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Impact of target interactions on small-molecule drug disposition: an overlooked area

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The selection of an appropriate drug target, as well as a solid understanding of drug pharmacokinetics and pharmacodynamics, are crucial for successful drug discovery[1]. Although it is generally accepted that the free (unbound) concentration of a drug is the driving force for its pharmacological effects (as only free drug molecules are available to interact with the therapeutic target), there are several misconceptions regarding the impact of drug binding on pharmacokinetics[2]. It is important to understand that the binding of a drug to constituents of the blood or tissues does not alter the overall unbound drug concentration following chronic drug administration, as this is primarily determined by intrinsic clearance[2]. However, drug binding can highly influence the pharmacokinetic profile, owing to the effects on the volume of distribution (*V*) and total drug clearance (*CL*), which are based on measurements of the total plasma concentration.

The major nonspecific drug-binding constituents are albumin (which is found in the blood and interstitial fluids) and acidic phospholipids (which are predominantly found in the tissues). Although drugs have only moderate affinity (potency) for these constituents, the impact of these constituents on drug pharmacokinetics is high because they are present at high concentrations. However, the impact of the drug binding to the target itself is often not discussed, although the affinity of drugs for their targets will often be much higher than the affinity of drugs for nonspecific blood and/or tissue constituents. Consequently, many small-molecule drug discovery projects are mounted with a firm biological rationale for the target, yet have little knowledge of the actual concentration of this protein, whether in the initial *in vitro* screens, in the subsequent *in vivo* animal studies, or in healthy subjects or patients. The potential consequences of this lack of knowledge can range from unreliable conclusions about compound potency to unusual and even highly variable pharmacokinetics in humans. In this article, we briefly highlight the underappreciated impact of target-mediated drug disposition (TMDD)[3] on small-molecule drug discovery, as well as approaches to address this issue.

Impact of TMDD

Impact on* in vitro *studies. Nowadays, a large proportion of early discovery assays overexpress the pharmacological target to create a viable *in vitro* assay. The general assumption is that the concentrations of target proteins are considerably lower than the affinity (equilibrium dissociation constant (K_d)) of the compounds being tested. With nanomolar and even picomolar affinities being routinely achieved in drug discovery, however, this assumption may be incorrect. As such, with a fixed high concentration of receptor in the assay, the apparent affinity of compounds will reach a lower limit approximating to the concentration of receptor. Thus a series of compounds with a range of potencies would appear equipotent if their affinity values were screened in an assay with a receptor concentration greater than those values. Clearly, this may mean underestimation of compound affinity and selectivity, as well as overestimation of the predicted clinical dose. Similarly, it could result in futile lead optimization cycles with the same or different chemical series.

Impact on* in vivo *studies. *In vivo*, different considerations are relevant for highly expressed targets. The binding of the compound to its target can have a major influence on both the distribution and the *CL*, and can make a large contribution to nonlinear pharmacokinetics. Although such TMDD is typically considered for large-molecule drugs such as monoclonal antibodies, it is often overlooked in small-molecule drug research. In many cases, this is not an issue, as there is indeed little influence of the target on the overall distribution of small molecules, because the mass of the targets comprises only a tiny fraction of the mass of the body constituents that bind the drug. However, for some small-molecule drugs, the target represents a higher proportion of these body constituents and thus the effect on the pharmacokinetics can be dramatic.

Typically, the impact of a target on the pharmacokinetics of a drug depends on: the affinity of the drug for the target; the total binding capacity of the target within the body; the affinity and binding capacity of other competing body constituents; the location of the target; and the dose and corresponding concentration of the drug. Some of these factors are interdependent, but they can be categorized into broad effects.

- If the target is tissue-based and is a major binding constituent within the body, it will influence local tissue distribution and *V* globally, but will have no effect on *CL*. Typically, *V* will decrease with increasing dose as a result of the saturation of target binding sites, and the half-life of the drug will decrease proportionally.
- If the target is plasma-based, saturation of the target with increasing dose will result in lower plasma binding, which will be particularly marked when the target is the major binding protein. This will typically be associated with an increase in both *V* and *CL* with a change in half-life, unless *V* and *CL* change to the same extent. For plasma-based (or blood-based) targets, there is a high probability that some influence of target binding will be observed *in vivo*. The influence of the target may be missed in *in vitro* studies unless plasma protein binding-type assays are conducted over a concentration range commencing at a value below the K_d of the drug for its pharmacological target.
- When the target is the predominant binding constituent in the body (or in the blood) and the therapeutic unbound drug concentration approximates to the target concentration, the pharmacokinetics of the drug will show pronounced nonlinearity over relatively small dose increments due to saturation of target binding. Multiple dose studies at doses giving rise to similar concentrations as the target may demonstrate this aspect of nonlinearity as an unanticipated accumulation profile of the drug.
- Given that relatively high doses are often used in preclinical pharmacokinetic and toxicology studies, it is likely that saturation of the target will prevail at this stage and obscure TMDD, meaning that TMDD may first be encountered during clinical development.

Of course, other processes such as saturation of metabolism or transporter-dependent clearance may contribute to nonlinearity in addition to target binding, which will add complexity to interpretation. Several examples of small molecules that exhibit substantial TMDD have been reported, including

the endothelin receptor antagonist bosentan, the angiotensin-converting enzyme inhibitor enalaprilat, the aldose reductase inhibitor imirestat, the dipeptidyl peptidase 4 inhibitor linagliptin, the monoamine oxidase B inhibitor selegiline and the vitamin K epoxide reductase inhibitor warfarin[4]. A recent example is the potent 11β-hydroxysteroid dehydrogenase type 1 inhibitor ABT-384, which showed marked nonlinear pharmacokinetics in humans that was attributed to TMDD[4]. At low doses (1, 2 or 4 mg) ABT-384 appeared to have a higher *V* than at higher doses (8, 30 or 100 mg). Of note, after repeated dosing, disproportionately higher accumulation of ABT-384 occurred at the lower doses than predicted based on half-life and dosing frequency, but not at the higher doses. These results are consistent with the notion of a strong influence of the target on the pharmacokinetics, which becomes particularly obvious at low doses. Once the target is saturated by administration of a high dose or multiple low doses, the pharmacokinetics are again linear. However, depending on the extent of target saturation, the influence on the pharmacokinetics can be highly complex[5]. A simulation illustrating the effects of TMDD for a hypothetical small molecule is shown in FIG. 1.

Impact on microdosing studies. TMDD also has implications when considering human microdosing approaches, which are increasingly being used in cases where the extrapolation of pharmacokinetics from animals and *in vitro* measurements to humans is problematic. Such studies can yield valuable information regarding human pharmacokinetic properties at an early stage in drug development, but success depends on pharmacokinetic linearity, which is violated when TMDD occurs. For example, warfarin is very highly bound to albumin but even more highly bound to its high-affinity, low-capacity therapeutic target vitamin K epoxide reductase. Accordingly, due to TMDD, warfarin has a much larger volume of distribution and longer half-life following a microdose compared with a therapeutic dose[6]. This clearly indicates TMDD can confound microdosing studies, potentially leading to incorrect expectations for the pharmacokinetics at therapeutic doses.

Understanding the potential for TMDD

In order to gain a better understanding of the potential for target-mediated effects on small-molecule drug pharmacokinetics, several preclinical and clinical activities can be considered.

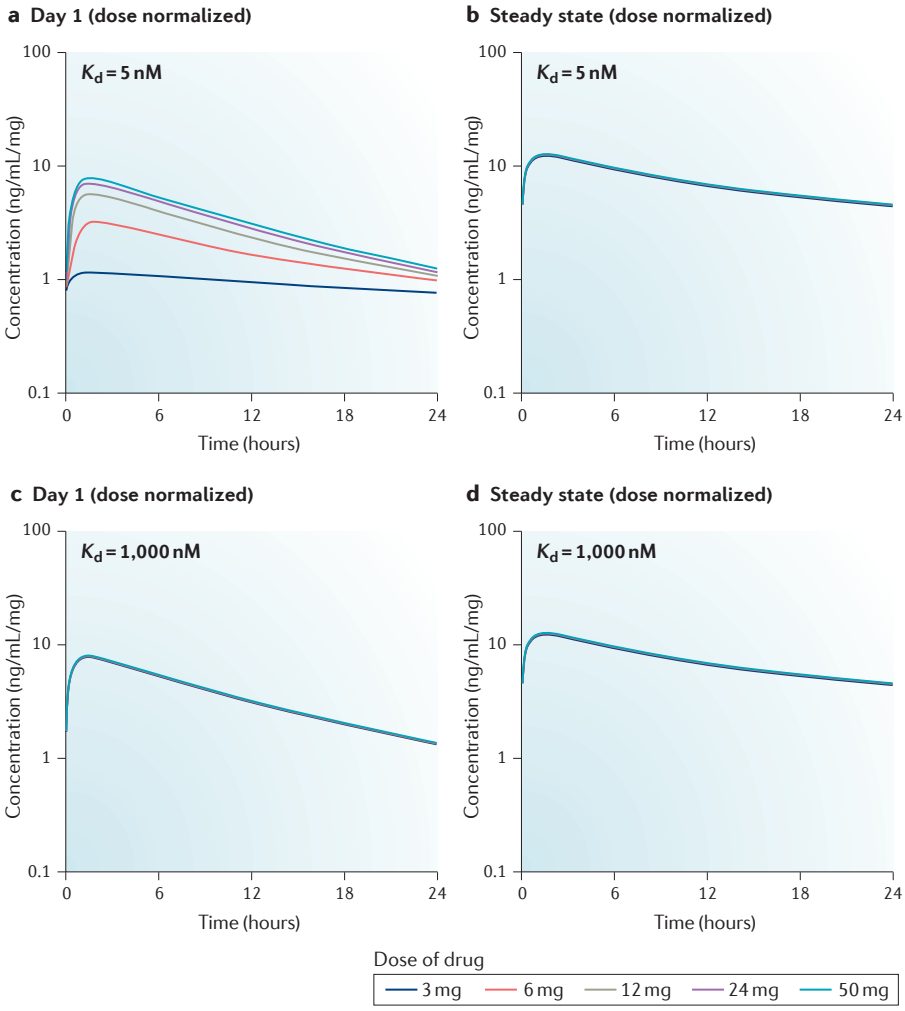

Figure 1 | **A simulated example of target-mediated drug disposition.** The top panels of the figure illustrate the pharmacokinetic consequences following single-dose (part **a**) and repeated-dose (part **b**) administration of a hypothetical small-molecule drug that has a high affinity (dissociation constant (K_d) = 5 nM) for a tissue-based target that results in target-mediated drug disposition (TMDD). Dose-normalized plasma concentration–time profiles are shown. Increasing the dose of the drug from 3 to 50 mg causes saturation of the target, resulting in a marked reduction in the volume of distribution (*V*), and a corresponding decrease in half-life ($t_{1/2}$); as clearance is unaffected, this translates into clear dose-nonlinearity on day 1 (part **a**). After multiple administrations, the plasma concentration at steady state, being dependent on clearance, shows no evidence of nonlinearity (part **b**). The lower panels of the figure illustrate the same protocol for a less potent compound (with a 200-fold higher K_d), for which TMDD and changes in *V* are not observed (parts **c**,**d**).

- Knockout (and knock-in) mouse studies. These are an elegant way of demonstrating whether the target can have a profound effect on the pharmacokinetics[7].
- Co-administration of a selective inhibitor for the pharmacological target. This will typically result in a decrease in *V* with no effect on *CL* with increasing dose of the inhibitor when the target is expressed in tissues, and an increase in both *V* and *CL* when the target is primarily expressed in blood[8].
- Determining tissue to plasma partition after a dose-ranging study in preclinical species. For tissues in which targets are highly expressed, the tissue/plasma ratio is expected to decrease with increasing dose[9].
- Comparison of single with multiple ascending doses. This provides further insight into TMDD as well as ruling out other sources of nonlinear pharmacokinetics, such as saturation of clearance mechanisms. Typically, the nonlinear pharmacokinetic effects will be diminished after repeated dosing (FIG. 1).
- Radioactive tracer studies. For example, the half-life of a tracer dose of ^{14}C-warfarin was substantially shortened when co-administered with a high dose of

unlabelled drug that saturated the target, owing to a substantial decrease in V[10].

- Intravenous studies. These are most suited to assess TMDD, as after oral administration, certain absorption and first-pass clearance processes could mask TMDD.
- *In vitro* binding assays. For plasma-based targets, *in vitro* binding assays for the drug conducted over the therapeutic concentration range can indicate whether any nonlinear effects are occurring.

Outlook

TMDD for small molecules has not received much attention and often only becomes apparent during clinical trials, with the potential for data misinterpretation. TMDD can have a major influence on drug distribution and sometimes clearance, resulting in unpredictable, nonlinear pharmacokinetics. As such, consideration of TMDD is crucial for meaningful interpretation of clinical data and could have an impact on the probability of success in drug discovery and development, particularly with the drive for increasing potency of drugs. Based on recent reports[4] as well as observations at Roche, we consider TMDD for small molecules to be more common than is appreciated and it might in fact often be overlooked. For drugs where it is indeed important, pharmacokinetic and pharmacodynamic modelling and simulation can provide useful insights as to the appropriate dosage regimen.

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doi:10.1038/nrd.2018.26
Published online 23 Feb 2018

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Competing interests
The authors declare competing interests: see Web version for details.