Utilising computational power to improve drug safety: predicting and understanding tissue selectivity

H.C. (Anna) Vlot
Supervisors: W.E.A. de Witte & G.J.P. van Westen
Division of Pharmacology & Division of Medicinal Chemistry − LACDR
Leiden University
h.c.vlot@umail.leidenuniv.nl

ABSTRACT
Increasing tissue selectivity of compounds may aid the development of safer drug treatments by decreasing side effect prevalence. To enable this, improved insight into the mechanisms underlying tissue selectivity is required. In this article the influence of receptor concentration, drug-target affinity and binding kinetics on tissue selectivity is described. Simulations were performed in a physiological model with drug-target binding, informed by *in silico* predicted physicochemical properties. Lower tissue selectivity was observed for high affinity ligands than for low affinity ligands. This observation moves against the current paradigm in which high affinity ligands are assumed to be better drug candidates.

Keywords
Tissue selectivity, physiologically-based pharmacokinetic modelling, target mediated drug disposition, adenosine A_1-receptor, receptor concentration, binding kinetics.

INTRODUCTION

The discovery of side effects in late-stage drug development or even after marketing is one of the leading causes of high drug attrition rates.[1,2] This contributes to both increased financial risks and health risks.[3] To reduce these risks side effects should be predicted in the earliest stages of drug development. This prediction requires a thorough understanding of the underlying mechanisms.

An important cause of side effects is a lack of tissue selectivity (i.e. differential drug effect on the same receptor in different tissues). Tissue selectivity of adenosine A_1-receptor ligands in mice has been quantified by Van Schaik *et al*. and Van der Graaf *et al*.[4,5] In these studies, the half-maximal anti-lypolytic effect was observed at lower ligand concentrations than the half-maximal haemodynamic effect. The authors propose the receptor concentration as the determinant of the observed tissue selectivity. The relation between receptor concentration and drug effect is intuitive and commonly assumed. However, this assumption is a simplification of the underlying system, since many other factors may influence tissue selectivity. These factors include differential blood flow, tissue partitioning and tissue-specific ligand depletion rates. Since the interactions between these processes have not yet been fully investigated, our understanding of tissue selectivity is limited and additional research is required.

In addition to the influence of receptor concentration on tissue selectivity, the influence of binding kinetics should be taken into account to predict how tissue selectivity changes over time. The potential role of binding kinetics in drug selectivity is supported by the increased recognition of binding kinetics as an important mediator of drug effect.[6–10] One of the main contributors to this recognition is the success of the residence time (RT) concept, which has been proven to be a better predictor of drug effect *in vivo* than drug-target affinity (K_D). RT is dependent on a binding kinetic parameter: the dissociation constant (k_{off}).

To include binding kinetics into the prediction of selectivity, target mediated drug disposition (TMDD) models can be applied.[11–14] The inclusion of target concentration in TMDD modelling makes it a useful tool for studying the influence of target concentration on drug-target binding. Using physiological target concentration values enables the development of a predictive model. These values can be extracted from online databases. Tissue specific drug distribution properties can be described by tissue specific parameters. For this cause, physiologically based pharmacokinetic (PBPK) models have previously been developed.[15] In PBPK models different compartments are assigned to specific organs/organ systems. Parameters such as blood flow and the tissue-blood partition ($P_{t:b}$) coefficient are used to define the physiological processes per tissue. $P_{t:b}$ describes the amount of ligand that is distributed into a certain tissue and can be predicted from the physicochemical properties of the ligand. These physicochemical properties can be predicted *in silico*.[16] Integrated TMDD and PBPK modelling forms a PBPK-TMDD model, which enables the simulation of drug-target binding in specific tissues.

In this study we combine the available resources to predict drug and receptor concentrations in different tissues and the binding to their targets. We use these combined predictions to derive new insights about tissue selectivity and compare our predictions to literature data.

METHODS

Overview

All simulations were performed in RStudio Version 0.99.893 - © 2009-2016 RStudio, Inc. Physicochemical parameters were predicted using Pipeline Pilot Version 9.0.2.1 Accelrys Software Inc., San Diego (2014).

Model

A schematic overview of the applied model is depicted in **Figure 1**. The interactions between the descriptive parameters were described by differential equations.

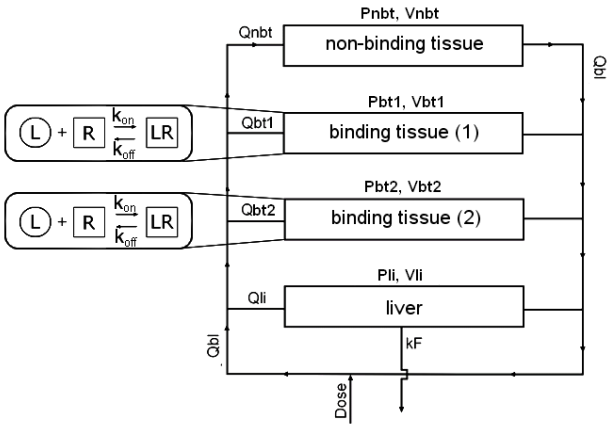

Figure 1. Schematic overview of the used PBPK-TMDD model. L = ligand concentration (nM), R = receptor concentration (nM), LR = ligand-receptor complex concentration (nM), k_{on} = association constant ($nM^{-1}h^{-1}$), k_{off} = dissociation constant (h^{-1}), Q = blood flow (L/h), V = tissue volume (L), nbt = non-binding tissue, bt = binding tissue, li = liver, P = tissue-blood partition coefficient, kF: forward rate constant of elimination (h^{-1}).

Parameters

$P_{t:b}$ was set to 0.95 for all tissues in all simulations. Blood flow in binding tissues ($Q_{bt1/2}$) was set to 20 L/h in all simulations. Binding tissue volume ($V_{bt1/2}$) was set to 1 L for receptor concentration (Rtot) simulations. For K_D simulations, one set of simulations was performed with $V_{bt1/2}$ set to 1 L and another with $V_{bt1/2}$ set to 20 L. kF was set to 100 h^{-1}. Dose was scaled so that similar receptor occupancies were obtained in all simulations.

Simulations

In order to investigate the influence of receptor concentration on tissue selectivity, simulations were performed using different values of Rtot for each of the two binding tissues. The simulations were performed for four different K_D values. Rtot and K_D values are specified in the figure legend and captions (**Figure 2**).

To further investigate the influence of binding kinetics on tissue selectivity, simulations were performed for different K_D values. These K_D values were obtained via different combinations of association constant (k_{on}) and k_{off}. Simulations were performed for K_D = 0.001, 0.01, 10, and 1000 nM. k_{off} values were set to 1, 0.1, 0.01, and 0.001 h^{-1} for all simulations. k_{on} values were then obtained by applying **Equation 1**. Values per simulation are displayed in the figure legends and captions (**Figure 3 and 4**).

$$(1) \qquad K_D = \frac{k_{off}}{k_{on}}$$

RESULTS AND DISCUSSION

For high affinities ($K_D = 1*10^{-5}$ or 0.001 nM) combined with low k_{off} rates (0.001 h^{-1}) (**Figure 2a and 2b**), Rtot influences the extent, but not the duration of receptor occupancy (RO). This results in tissue selectivity for the tissue in which Rtot is the lowest. As drug-target affinity decreases, the RO values in the binding tissues grow more similar, until from K_D = 1 onwards tissue selectivity is no longer observed (**Figure 2a-c**). When k_{off} increases for low drug-target affinity (K_D = 100), the duration of RO becomes more sensitive to differences in Rtot (**Figure 2d**). These results illustrate an important role for K_D as well as Rtot in determining the extent of both RO and tissue selectivity.

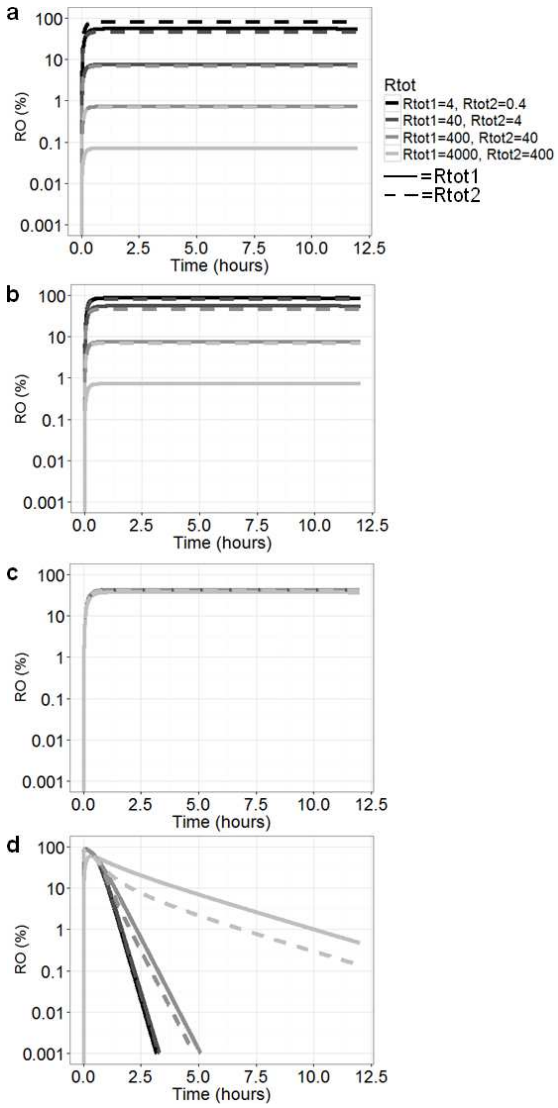

Figure 2. The influence of receptor concentration on receptor occupancy at different K_D values. a: $K_D = 1*10^{-5}$ nM, k_{on} = 100 $nM^{-1}h^{-1}$, k_{off} = 0.001 h^{-1}, b: K_D = 0.001 nM, k_{on} = 1 $nM^{-1}h^{-1}$, k_{off} = 0.001 h^{-1}, c: K_D = 1 nM, k_{on} = 0.001 $nM^{-1}h^{-1}$, k_{off} = 0.001 h^{-1}, d: K_D = 100 nM, k_{on} = 1 $nM^{-1}h^{-1}$, k_{off} = 100 h^{-1}.

In order to further investigate the influence of binding kinetics on RO, simulations were performed for different K_D values obtained via different combinations of k_{on} and k_{off} (**Figure 3**). In these simulations, selectivity was observed for the tissue with a low Rtot value (10 nM) when drug-target affinity is high (K_D = 0.001 or 0.01 nM) (**Figure 3a and 3b**). This selectivity decreases more rapidly for faster binding kinetics. However, the influence of binding kinetics decreases as drug-target affinity increases (**Figure 3a**). Selectivity for the tissue in which Rtot is the highest, even if only marginal, is observed when binding kinetics are fast and K_D is equal to the highest Rtot (**Figure 3c**).

The observations described above indicate that high drug-target affinity does not guarantee high tissue selectivity for the target tissue. In fact, quite the opposite seems to be true. The desired effect of a ligand at the receptor is most commonly targeted at the tissue in which the Rtot is the highest. Therefore, the lower the RO in the tissue with the lower Rtot, the better. In this study, higher RO was observed in a tissue with an Rtot of 0.01 nM than in a tissue with an Rtot of 10 nM for the higher drug-target affinities (**Figure 2 and 3**). This suggests selectivity for the off-target tissue rather than the target tissue.

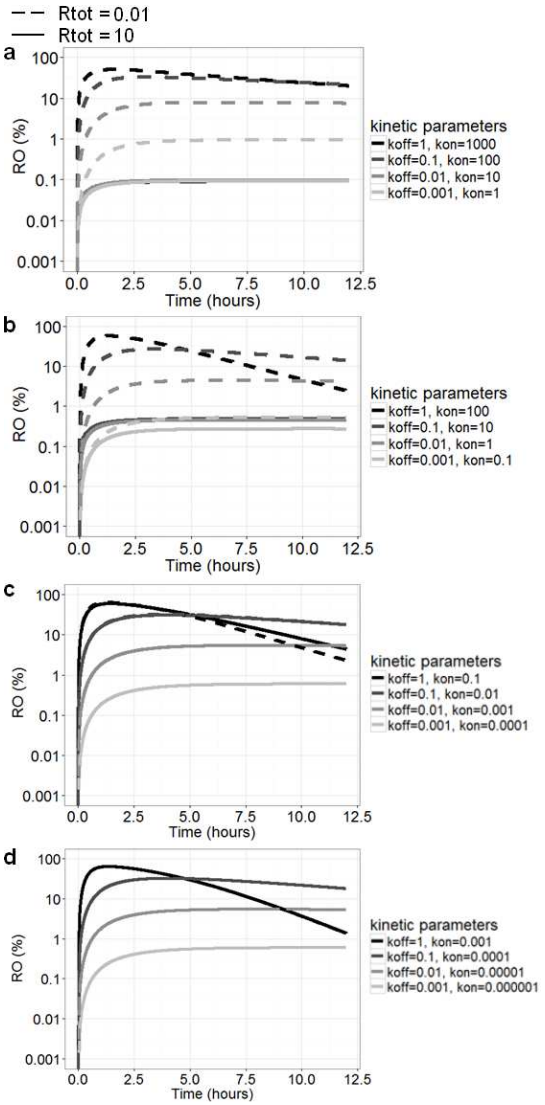

Figure 3. Increased receptor occupancy in tissues with lower receptor concentrations for ligands with a higher drug-target affinity as compared to ligands with a lower drug-target affinity. Q = 20 L/h, V = 20 L, k_{on} is in $nM^{-1}h^{-1}$, k_{off} is in h^{-1}. a: K_D = 0.001 (nM), b: K_D = 0.01 nM, c: K_D = 10 nM, d: K_D = 1000 nM.

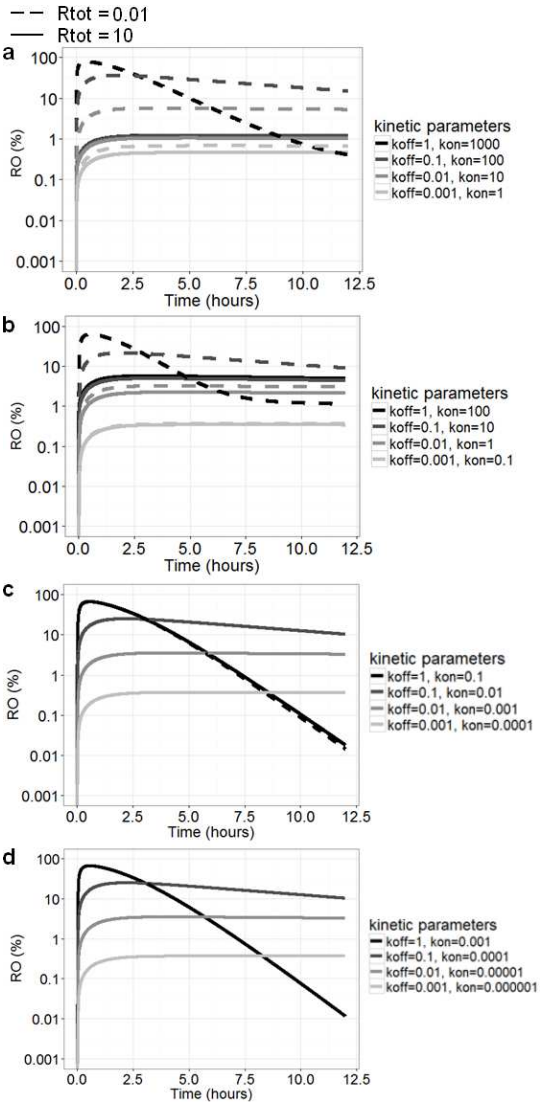

Figure 4. Influence of binding kinetics and receptor concentration on tissue selectivity when blood flow-tissue volume ratio is 20. Q = 20 L/h, V = 1L = 20, k_{on} is in $nM^{-1}h^{-1}$, k_{off} is in h^{-1}. a: K_D = 0.001 (nM), b: K_D = 0.01 nM, c: K_D = 10 nM, d: K_D = 1000 nM.

The ratio of Q_{bt}:V_{bt} was set to 1 in the simulations presented **Figure 3**. This value is lower than the values observed for most organs in the human body. Low Q_{bt}:V_{bt} values limit the elimination of ligand from the tissue, prolonging the period during which drug-target binding may occur. This leads to an increase in the extent and duration of RO. When this occurs, Q is rate-limiting for the decline of RO over time. This effect may be observed for all values of Rtot, but is most pronounced for low Rtot values, since high values of Rtot limit the distribution of drug out of the tissue.

In order to further clarify the influence of Q_{bt}:V_{bt} on tissue selectivity, the same simulations as presented in **Figure 3** were performed for a Q_{bt}:V_{bt} value of 20 (**Figure 4**). At this value, Q_{bt}:V_{bt} is expected not to have a rate-limiting effect on RO. Roughly the same pattern of RO was observed as in **Figure 3**, but there are a couple of notable differences. As expected, an accelerated decline of RO over time was observed, mainly for the tissue with the lower target concentration. This accelerated decline of RO and drug-target dissociation is most pronounced for faster binding kinetics (**Figure 4a and 4b**). This effect lasts until selectivity is reversed and is observed for the tissue with an Rtot of 10 nM.

The developed model was applied to the ligands studied by Van Schaick *et al*[4], in order to validate the predictive potential of the developed model and the relevance of this research's results. The required humane Rtot values were obtained from online expression databases, physiological parameters and K_D were obtained from literature, high dissociation (k_{off} = 10 h^{-1}) was assumed, and the $P_{t:b}$ values per humane tissue per ligand were predicted *in silico*.[4,17–24] The RO-based selectivity profile predicted by the developed model did not comply with the selectivity profile observed by Van Schaick *et al*.[4] However, when predicting the absolute ligand-receptor binding based selectivity profile, the predicted profile does comply with the effect based selectivity as observed by Van Schaick *et al*. This implies an important role for absolute receptor binding on *in vivo* drug effect, confirms the predictive potential of our model and confirms the relevance of our observed results.

Altogether, the results described in this article suggest that the quest for ligands with a high drug-target affinity at the receptor may not yield the safest and most efficacious therapeutic entities. In order to apply this new knowledge to the field of drug discovery, future research will be performed to quantify the influence of binding kinetics and

physiological features on tissue selectivity. It will be attempted to create easy-to-use formulas to enable prediction of tissue selectivity. Furthermore, the model will be validated for a set of ligands of which binding kinetic data at the receptor is available, as well as tissue specific receptor concentrations and receptor occupancy measurements. Ultimately, the goal of future research is the development of an integrated model in which *in silico* predictions of binding kinetic are used to inform the PBPK-TMDD model in order to increase predictability of tissue selectivity.[25]

CONCLUSION

A combined effect of physiological properties and binding kinetics on tissue selectivity was observed. Most notable is the observation that high drug-target affinity may result in lower target tissue selectivity. Moving against the current paradigm in which high drug-target affinity is considered desirable, this research triggers the further investigation of the exact role of binding kinetics in tissue selectivity.

ROLE OF THE STUDENT

This article is the result of H.C. (Anna) Vlot's final BSc research project, which was performed under the supervision of W.E.A. de Witte and Dr. G.J.P. van Westen at the Leiden Academic Centre for Drug Research. The topic was proposed by the supervisors and a general PBPK-TMDD model was provided by W.E.A. de Witte. The student formulated a research proposal, extended the basic model to incorporate physicochemical information, and performed all experimental work. The results were processed and interpreted by the student, who then formulated the conclusions, and wrote a report and this paper. All work was discussed with W.E.A. de Witte on a regular basis.

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