_{on} + k_{out} \cdot (1-BF)} \quad (B.8)$$

Appendix C: Differential equations of the four different models as used for the simulations.

The concentrations in the depot and the central compartment were modeled in the same way for all four models according to equation C.1 and C.2, respectively. In these equations, $[DEP]$ is the drug concentration in the depot compartment, $[C]$ is the drug concentration in the central compartment, k_a is the first-order absorption rate constant and k_{el} is the first-order elimination rate constant.

$$\frac{d[DEP]}{dt} = -k_a \cdot [DEP] \quad (C.1)$$

$$\frac{d[C]}{dt} = k_a \cdot [DEP] - k_{el} \cdot [C] \quad (C.2)$$

The concentration in the binding compartment is only explicitly simulated in Model 1 and Model 2 according to equations C.3 and C.4, respectively. Here, $[B]$ denotes the drug concentration in the binding compartment, $[N]$ denotes the unbound target concentration (which is calculated by assuming the total target concentration is constant), $[LR]$ denotes the drug that is bound to the target, k_{out} is the first-order distribution constant between the binding compartment and plasma, k_{on} is the second-order association rate constant and k_{off} is the first-order dissociation rate constant.

$$\text{Model 1:} \quad \frac{d[B]}{dt} = k_{out} \cdot ([C] - [B]) - k_{on} \cdot [B] \cdot [N] + k_{off} \cdot [LR] \quad (C.3)$$

$$\text{Model 2:} \quad \frac{d[B]}{dt} = k_{out} \cdot ([C] - [B]) \quad (C.4)$$

The concentration of the target-bound drug is calculated identically for Model 1 and Model 2 according to equation C.5:

$$\text{Model 1, Model 2:} \quad \frac{d[LR]}{dt} = k_{on} \cdot [B] \cdot [N] - k_{off} \cdot [LR] \quad (C.5)$$

For Model 3 and Model 4 the target bound drug is calculated according to equation C.6 and C.7, where BF denotes the fraction of the target that is bound to the drug.

$$\text{Model 3:} \quad \frac{d[LR]}{dt} = (k_{on} \cdot [C] \cdot [N] - k_{off} \cdot [LR]) \cdot \frac{k_{out}}{k_{out} + k_{on} \cdot N} \quad (C.6)$$

$$\text{Model 4:} \quad \frac{d[LR]}{dt} = (k_{on} \cdot [C] \cdot [N] - k_{off} \cdot [LR]) \cdot \frac{k_{out} \cdot (1-BF)}{k_{off} + k_{out} \cdot (1-BF) + k_{on} \cdot N} \quad (C.7)$$

References

1. Johnson M, Kozielska M, Pilla Reddy V, et al. Mechanism-based pharmacokinetic-pharmacodynamic modeling of the dopamine D2 receptor occupancy of olanzapine in rats. *Pharm Res* 2011;28(10):2490–504
2. Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* 2006;5(9):730–9
3. Yassen A, Olofsen E, Kan J, et al. Animal-to-human extrapolation of the pharmacokinetic and pharmacodynamic properties of buprenorphine. *Clin Pharmacokinet* 2007;46(5):433–47
4. Dahl G, Akerud T. Pharmacokinetics and the drug-target residence time concept. *Drug Discov Today* 2013;18(15–16):697–707
5. Vauquelin G, Charlton SJ. Long-lasting target binding and rebinding as mechanisms to prolong in vivo drug action. *Br J Pharmacol* 2010;161(3):488–508
6. Vauquelin G. Impact of target binding kinetics on in vivo drug efficacy: k_{off} , k_{on} and rebinding. *Br J Pharmacol* 2016;173(15):2319–34
7. de Witte WEA, Danhof M, van der Graaf PH, et al. In vivo Target Residence Time and Kinetic Selectivity: The Association Rate Constant as Determinant. *Trends Pharmacol Sci* 2016;37(10):831–42
8. Goldstein B, Dembo M. Approximating the effects of diffusion on reversible reactions at the cell surface: ligand-receptor kinetics. *Biophys J* 1995;68(4):1222–30
9. Coombs D, Goldstein B. Effects of the geometry of the immunological synapse on the delivery of effector molecules. *Biophys J* 2004;87(4):2215–20
10. De Meyts P. Cooperative properties of hormone receptors in cell membranes. *J Supramol Struct* 1976;4(2):241–58
11. Perry DC, Mullis KB, Oie S, et al. Opiate antagonist receptor binding in vivo: evidence for a new receptor binding model. *Brain Res* 1980;199(1):49–61
12. Frost JJ, Wagner HN. Kinetics of Binding to Opiate Receptors In Vivo Predicted From In Vitro Parameters. *Brain Res* 1984;305(1):1–11
13. Cleton A, de Greef HJ, Edelbroek PM, et al. Application of a combined “effect compartment/indirect response model” to the central nervous system effects of tiagabine in the rat. *J Pharmacokinet Biopharm* 1999;27(3):301–23
14. Groenendaal D, Freijer J, Rosier A, et al. Pharmacokinetic/pharmacodynamic modelling of the EEG effects of opioids: the role of complex biophase distribution kinetics. *Eur J Pharm Sci* 2008;34(2–3):149–63
15. Francheteau P, Steimer JL, Merdjan H, et al. A mathematical model for dynamics of cardiovascular drug action: Application to intravenous dihydropyridines in healthy volunteers. *J Pharmacokinet Biopharm* 1993;21(5):489–514
16. Nolan ER, Feng MR, Koup JR, et al. A novel predictive pharmacokinetic/pharmacodynamic model of repolarization prolongation derived from the effects of terfenadine, cisapride and E-4031 in the conscious chronic av node--ablated, His bundle-paced dog. *J Pharmacol Toxicol Methods* 2006;53(1):1–10
17. Yassen A, Olofsen E, Dahan A, et al. Pharmacokinetic-Pharmacodynamic Modeling of the Antinociceptive Effect of Buprenorphine and Fentanyl in Rats : Role of Receptor Equilibration Kinetics. *J Pharmacol Exp Ther* 2005;313(3):1136–49
18. Äbelö A, Andersson M, Holmberg AA, et al. Application of a combined effect compartment and binding model for gastric acid inhibition of AR-HO47108: A potassium competitive acid blocker, and its active metabolite AR-HO47116 in the dog. *Eur J Pharm Sci* 2006;29(2):91–101
19. DeLisi C. The biophysics of ligand-receptor interactions. *Quarterly Rev Biophys* 1980;13(2):201–30
20. Vauquelin G. Rebinding : or why drugs may act longer in vivo than expected from their in vitro target residence time. *Expert Opin Drug Discov* 2010;5(10):927–41
21. R Core Team. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria URL <http://wwwR-project.org/> 2013;
22. Soetaert K, Petzoldt T, Setzer RW. Solving Differential Equations in R: Package deSolve. *J Stat Softw* 2010;33(9):1–25