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

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Chapter 9. Mechanistic modelling of drug target binding kinetics as determinant of the time course of drug action *in vivo*

Discussion, perspectives and conclusion

abbreviations: BF: Target fraction bound, k_{off}: drug-target dissociation rate constant, k_{on}: drug-target association rate constant, k_{el}: drug-target elimination rate constant, $t_{1/2z}$-pl: terminal plasma, elimination half-life, $t_{1/2}$-diss: drug-target dissociation half-life

For any drug that is administered to patients or that is being developed, is essential that the time course of its effects can be predicted to ensure rational drug therapy and drug development. After its administration, the time course of the effect of a drug can be influenced by all processes that constitute the complex system of the human body. The most common processes that determine the time course of drug action can be categorised as related to either target site exposure, target binding, signal transduction or homeostatic feedback mechanisms, as indicated in *Figure **1***. For the development of new drugs, it is critical to predict the time course of drug action as early as possible. To this end, the *in vitro* measurement and *in silico* prediction of the critical process of target binding provides a valuable selection criterion to identify potential drug candidates.

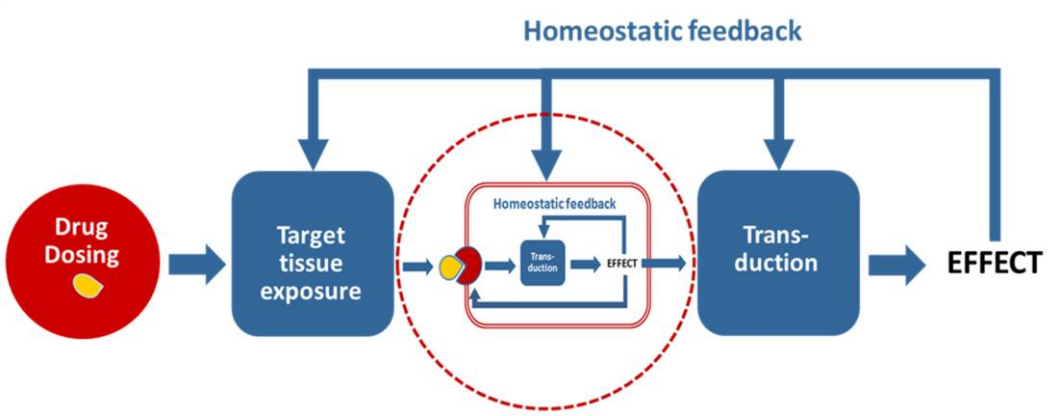

Figure 1. The causal chain from drug dosing to drug effect. The drug is indicated by the yellow shape and the drug target is indicated by the complimentary red shape. Adapted by E.C.M. de Lange from Danhof, 2016.[1]

To use target binding as selection criterion in drug discovery, the measurement of target binding under equilibrium conditions has been traditionally used to determine a single drug-specific parameter for the drug-target affinity, being the drug target dissociation constant K_D. However, the kinetics of this target binding (drug-target binding kinetics), has often been demonstrated to influence the time course of drug action.[2–10] More than half a century ago, it has even been postulated that the strength of a drug effect is proportional to the rate of drug-target dissociation (rate theory), rather than to the occupancy of the target (occupancy theory).[11] Together with new technologies to determine drug-target binding kinetics and new compound series with varying binding kinetics, this has sparked a new interest in the application of especially the drug target dissociation rate constant k_{off} as selection criterion in drug discovery.[12] However, drug-target binding kinetics is only a single step in the long chain of events from drug dosing to drug effect and many other processes influence the kinetics of drug action *in vivo* as discussed above. To understand the role of drug-target binding kinetics, to predict its influence on the time course of drug action, and to use it to develop better drugs, one should study drug-target binding kinetics in relation to the other determinants of the time course of drug action.

In this thesis, we studied a wide range of pharmacokinetic-pharmacodynamic (PKPD) models that include expressions to describe target binding kinetics by simulation for a wide range of their parameter values and, where possible, application to *in vitro* and *in vivo* pharmacokinetic and pharmacodynamic data. The main question in these studies was how the drug-target binding kinetics, in conjunction with plasma pharmacokinetics, tissue distribution kinetics, endogenous ligand competition, signal transduction kinetics and homeostatic feedback determine the *in vivo* time course of drug action. In this chapter, we first discuss how our findings contribute to our understanding of the influence of drug-target binding kinetics on the time course of drug action. Next, we discuss how our findings can be applied in drug discovery, drug development and in clinical practice. Finally, we provide suggestions for future research and conclude this thesis.

The added value of drug target binding kinetics as selection criterion in drug discovery is relatively new and subject to an ongoing debate.[13] As described in **chapter 1 and 3**, the considerations regarding the role of drug-target binding kinetics, especially the dissociation rate constant k_{off}, on the time course of drug effect *in vivo* fall into four categories:

I) a low k_{off} value can result in prolongation of target occupancy [3,14,15],
II) a low k_{off} value for the therapeutic target compared to the secondary-targets can give rise to an increase in selectivity over time [3,16,17],
III) a low k_{off} value will lead to a more constant blocking of endogenous ligand binding and thus block the endogenous signalling more effectively [18,19] and
IV) a low k_{off} value can yield a more efficient coupling to signal transduction, leading to a higher efficacy.[20,21]

In this thesis, we have investigated the validity and the limiting conditions of the first three of the considerations that support the relevance of drug-target binding kinetics. The fourth consideration is beyond the scope of this work. Below, we will shortly summarise and discuss our findings for each of these three arguments.

I. Target dissociation kinetics as determinant of the time course of target occupancy.

The first consideration on the influence of the k_{off} value on the duration of target occupancy was investigated in relation to i) plasma pharmacokinetics, ii) tissue distribution kinetics and iii) the concentration of the target, in **chapter 2, 4 and 5**. Simulations on the basis of a one-compartment pharmacokinetic model with target binding showed that the time course of target occupancy is only affected by the value of k_{off} if both the k_{off} is lower than the product of the elimination rate constant (k_{el}) and unbound target fraction (1-*BF*) and the k_{on} is lower than the ratio of k_{el}/R_{tot} as illustrated in *Figure* ***2***. If the k_{off} is lower than the product of the elimination rate constant and unbound target fraction and the k_{on} is higher than the ratio of k_{el}/R_{tot}, the duration of target occupancy is equally influenced by the k_{off} and the k_{on}.

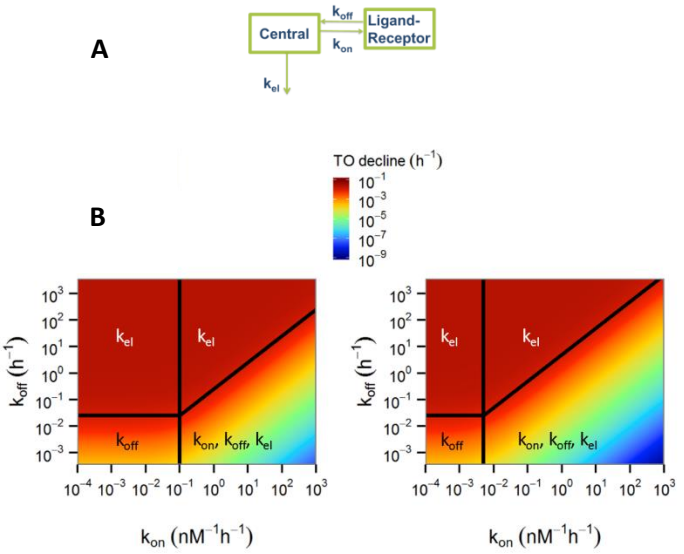

Figure 2. Approximation of the decline in target occupancy as function of k_{off} and k_{on} using a simple one compartment model with target binding. A: schematic representation of the approximated model. B: Approximation results for a total target concentration 1 nM (left panel) and 20 nM (right panel) an elimination rate constant of 0.1/h and a target fraction bound of 0.75, to represent a clinically relevant degree of target occupancy. Colours represent the decrease of target occupancy. The vertical line is given by $K_{RLon} = k_{el} / (R_{tot} * k_{on}) = 1$, the horizontal line is given by $K_{RLoff}(BF) = k_{el} * (1-BF)/k_{off} = 1$ and the diagonal line is given by the equation $k_{off} = k_{on} * R_{tot} * (1-BF)$. In these equations, k_{el} is the elimination rate constant, R_{tot} is total target concentration and BF is the bound fraction of the target. The annotations indicate which parameters influence the decrease in target occupancy in the corresponding segment of the plot.

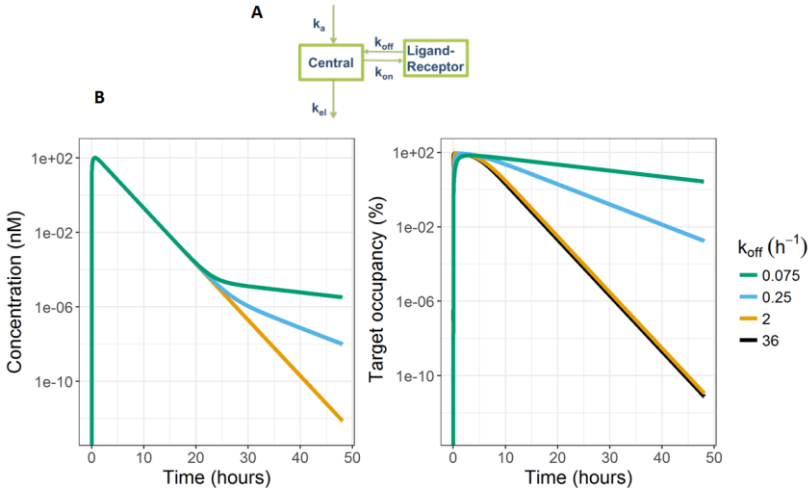

Figure 3. Simulations with a one compartment pharmacokinetic model with drug target binding demonstrate the parallel terminal phase of the pharmacokinetic and target occupancy curves. A: schematic representation of the model that was used for these simulations. For these simulations, the first order absorption rate constant k_a was 3 hr^{-1}, the first order pharmacokinetic elimination rate k_{el} was 0.693 hr^{-1}, the target concentration was 0.001 nM and the K_D was 10 nM.

When drug distribution out of the target site is slow compared to its elimination from plasma, the distribution can also become the rate-limiting step in the decline of target occupancy, which leads to an equal influence of k_{on} and k_{off} on the duration of target occupancy. These findings contrast with studies that suggested that the role of the k_{off} value is independent of the K_D and the concentration of the target[4,15,22], but are in line with studies on rebinding, in which also a clear influence of the target concentration on the duration of target occupancy has been observed.[14,23,24] In **chapter 4**, we have shown that k_{off} values, even when these are much lower than the pharmacokinetic elimination rate constant, lead to equilibrium between free target site and target-bound drug concentrations for high target concentration/K_D ratios. This finding is in line with the equilibrium binding and steady-state assumptions, which require free and bound drug concentration to be in equilibrium, which are often successfully incorporated in drug target binding models.[25–27] Moreover, our findings in **chapter 4** showed that the decline of plasma concentrations eventually parallels the decline of target occupancy on semi-log scale, even if drug-target dissociation is the rate-limiting step for the decline of target occupancy. This parallel decline is illustrated in *Figure **3*** for an extremely low target concentration of 0.001 nM and a K_D of 10 nM. This low target concentration/K_D ratio causes this parallel decline to appear only at the late time points and at an extremely low plasma concentration, but these simulations show that even in this case both plasma pharmacokinetics and target occupancy curves are eventually parallel.

This parallel decline in drug concentration and target occupancy makes that the comparison of the terminal plasma half-life ($t_{1/2z}$-pl) and target dissociation half-life ($t_{1/2}$-dis) is only informative if the $t_{1/2}$-dis is much shorter than the $t_{1/2z}$-pl. If both half-lives have similar values, this does not necessarily dispute the influence of k_{off} on the decline rate of target occupancy. This influence of the drug-target dissociation rate on the $t_{1/2z}$-pl might not be observed, because of the lower limit of quantification of the assays for determining drug concentrations in plasma. However, Dahl et al. [15] calculated the ratio of the $t_{1/2z}$-pl and the $t_{1/2}$-dis for a series of marketed drugs and observed that the $t_{1/2}$-dis is often shorter than the $t_{1/2z}$-pl. While this observation supports the conclusion of the authors that the $t_{1/2}$-dis does not determine the duration of target occupancy, it should be noted that several of the studied drugs had a $t_{1/2}$-dis in the same order of magnitude as the $t_{1/2z}$-pl. As described above, if the target occupancy duration is determined by the $t_{1/2}$-dis, the $t_{1/2z}$-pl will be identical to the $t_{1/2}$-dis. Thus, the duration of target occupancy for the drugs with a $t_{1/2}$-dis in the same order of magnitude as the $t_{1/2z}$-pl in the study of Dahl et al. could have been determined as well by the $t_{1/2}$-dis.

So far, our description of the influence of plasma elimination, target site distribution and the target concentration on the duration of target occupancy did not take into account the role of target synthesis and degradation, distribution to non-target binding tissues, and protein binding. These latter factors need to be addressed as well, as they can influence the duration of target occupancy (**chapter 1**). While extensive distribution to non-target binding tissues and plasma protein binding can reduce the effective elimination rate of the drug from the plasma and thereby prolong drug-target binding, a fast target turnover can reduce the duration of target occupancy, even for a slow drug-target dissociation compared to the plasma drug elimination or target site distribution. Thus, whereas the pharmacokinetic rate of elimination of the drug functions as an upper limit for the values of k_{off} that influence the duration of target occupancy, the degradation rate constant of the drug-target complex functions as a lower limit for the values of k_{off} that influence the duration of target occupancy.

In summary, a low k_{off} value can prolong the duration of target occupancy, but this prolongation can only be predicted in conjunction with the pharmacokinetics, target concentration and target turnover.

II. The relation between the k_{off} value for different targets, target selectivity and tissue selectivity

The second consideration for the relevance of the k_{off} value for the time course of drug action is based on the differential duration of target occupancy between the therapeutic target and the off-target(s), as can be caused by different k_{off} values. In **chapter 6,** we distinguish between selective binding to the therapeutic target relative to off-targets caused by differential k_{off} values, which is commonly referred to as "*kinetic selectivity*" [3,16,17], and selective binding to a target in the therapeutic tissue, relative to tissues that mediate side effects, which is commonly referred to as "*tissue selectivity*"[28,29]. *Kinetic selectivity* is closely related to the time course of target occupancy for each target. Therefore, the principles for single target binding in one tissue as identified in **chapter 4** are likely to hold for *kinetic selectivity* as well. Our simulations in **chapter 6** were in line with **chapter 4**: a high target concentration/K_D ratio leads to a prolonged duration of target occupancy, which is caused by a slow decline of the drug concentration at the target site. As a consequence, all targets at the same target site will be exposed to drug concentrations that decline slowly and their duration of target occupancy will be equally long. On the other hand, any tissues with much lower target concentrations than the therapeutic tissue will be exposed to faster decline of drug concentrations and the target occupancy in those tissues will also decline faster. This results in *tissue selectivity*. This is illustrated in *Figure **4***, where target 2 has a much longer target occupancy duration in tissue 1 compared to tissue 2, while both tissues have the same distribution rate constants, the same concentration of target 2 and the same binding kinetics to target 2. The long duration of target occupancy in tissue 1 is caused by the high concentration of target 1, which causes retention of the drug in tissue 1, as reflected by the concentration profiles.

However, *tissue selectivity* decreases when K_D is extremely low and the concentration of the target is higher than the K_D in both therapeutic and non-therapeutic tissues, as compared to tissues with reasonably high K_D values. These results demonstrate that a high K_D value may result in a decrease in both *kinetic selectivity* and *tissue selectivity*. Moreover, the combination of target and tissue selectivity may lead to a reversal of selectivity over time if one of the targets has a high target concentration. We have shown that the use of mechanistic modelling and simulation combined with statistical Quantitative Structure Activity Relationship (QSAR) modelling can help to predict both target and tissue selectivity in the earliest stage of drug discovery. However, we also found that these predictions are dependent on the effective distribution of the target site and the target concentration. These latter parameters might be difficult to obtain with high precision, especially in the earliest phase of drug discovery.

In short, a low k_{off} value can result in *kinetic selectivity*, but only when the target concentration/K_D ratio is not high enough to induce a slow decline of local drug concentrations. Moreover, a high target concentration or low K_D value can increase tissue selectivity.

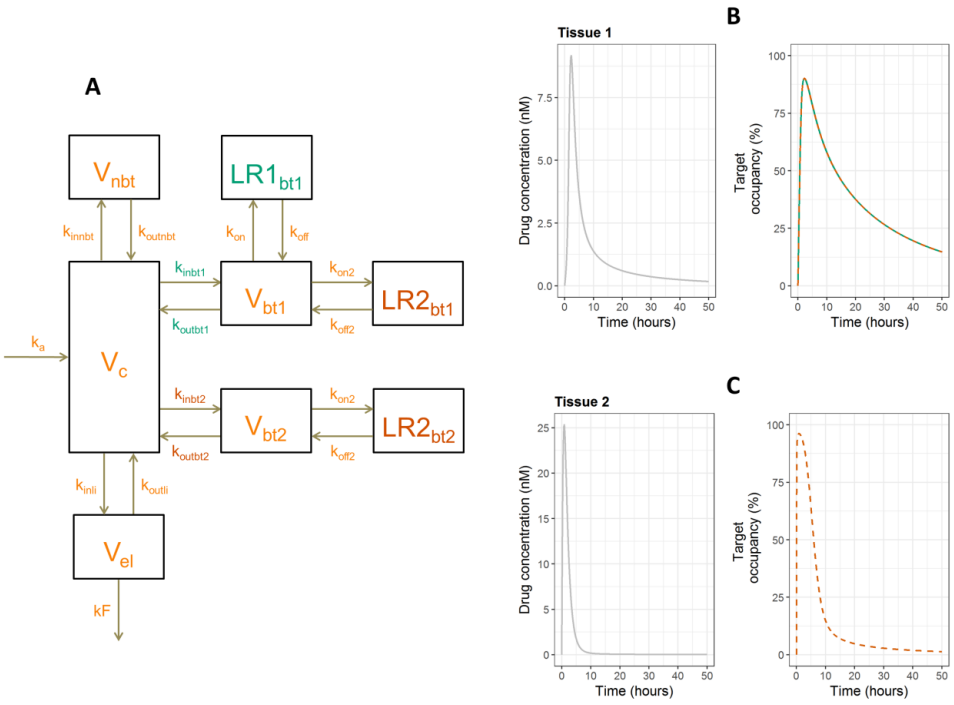

Figure 4. Simulation of simultaneous target and tissue selectivity and the influence of the target concentration. A: schematic representation of the applied model structure for these simulations. k_a = absorption rate constant, k_{in} = inwards distribution rate constant, k_{out} = outwards distribution rate constant, k_{on} = association rate constant, k_{off} = dissociation rate constant, kF = forward rate of elimination constant, LR = ligand-receptor complex, V = tissue volume (L), nbt = non-binding tissue, c = plasma compartment, bt = binding tissue, nbt = non-binding tissue, el = elimination tissue. The values for k_{on} and k_{off} were 10 nM^{-1} h^{-1} and 10 h^{-1}, respectively, for both targets. The values for k_{inbt} and k_{outbt} were 2.6 and 0.88 h^{-1}, respectively, and V_{bt} was 2 L for both tissues, the target concentration for target 1 was 100 nM and was 1 nM for target 2 in both tissues. The values of the other parameters were: k_a = 3 h^{-1}, k_{innbt} = 60 h^{-1}, k_{outnbt} = 5.5 h^{-1}, k_{inli} = 17 h^{-1}, k_{outli} = 54 h^{-1}, kF = 100 h^{-1}, V_c = 6 L, V_{nbt} = 66 L, V_{li} = 1.9 L B: Drug concentration and target occupancy of target 1 (solid line) and target 2 (dashed line) in tissue 1. Both target occupancy profiles are identical. C: Drug concentration and target occupancy of target 2 (dashed line) in tissue 1.

III. The relation between the k_{off} value, resilience to endogenous ligand binding, signal transduction and homeostatic feedback.

The third consideration that supports the relevance of the k_{off} value for the time course of drug action is related to the resilience to endogenous ligand competition. This idea has been raised in relation to dopamine D_2 antagonists [18], but the principle also holds for any other target where endogenous competition is important. In short, an endogenous signal in the form of a steep increase and decrease in the endogenous ligand concentration would normally lead to endogenous ligand binding to the target and further signal transduction. In the presence of a drug with a high k_{off} value that is bound to the target, this rise in endogenous ligand would still lead to binding of the endogenous ligand, albeit to a lesser degree depending on the concentration of the drug and its K_D. For a drug with a low k_{off} value, the endogenous

ligand would not have enough time to displace the drug from the receptor before its concentrations go down to the basal level. As a result, for a drug with a relatively high k_{off} value, part of the physiological signalling is maintained, whereas for a compound with a relatively low k_{off} value the signalling would be completely blocked. In the case of the dopamine D_2 receptor, this extensive blocking of the dopaminergic signalling is considered to lead to side effects, and a low k_{off} value is therefore considered to be a disadvantage of a D_2 antagonist. In **Chapter 8**, we found that a low k_{off} value can indeed prolong the drug-target occupancy with fluctuating endogenous ligand concentrations. However, we also found that this influence of the drug k_{off} only occurs when the endogenous ligand k_{off} is high enough to result in rapid endogenous ligand binding. Moreover, if the turnover of the signal transduction molecules is not fast enough, the rapid increase and decrease of endogenous ligand target occupancy does not lead to rapid fluctuations in the concentrations of the signalling molecules. This limited influence of the k_{off} value on the drug effect to frequently fluctuating endogenous ligand concentrations for high k_{off} values was not identified in a previous study that did not take the endogenous ligand k_{off} and the signal transduction kinetics into account.[19] The limited translation of fluctuating endogenous ligand concentrations into fluctuating second messengers is in line with the concept of frequency encoding, which explains that the strength of biological signals can be translate into the frequency of the fluctuations in signalling molecules and *vice versa*. These fluctuations are therefore only representing the strength of a signal and eventually not translated into a fluctuating effect, but into a stable effect, the extent of which is dependent on the fluctuation frequency of the signalling molecules.[30]

In summary, the k_{off} value of a drug (especially an antagonist) is only relevant for the resilience to endogenous signalling if both the endogenous ligand k_{off} and the turnover of the signalling molecules are high enough to translate the endogenous ligand fluctuations into fluctuations in signalling strength.

Modelling the delay between pharmacokinetics and pharmacodynamics and the relevance of drug-target binding.

One of the considerations that disputes the relevance of the k_{off} value for the time course of drug action is that, on one hand, the drug-target binding kinetics are not generally required in PKPD models that give a good description of the observed drug concentration and effect data. On the other hand, target binding models are often required to adequately describe antibody pharmacokinetics, in so called Target Mediated Drug Disposition (TMDD) models, which can and have been applied to small molecules as well.[25,31,32]

The effect compartment model is typically used to explain hysteresis, rather than a target binding model.[33] However, this does not necessarily mean that drug-target binding kinetics does not influence the time course of drug action. In **Chapter 7**, we compared the target binding model and the more popular effect compartment model and found that these models do not lead to a different time course of the drug effect for all parameter values combinations that result in a delay between drug concentrations and drug effect. In other words, hysteresis between plasma drug concentrations and effect can be described equally well by an effect compartment and a target binding model for many of the parameter value combinations used in this study. Although this is not a finding that directly supports the relevance of drug target binding for the time course of drug action, it does suggest that the drug-target binding model should be tested more often to allow *prediction* of the time course of drug action, by the incorporation of *in vitro* data, and therewith to improve the *in vitro-in vivo* translation in drug discovery.

Perspectives for the development and application of pharmacotherapy

The centrality of the drug-target binding event in drug treatment makes our findings applicable across the whole range of pharmacotherapy, from drug discovery to clinical practice. The most general application of our work is that we obtained a better understanding of drug-target binding kinetics and its role in the complex chain between drug dosing and drug effect. Below, we discuss more specifically how our insights can be applied in drug discovery, drug development and clinical practice.

In drug discovery, the selection of the best drug candidates is essential because of the limited resources and the large number of molecules that enter the drug discovery phase, while only a limited number of tests can be performed. Therefore, these tests need to yield information on the most critical drug properties which can be easily translated into selection criteria for the best drug candidates. The current understanding of the value of drug-target binding kinetics as a selection criterion in drug discovery is limited and mostly focussed on obtaining drugs with low k_{off} values. In this thesis, we have shown that a low k_{off} value is only a beneficial drug property if the whole PKPD context favours the influence of the k_{off} on the time course of drug action. Therefore, our findings suggest that for any new disease/therapeutic indication, detailed knowledge on the system-specific parameters is required before knowledge of drug-target binding kinetics can be applied meaningfully. Such parameters include the concentration of the target, its degradation and synthesis rate constants, the perfusion of the target site and the concentration and binding kinetics of endogenous ligands. Subsequently, mechanistic PKPD modelling should be applied to identify the optimal drug properties, including the drug target k_{off} and K_D.

As a rule of thumb, targets that are expressed at a higher concentration than the K_D value of the drug and targets with a faster degradation rate constant than the k_{off} value of the drug are not expected to favour the relevance of the k_{off} value.

In drug development, the *in vitro – in vivo* translation of drug effects and the translation across animal species is essential to get the best drug candidates to the market. For this, the combination of *in vitro-in vivo* extrapolations (IVIVE) with physiologically based pharmacokinetic models can be used to predict drug effects across animal species and humans.[34,35] However, drug target binding kinetics are often not incorporated in these models. Our results suggest that the target concentrations, the perfusion of the target tissue and active processes in drug distribution to the target site are important and need to be included in these models to enable translation between species, especially for high affinity compounds.

In clinical practice, the prediction and understanding of the time course of drug action can be critical for effective and safe drug treatment. Our findings in **chapter 4 and 6** demonstrate that a delayed onset and offset of drug action can be caused by slow drug-target binding kinetics or binding to a target with a high target concentration. Importantly, if these mechanisms drive a delayed onset of the drug effect, this can be avoided by using higher drug doses. Thus, the combination of a high initial drug dose (loading dose) combined with lower subsequent doses (maintenance dose) can be used to achieve rapid drug action while still minimizing toxicity. Since our findings demonstrate the influence of the target concentration on the time course of drug action, these findings can also be used to individualize drug dosing based on target concentrations. This might be especially relevant for high target concentrations that are also highly variable between patients, such as HER2 concentrations in HER2-positive breast tumours.[36]

Future research

The findings described in this thesis present an improved understanding of the influence of drug-target binding kinetics on the time course of drug action in relation to the most important determinants of the time course of drug action. However, the complete biological system that determines drug action is too complex to understand completely or even to describe all elements and their relation with the role of drug-target binding kinetics. The main questions that remain elusive after the studies in this thesis are described below.

As mentioned in **chapter 1** and in the discussion above, the turnover of the target can be an important factor that influences the role of drug-target binding kinetics. Although target turnover is often referred to as a single parameter, its relation with drug-target binding kinetics is complex since the turnover of the unbound target can be different than the turnover of drug-target complex. This is schematically represented in *Figure 5*. The analysis of target turnover can be simplified by assuming that the turnover of the unbound target (k_{degT}) and the drug-target complex (k_{degC}) are equal, but differences between these two parameters of more than tenfold have been estimated from *in vivo* data.[37–39] In these studies k_{degT} has been observed to be both more than tenfold larger and more than tenfold smaller than k_{degC}. A consequence of a difference in k_{degT} and k_{degC} is that the total target concentration is not constant and depends on the amount of target binding. This makes the level of target occupancy and the drug-target affinity constant K_D less informative parameters [40] and makes mathematical analysis of the model less straightforward. Moreover, the pharmacological entity that drives the drug effect depends on the disease and the target: for an enzyme inhibitor, the concentration of the unbound target determines the drug effect, while for a receptor agonist, the concentration of the drug-target complex drives the drug effect. These complexities have not been investigated in this thesis and it would require further research to understand their relationship with drug-target binding kinetics.

Figure 5. schematic representation of target turnover in relation to drug-target binding kinetics and pharmacokinetics. k_{el}, k_{degT}, k_{degC} and k_{off} represent the first order rate constant of elimination of the drug from plasma, degradation of the unbound target, degradation of the drug-target complex and dissociation of the drug-target complex, respectively. k_{on} represents the second order drug-target association rate constant, k_{syn} represents the zero-order target synthesis rate constant.

In addition to the influence of drug-target turnover, the translation from target occupancy to drug effect also requires further exploration. First of all, several authors have observed a correlation between the k_{off} and the efficacy of agonists.[21,41,42] Interestingly, these correlations all show a higher efficacy for agonists with lower k_{off} values, which is opposite to what Paton postulated in his rate theory in 1961.[11] The higher efficacy of slowly dissociating drugs can be explained by a more efficient coupling of activated receptors to signal transduction if they are active for a longer continuous period of time, which reduces the fraction of aborted signalling events.[21] This correlation between k_{off} and efficacy should be supported with more compound series for various targets and with the analysis of mechanistic signal transduction models.

Secondly, the occurrence of a non-linear target occupancy *versus* effect relationship (transducer function) can reduce the impact of a change in target occupancy levels and its rate.[43] If the transducer function, for example, has the classical sigmoidal shape, this means that a fast declining target occupancy does not lead to a fast decline of the drug effect if the target occupancy is close to 100%. In a clinical setting of continuously high target occupancies, this would make the decline rate of target occupancy less relevant for the duration of drug effects. It should be noted that this nonlinearity can have a similar influence on the time course of drug action as the nonlinearity between drug concentrations and target occupancy, as described in **chapter 2**. In addition, the occupancy versus effect relationship can have various profiles, including a parabolic profile [44,45], which further complicates the translation from target occupancy kinetics to drug effect kinetics. The signal transduction system that was analysed in **chapter 8** included the turnover of secondary messengers and regulation via a negative feedback loop. While we did observe the influence of the turnover of the feedback molecule on the transduction of fluctuating endogenous ligand concentration, we focused mainly on the fluctuation amplitude of the response in steady-state. The presence of homeostatic feedback mechanisms can also influence the initial response to a drug after its first administration and lead to a system with multiple steady-states.[46] Such a system can be sensitive to the rate of administration of a drug[47] which makes it more likely for the rate of drug-target association to influence which of the steady-states will be reached. Finally, signal transduction is often interlinked with an extensive signalling network with signalling cascades that are branched and result in simultaneous signal transduction at multiple levels, as identified for GnRH analogues.[48] Analysing the influence of drug-target binding kinetics on the drug effect in such complex networks would require additional research. The simulation-based frequency response analysis that was applied in **chapter 8** could also be applied to such signalling networks to unravel their dynamic behaviour and its determinants.

Conclusions

The research in this thesis has improved our understanding of the influence of drug-target binding kinetics on the time course of drug action. We have especially elucidated and quantified how drug-target binding kinetics relate to the other determinants of the time course of drug action, including pharmacokinetics, target turnover, endogenous competition and signal transduction. This research does not provide a complete understanding of all these factors and further research is especially required on the interaction between target turnover, signal transduction and binding kinetics. Nonetheless, our insights can be applied to the selection of better drug candidates, to improve translational research and to optimize and personalize clinical practice of pharmacotherapy.

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