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

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Chapter 3. Mechanistic modelling of drug target binding kinetics as determinant of the time course of drug action *in vivo*

Scope and intent of the investigations

General introduction

Drug-target binding kinetics refers to the kinetics of the central event in pharmacology: target engagement. The kinetics of this event can be described in its simplest form according to equation 1, in which k_{on} is the second order association rate constant, k_{off} is the first order dissociation rate constant, L is the concentration of the ligand (i.e. a drug or an endogenous ligand), R is the concentration of the target and LR is the concentration of the bound ligand-target complex. The equilibrium dissociation constant (K_D) is given by the ratio k_{off}/k_{on}.

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} LR \qquad \text{Eq. 1}$$

The mathematical description of drug target binding according to equation 1 is regularly incorporated in the models that describe the pharmacokinetic profile of monoclonal antibodies and their associated pharmacodynamic profiles in so-called target mediated drug disposition (TMDD) models.[1–3] For small molecules however, the role of drug-target binding kinetics has also been incorporated [4–7] but this effect is most often assumed to be negligible in both PKPD modelling practices and in the design and development of new molecules.

With the publication of Copeland et al. in 2006 [8], a new interest was initiated to utilize drug-target binding kinetics and especially the drug-target dissociation rate constant (k_{off}) or its reciprocal value that expresses the mean target residence time (RT) of each drug molecule on the target after drug-target association. In this regard, especially a low k_{off} value was proposed to be a desirable property for new drug candidates.

The reasons why a low k_{off} value is considered as a desirable drug property are diverse:

- A lower k_{off} can lead to a slower decline of target occupancy and as a result, a prolongation of the drug effect, compared to a drug with rapid target equilibration kinetics.[9,10]
- Differences in target equilibration kinetics at different targets may result in an improved selectivity of action over time, a phenomenon which is often referred to as kinetic selectivity.[8,11]
- The value of k_{off} may affect fluctuations in target occupancy upon repeated dosing and/or in the situation of fluctuating endogenous ligands concentration, with potentially a more favourable efficacy/safety balance.[12]
- A low k_{off} gives rise to longer binding of each drug molecule to the target molecule, which could improve the efficacy of signal transduction.[13,14]

In this thesis, we focus on the first three points, which are all focused on the duration of target occupancy as determinant of the duration of drug effect. As outlined in **chapter 1**, the duration of target occupancy is not only influenced by the drug-target binding kinetics but also by all other kinetic factors that connect drug dosing to drug effect [15]:

First of all, after a drug enters the body and is absorbed into the blood circulation, the elimination and distribution of a drug are the main determinants for how quickly the drug concentrations decline. If the drug concentration is declining slowly compared to the k_{off}, the duration of target occupancy is hardly influenced by the value of k_{off}.

Secondly, the distribution into and out of the tissue where the target resides can also affect the time course of target occupancy and, similar to a slow elimination from plasma, slow distribution out of the target tissue compared to drug-target dissociation reduces the influence of the k_{off} value on the duration target occupancy.

Thirdly, the concentration profile and target binding kinetics of endogenous ligands can also influence the time course of drug-target occupancy. If a drug is bound to the target, a steep increase in the endogenous ligand concentration would lead to faster displacement of the drug by endogenous ligand binding for drugs with a higher k_{off} value compared to drugs with a lower k_{off} value.

Fourthly, the target synthesis and degradation rate constants can influence the time course of target occupancy, especially when the target turnover is fast, compared to drug target dissociation. In essence, target turnover functions as an additional dissociation mechanism (since drug-bound target is degraded and unbound target is synthesized) and determines the duration of target occupancy if it is much faster than the drug-target dissociation. On top of this influence of target turnover on the effective drug-target dissociation, the degradation of target-bound drug also functions as additional elimination mechanism of the drug, which is described in Target-Mediated Drug Disposition models.[2]

Finally, signal transduction and homeostatic feedback kinetics can influence the time course of drug action, as well as the factors that affect the time course of target occupancy described above. Especially a slow turnover of one of the signalling molecules in a signalling cascade, compared to the fluctuations in target occupancy, will decrease the fluctuations of the drug effect compared to a fast turnover of all signalling molecules.

In this respect, it is important to emphasize that prolonged target occupancy compared to the plasma pharmacokinetics is not defined by a lower log-linear tangent of the target occupancy versus time curve at a given time point, compared to the log-linear slope of plasma concentrations. As explained in **chapter 2**, if the target occupancy is close to 100 %, the log-linear tangent of target occupancy *versus* time profile is lower than the log-linear slope of the plasma concentration *versus* time profile, even if target binding is fast and results in an immediate binding equilibrium. This is a result of the non-linear drug concentration *versus* equilibrium target occupancy relationship.

The aim of our studies was to investigate how the influences of drug-target binding kinetics on the time course of the target occupancy (**Section II**) and the drug effect (**Section III**) depend on the other kinetic processes that constitute the PKPD context of drug-target binding kinetics.

Section II. Simulations, model analysis and experimental validation of the influence of binding kinetics on the time course of target occupancy

In **Section II**, we first investigate how, upon single dose administration, the time course of the target occupancy depends on one hand on the drug-target binding kinetics and on the other hand also on the pharmacokinetics, in particular the elimination rate constant and the tissue distribution kinetics. Secondly, we apply this insight and study how this affects the selectivity of action of a drug and the ability to discriminate between a drug-target binding model and a biophase distribution model to account for hysteresis in PKPD modeling.

Kinetics of *in vivo* target occupancy: impact of drug distribution and elimination *versus* target binding kinetics

To understand the influence of the drug-target dissociation rate constant (k_{off}) on the time course of target occupancy and its dependence on the plasma elimination rate constant (k_{el}), one cannot just compare the k_{off} with the k_{el} and conclude that the lowest rate constant determines the decline rate of target occupancy, as suggested previously [10]. The problem with this approach is that it does not take into account that the decline of drug concentrations over time are influenced by drug target binding, as is well documented for biologics [1,2] and has been described as rebinding for small molecules on a local scale [9]. In **chapter 4**, we therefore investigate when, on the one hand the elimination rate constant and the tissue distribution kinetics and on the other hand, the drug-target dissociation become the rate-limiting step in the decline of target occupancy, taking into account the important role of the total target concentration. This rate-limiting step refers to the slowest step in a chain of reactions that determines the overall rate of the whole chain of reactions, which is a concept often applied in chemistry.[16]

To explore the interrelationships between the pharmacokinetics and the target association and dissociation kinetics as determinants of the time course of target occupancy, we approximate two minimal pharmacokinetic target binding models, with first order elimination from the central compartment, in which the target binding either takes place in the only pharmacokinetic (plasma) compartment or in a peripheral tissue compartment connected to the plasma by linear distribution kinetics. These approximations are obtained by formalizing the rate-limiting step concept as described in the previous paragraph to obtain algebraic equations for the tangent of the target occupancy *versus* time curve for a given value of target occupancy.

The obtained approximations are subsequently used to visualise the influence of the values of k_{on} and k_{off}, relative to the elimination rate constant, on the time course of target occupancy for a wide range of k_{on} and k_{off} values and to calculate the influence of the elimination rate constant, the tissue distribution rate constants and the target concentration on the duration of target occupancy. With this analysis, we show that at concentrations of a drug target which largely exceed the K_D value, the values of k_{off} and the k_{on} have an equal effect on the duration of target occupancy, which is thus driven by the K_D and in equilibrium under these conditions.

Chapter 5 focuses on the role of rebinding as determinant of the duration of target occupancy and the mathematical approximation of this phenomenon. More specifically, we compared the rate-limiting step approximation, with the steady-state approximation. The rate-limiting step approximation assumes that the slowest step in the chain from drug-target dissociation, drug distribution and drug elimination determines the duration of target occupancy, as described in chapter 4, whereas the steady-state approximation is based on the assumption that the target site concentrations are in steady-state and dependent on the target-bound concentration.[17–19]. In **chapter 5**, we investigate how the rate limiting step approximation differs from the steady-state equation. To do so, we rewrite our rate-limiting step approximation equations in the same format as the steady-state approximation equations, compare these equations and perform simulations to investigate which approximation provides the most similar results to the full pharmacokinetic-target binding model. We demonstrate that the rate-limiting step approximation performs best in most situations and performs much better if the steady state assumption does not hold: if the drug-target dissociation is fast compared to the distribution out of the target site and the drug-target association. Based on these findings, we propose that either the rate-limiting step approximation or the full differential equation model is used, rather than the steady-state approximation.

Selectivity of action: target selectivity *vs* tissue selectivity

As explained before, one of the proposed benefits of a low k_{off} value for a small molecule drug is that it could dissociate slower from the therapeutic target compared to the secondary-target (kinetic selectivity). However, the results in chapter 4 indicate that for drugs with a high target concentration compared to the K_D value, a low k_{off} value would still lead to equilibrium target binding. This implies that kinetic selectivity would be decreased or absent for drugs with a high target concentration and low K_D value. On the other hand, if a high target concentration compared to the K_D value leads to an increased target site concentration compared to the plasma concentration, this could lead to increased tissue selectivity for tissues with high target concentrations. The aim of **chapter 6** is therefore to explore the influence of tissue distribution kinetics, drug-target binding kinetics, and target concentrations on the time course of target and tissue selectivity. To that end, we perform simulations in three minimal physiologically-based pharmacokinetic models and combine those with target binding kinetics models. The first model is designed to investigate target selectivity, where we lump all tissues without drug-target binding except the eliminating organ and the plasma, and incorporate one tissue with drug-target binding to two different targets. The second model is designed to investigate tissue selectivity and differs from the first model in that it has two tissues with drug-target binding and that only one target type is incorporated in both tissues. The third model is a more specific case study that combines drug-target binding to 4 different targets in 3 different brain areas.

With these simulations, we describe that the characteristics that determine the decline of target occupancy, as identified in chapter 4, could similarly be identified in the more complex models for target and tissue selectivity. We describe the influence of target expression levels and the affinity constant K_D on target and

tissue selectivity. The identification of a context-dependent optimal K_D value rather than aiming to minimize the K_D increases the value of K_D predictions in early drug discovery. Therefore, we developed a Quantitative Structure Activity Relationship (QSAR) with a random forest model based on public affinity values for the CB1, 5-HT1a, mGlu5 and TRPV1 targets. Combined with the target concentrations for these targets in 3 different brain areas, we simulate the target occupancy profiles for Rimonabant, CP-55490 and Δ^8 THC, CB1 ligands which have approximately tenfold different K_D values for the CB1 receptor. In these studies, the dose was adjusted to obtain similar equilibrium CB1 occupancy for all three compounds. When steady state occupancy was reached, all compounds showed selectivity for the different targets according to the difference in their K_D values for the different targets. We also describe that the higher affinity compounds Rimonabant and CP-55490 showed a much slower approach to steady-state occupancy in the brain regions with the highest target expression compared to Δ^8 THC, which led to a change in target selectivity across tissues in the first week of treatment. We thus show the advantage and propose the application of combined computational methods to predict target and tissue selectivity in the earliest phase of drug discovery and generate understanding of multiple target binding in multiple target tissues.

SECTION III. Simulations, model analysis and experimental validation of the influence of binding kinetics on the time course of drug action

In chapter 4, 5 and 6, we show how the influence of drug-target binding kinetics on the time course of target occupancy is dependent on the pharmacokinetics and target concentrations. This invokes the question when a drug-target binding model can be discriminated from linear target site distribution models and when these models provide similar drug effect profiles. To investigate this, in **chapter 7** we fit an effect compartment model, a target binding model and a combined effect compartment-target binding model to a historical morphine PKPD dataset in rats. This dataset contained plasma and brain extracellular fluid (ECF) morphine concentrations and EEG amplitudes as pharmacodynamic endpoint. In addition, we perform simulations with a one compartment PK model with drug-target binding to identify for what parameter values the time to the maximal target occupancy ($Tmax_{TO}$) changed significantly with a tenfold increase in the dose. We describe that the differentiation between the target binding and effect compartment model is difficult, both if the plasma concentrations or the brain concentrations are assumed to be directly linked to the target binding/effect compartment. Moreover, our simulations show that the shift in $Tmax_{TO}$ with increasing dose is only observed for intermediate k_{off} values around the elimination rate constant and for low K_D values relative to the target concentrations. We conclude that successful target binding or effect compartment model fits are not supportive for the relevance of target binding or target site distribution, respectively. The target binding model should be considered more often as alternative to the effect compartment model to obtain the best model and to generate the possibility to inform the *in vivo* model with *in vitro* parameters.

So far, we have focused on the role of target binding kinetics as a determinant of the time course of the target occupancy and the selectivity of action in a stationary system without homeostatic feedback, where a constant drug concentration leads to a constant drug effect. In reality, fluctuations of the drug effect may occur, also with constant drug concentration. Such fluctuations may result from fluctuations in the release of neurotransmitters or from homeostatic feedback mechanisms. In Section III, we study how the influence of drug-target binding kinetics depends on the concentration profile and target binding kinetics of endogenous ligands, on the signal transduction and on homeostatic feedback. We investigate this question in **chapter 8** based on the *in vitro* binding kinetics and cAMP response data for 17 dopamine D_2 antagonists. The relation between drug-target binding kinetics, endogenous competition and signal transduction is especially relevant for D_2 antagonists since it has been postulated in the "fast-off" hypothesis that a high antagonist k_{off} value would reduce their side effects by partially allowing the endogenous dopamine binding to the D_2 receptor.[20] This hypothesis has been investigated with a more detailed simulation study for fluctuating dopamine concentrations, but the signal transduction and feedback kinetics were not taken into

account in that study.[12] Therefore, we firstly develop a minimal mechanistic model that included antagonist and dopamine binding kinetics to the D_2 receptor, the synthesis and degradation of cyclic adenosine monophosphate (cAMP) and the synthesis and degradation of phosphodiesterase (PDE), which provides negative feedback on cAMP concentrations. This model is fitted to the *in vitro* cAMP response data of all 17 D_2 antagonists and subsequently used to simulate the response to fluctuating dopamine concentrations with a wide variety of fluctuation frequencies, as observed *in vivo*.[21] We find that the influence of the antagonist k_{off} on the amplitude of the induced cAMP fluctuations is restricted by both the cAMP degradation rate constant and the dopamine k_{off} which also means that an antagonist k_{off} higher than one of these values does not influence the cAMP fluctuations. This means that to study the relevance of the k_{off} value, the signal transduction kinetics, endogenous ligand binding kinetics and the homeostatic feedback kinetics need to be taken into account, especially for fluctuating endogenous ligand concentrations.

In **chapter 9**, we discuss how the findings in this thesis affect our understanding of the influence of drug-target binding kinetics on the time course of target occupancy and drug effect. We conclude that this is dependent on the pharmacokinetic and pharmacodynamic context and that modeling and simulation can be a valuable tool to increase the understanding of the complex biological system that determines the relevance of drug-target binding kinetics for the time course of drug action.

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