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REVIEW

Evolution of the drug-target residence time model

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ABSTRACT

Introduction

The pharmacological action of a drug is linked to its affinity for a specific molecular target as quantified by in vitro equilibrium measurements. However, it is clear that for many highly effective drugs, interactions with their molecular targets do not conform to simple, equilibrium conditions in vivo and this results in a temporal discordance between pharmacokinetics and pharmacodynamics. The drug-target residence time model was developed to provide a theoretical framework with which to understand cases in which very slow dissociation of the drug-target complex in vivo results in durable PD effects even after systemic concentrations of drug have waned.

Area covered

In this article, the author provides a brief description of the drug-target residence time model and focuses on the refinements that have been made to the original model to incorporate the influences of compound rebinding in cells and pharmacokinetic properties of drug molecules.

Expert opinion

There is now overwhelming evidence for the utility of the drug-target residence time model as a framework for understanding in vivo drug action. The in vitro measured residence time (τ_R) must be used in concert with equilibrium measures of drug-target affinity (e.g. IC_{50}) and with in vivo measures of pharmacokinetic half-life, to afford the researcher a powerful approach to compound optimization for clinical effect. Despite the significant use and refinement of this model, continued studies are required to better understand the dynamic interplay between residence time, target pathobiology, drug distribution and drug pharmacokinetics.

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Residence time; PK/PD relationship; durable pharmacology; drug discovery; drug optimization; drug selectivity

1. Introduction

> *… these [drugs] and similar bodies, in producing their specific effects form reversible chemical combinations with certain constituents – receptive substances – of the cells*
>
> • Archibald Vivian Hill, 1909

With these prophetic words, A. V. Hill – one of the founders of modern biochemistry and biophysics – ushered in a new era in our understanding of biological chemistry and molecular pharmacology [1]. The idea that metabolites and drug molecules engender their physiological effects by reversible binding to specific targets (i.e, receptors and enzymes – the receptive substances in Hill's vernacular), had already been introduced by Langley [2] and independently by Ehrlich [3], but Hill's work in 1909 and that of many others to follow [4], put the receptor theory of drug action on a firm experimental foundation.

These studies culminated in the now-familiar concept that in vivo target occupancy is the progenitor of all specific, pharmacodynamic effects (both desired, beneficial effects and sometime, untoward side effects as well). For most of the past century, drug discovery researchers have made two common assumptions, based on this foundational concept. First, that measures of in vitro drug-target affinity under equilibrium conditions (e.g. K_d, IC_{50}, EC_{50} and the like) are reasonable portents of in vivo target occupancy within the open system of the human body. Second, that the degree of target occupancy within the tissues of the body is temporally determined by the concentration of drug in systemic circulation, so that the time courses of drug pharmacokinetics (PK; i.e. the time course of drug concentration in plasma or whole blood) and of pharmacodynamics (PD; i.e. the pharmacological action of the drug) are for the most part coincident, as illustrated in Figure 1(A). These assumptions are supported by experimental results for many compounds. However, there are also numerous examples for which these assumptions break down, especially for many highly effective drugs.

In a number of cases, one observes a situation in which the PD effects of the drug continue long after systemic concentration of the drug has waned, as illustrated in Figure 1(B). If tissue target occupancy is indeed the antecedent of pharmacodynamics, the data in Figure 1(B) would indicate that in some cases the duration of target occupancy can be sustained well beyond the timeframe in which the systemic concentration of drug is above some pharmacological threshold. Swinney [5], in a landmark analysis of drug discovery success, made the point that many of the most effective drugs deviate from the simple one-step binding, one-step dissociation model of drug-target equilibrium. This is an important, yet

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often overlooked aspect of the proper analysis of in vitro drug-target affinity. Nevertheless, the analysis by Swinney does not offer a full explanation for the temporal disconnect between PK and PD that is illustrated by Figure 1(B). How then, might one rationalize such behavior of drugs in vivo?

To answer this question, one must think about in vivo target occupancy not only in terms of the strength of interaction (i.e. the affinity of the drug-target binding) but also in terms of the durability of the binary drug-target complex. This mind-set reflects the assumption that the duration of PD effects will be directly related to the sustainability of target occupancy – i.e. on the lifetime of the binary drug-target complex. This seems quite reasonable in the case of a pharmacological agent that inhibits the biological function of its target. In this situation, inhibition is only maintained while the binary inhibitor-target complex is intact. Once the inhibitor leaves the target, the target molecule is free to once again resume its biological function [6]. Although less obvious, a similar situation must occur when pharmacology is the result of target agonism; that is, the binary agonist-target complex must endure beyond some minimum lifetime, to ensure that the downstream effects of target agonism are realized within the cell. Viewing the problem through this lens led to our formulation of the drug-target residence time model [7–15], which is the topic of this review.

In the following pages I will briefly review the salient points of the model as originally stated, clarify some points that have been raised in the recent literature and describe recent studies that have helped to refine and expand this model. I shall also discuss recent applications of the model to drug discovery efforts that have been reported over the past five years, since our last major review of the model [14].

2. The drug-target residence time model

As originally formulated [7], the drug-target residence time model posits that the duration of in vivo PD is determined by the lifetime, or residence time (τ_R), of the binary drug-target complex. If we consider a typical in vitro experiment, in which a fixed concentration of drug molecules is mixed with a fixed concentration of target molecules, in a fixed volume of solution, the formation of a binary drug-target complex – in the simplest model of interaction – is determined by two kinetic processes: the rate of binding or association of the free drug with target (quantified by the rate constant k_{on}) and the rate of binary complex dissociation (quantified by the rate constant k_{off}). When the system reaches equilibrium, the strength of binding (i.e. the affinity) of the drug for its target is commonly quantified by the thermodynamic equilibrium dissociation constant, K_d (in Molar units) which is defined as the ratio of k_{off} (in units of s^{-1}) over k_{on} (in units of M^{-1} s^{-1}), as shown in Figure 2(A). Note that the rate of drug binding to its target is determined both by the value of k_{on} and by the concentration of drug present. The rate of binary complex dissociation, in contrast, depends only on k_{off}. The K_d value thus quantifies how tightly the drug binds to its intended target, but it does not give any information on the lifetime of the complex under nonequilibrium conditions.

If we now consider the fate of an enterally administered drug, we come to realize that a number of macroscopic processes (e.g. gastrointestinal absorption and tissue distribution from systemic circulation, both of which occur at much slower rates than that of typical bimolecular association) impede the rate at which a drug molecule reaches its intended, tissue-localized target. Additionally, drug metabolism and elimination processes continuously diminish the concentration of total drug within the body over time. Hence, in vivo, equilibrium conditions do not apply to drug-target interactions; instead, the concentration of drug impinging on its target is in constant flux. Under these conditions, it is the kinetic rates of binding and dissociation, rather than the equilibrium constant, that determine the duration of target occupancy in the tissue – i.e. the duration of drug residence on its target.

As we shall see in the next section, both k_{on} and k_{off} contribute to the overall residence time of the drug-target complex in cells. However, Copeland et al. have argued [7] that the best in vitro surrogate for intracellular drug-target complex lifetime is the reciprocal of k_{off}, which is defined as the residence time τ_R (more correctly, the in vitro residence time). Copeland et al. [7] emphasized the dissociation process

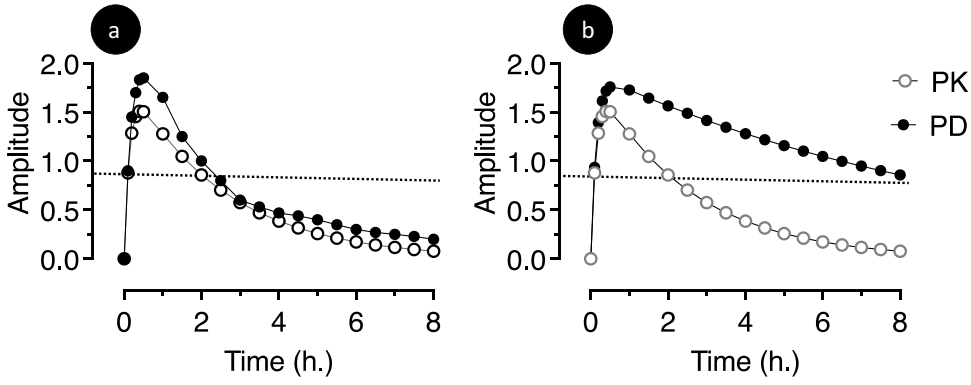

Figure 1. The temporal relationships between pharmacokinetics and pharmacodynamics for enterally administered drugs. **A**. Typical temporal relationship between the systemic concentration of drug (PK: open circles) and pharmacodynamic response (PD: black circles). **B**. The atypical situation in which a durable PD response continues even after drug concentration has waned from systemic circulation. Symbols as in **A**. In both graphs, the dashed, horizontal line indicates the threshold concentration of drug in systemic circulation required for significant target occupancy (TO), based on equilibrium measures of PD response in cells (typically, this threshold corresponds to the concentration of plasma protein-unbound drug at the IC_{90} of cellular PD response, but the actual threshold varies with target and disease state under study).

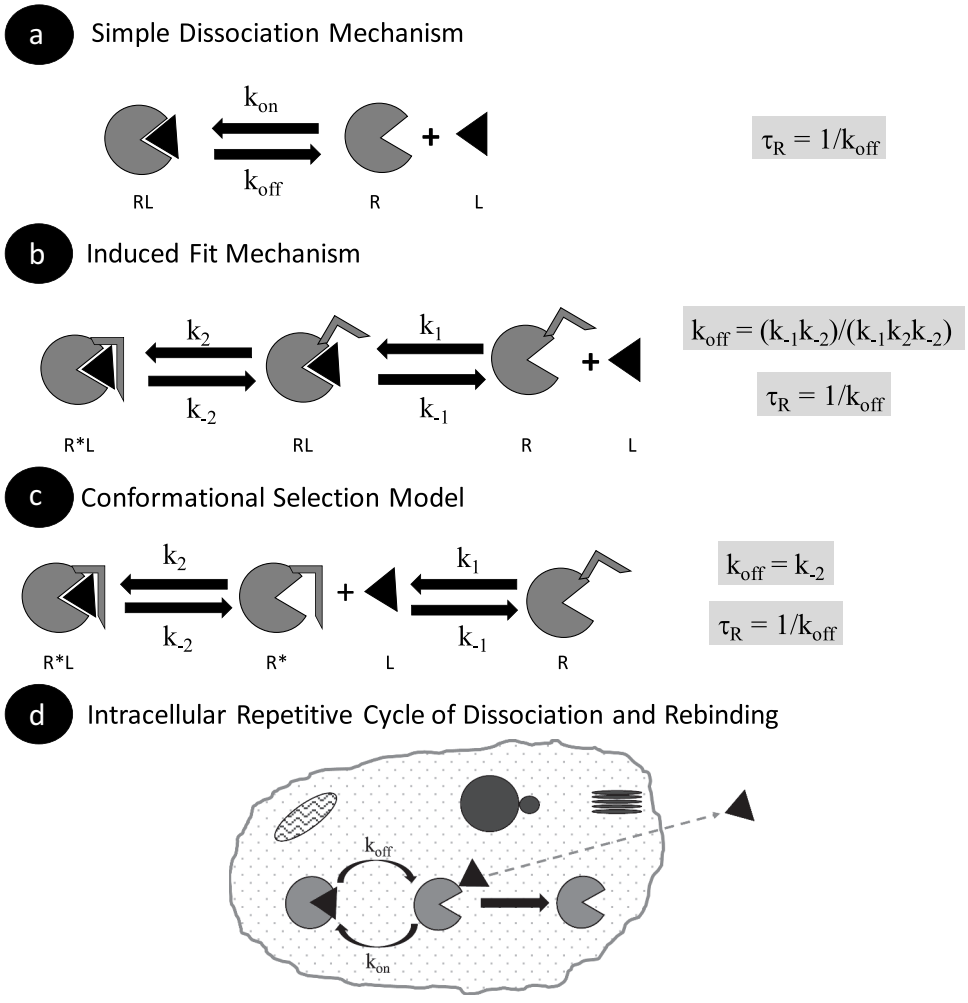

Figure 2. Dissociation mechanisms for drug-target complexes. **A**. The simple one-step binding and one-step dissociation mechanism. **B**. The induced fit mechanism in which the target isomerizes from a high drug affinity conformation in which the drug binding pocket is occluded from solvent, to one of lower drug affinity in which the binding pocket is now open to solvent, prior to dissociation of the drug molecule from the complex. **C**. The conformational selection mechanism in which drug dissociates from the high affinity, solvent occluded conformational state and the unbound target equilibrates between this conformational state and one in which the drug binding pocket is more solvent accessible. **D**. Cartoon illustrating a repetitive cycle of slow drug dissociation and rapid rebinding that significantly extends the intracellular residence time of the drug-target binary complex before the diffusional escape of drug molecule from the cell. In all of these illustrations the target is represented by a dark gray shape and the label R (for Receptor), the drug molecule is represented by a black triangle and the label L (for Ligand) and the mathematical definitions of residence time (τ_R) and k_{off} for mechanisms **A-C** are provided.

(over the association process) in defining drug-target residence time for three reasons. First, the molecular determinants of k_{on} (e.g. drug molecule desolvation, molecular orbital steering, etc.) do not lend themselves well to systematic modulation by medicinal chemistry, whereas the molecular determinants of k_{off} are mostly the same features (e.g. hydrogen bonding, π-π interactions, π-cation interactions, log P, van der Waals forces, and the like) that medicinal chemists are familiar with and know how to systematically modify through structure-activity relationships (SAR). Additionally, the rate of diffusion-controlled molecular encounters between a small molecule ligand (e.g. a drug molecule) and a macromolecular target (e.g. a protein) enforces a strict upper limit on the rate of compound association with its target (for small molecule ligand -protein interactions this limit is approximately 10^9 $M^{-1}s^{-1}$). In fact, a fast on-rate may not always be optimal for target occupancy, when drug binding and dissociation are both mediated by a slow conformational change of the target [5,6,11], as illustrated in Figure 2(B, C).

Second, surveys of the kinetics of bimolecular complex formation for high-affinity natural and synthetic ligands suggested that the value of k_{off} plays a predominant role in controlling the potency and selectivity of interactions (but see later studies summarized below). For example, in most of the cases examined, mutations within a receptor molecule that altered high affinity drug interactions did so by altering the value of k_{off}, with little change in k_{on} [8,14]. This trend was also observed for drug interactions with resistance-inducing mutants of target proteins and among compounds within a pharmacophore series binding to a common target molecule [14,16,17]. Hence, it seemed that in many cases, k_{off} – and by extension, residence time – is a predominant driver of drug-target affinity as well as the durability of the binary complex; this view has evolved with further studies of large compound libraries, as discussed later in this review. Third, from a pragmatic perspective, measuring the off-rate of a drug

from a target can be accomplished by a broad range of experimental methods that are available to the drug discovery researcher [6], while measurements of k_{on} often require more specialized methods (e.g. radiometric or fluorescent labeling of ligands, SPR and the like) [18]. For example, the impact of drug binding on the biochemical or biological function of a target can be rapidly reversed by large dilution of drug concentration, in cell-free experiments by 'jump-dilution' [19] or in cellular experiments by 'washout.' The accompanying change in target function with time after dilution can then be used to quantify k_{off} and residence time. Hence, as a practical matter, the researcher can quite readily determine measures of thermodynamic affinity (e.g. IC_{50}) and residence time for large numbers of compounds during the course of a drug discovery campaign.

Thus, according to this model, if one had two compounds that bind to a common target with similar affinity and similar PK properties, the compound with the longer residence time would show the most durable PD in vivo. This expectation has been experimentally realized multiple times, across a broad range of compounds and targets in the contemporary literature (see below).

Three important (and interdependent) points are often overlooked in studies of drug-target residence time, and these should be borne in mind when attempting to rationalize cellular and in vivo data by this model. First, as championed by Swinney [5] and others [6,11,14] target conformational dynamics almost always play a critical role in high affinity, highly effective drug-target interactions. Figure 2(A-C) illustrate three common mechanisms for dissociation of a drug-target binary complex to yield the free target and drug (in this figure R is used to represent the target or receptor, L to represent the drug or ligand, and R* to represent an alternative conformation of the target with highest affinity for the drug). Figure 2(A) illustrates the simplest case where complex dissociation occurs in a single step and drug association with its target also occurs in a single step. This model assumes no conformational alterations of the target protein attending formation of the drug-target complex. The model is attractive because of its simplicity and the ability to relate just two kinetic rate constants (k_{on} and k_{off}) to the thermodynamic constant K_d ($K_d = k_{off}/k_{on}$). Despite the attractiveness of its simplicity, this model is seldom representative of how effective drugs interact with their targets. In the majority of cases that have been studied, high affinity drug-target complex formation involves some conformational alterations to the target protein. Two extreme models of receptor-ligand binding have been put forth: the induced fit model (related to the dissociation pathway shown in Figure 2(B)) and the conformational selection model (related to the dissociation pathway shown in Figure 2(C)). These two models both result in the final, high-affinity binary complex R*L, but they differ in the rate-limiting step to final complex formation. In the induced fit model, the ligand binds rapidly to state R of the receptor (see Figure 2(B)) and then induces a slower conformational change from RL to R*L. In the conformational selection model (Figure 2(C)), the free receptor slowly interconverts between states R (which does not bind ligand) and the ligand-binding state R*. Once the R* state is populated, ligand binding to this conformational state occurs rapidly. In reality, the trajectory of ligand binding/dissociation to/from a receptor likely reflects some intermediate form that displays aspects of both the induced fit and conformational selection models (but see below). Second, models of drug association (binding) with a target may be useful in considering the onset of PD (although, as discussed above, this is usually delayed by macroscopic rates of absorption, distribution and the like), but they are not always appropriate for defining the duration of drug residence on a target. For this latter objective, one must shift one's view to focus on the trajectory of drug-target complex dissociation. For example, one can ask what is the nature of the conformational rearrangements that attend the shift from state R to state R* in Figure 2(B, C). In the majority of cases studied, this conformational shift involves movements of loops or other elements of protein structure to create a more solvent occluded binding pocket for the drug. A classic example of this is the flap closing of HIV-1 protease to occlude the enzyme active site from bulk solvent upon inhibitor (and presumably substrate) binding. Such conformational shifts, that occlude the binding pocket from solvent, create a steric barrier to compound dissociation. This barrier is relieved in the induced fit model by the conformational shift from R*L to RL. It is difficult, however, to structurally rationalize a drug escape trajectory for the conformational selection model, as this model anticipates drug dissociation directly from the occluded R*L conformational state. Finally, it is critical to remember that equilibrium measures of drug-target interactions are not germane to the open system of in vivo biology, as discussed above. Despite the essentially universal agreement that open system biology represents a non-equilibrium system with respect to drug concentration and drug-target interactions, the literature is replete with examples of attempts to describe in vivo target occupancy in terms of equilibrium interactions and use of the simple, but inappropriate, 1-step model of drug binding and drug-target dissociation, illustrated in Figure 2(A). For example, numerous research articles calculate K_d, k_{on}, or k_{off} from two of the three values and use of the simplifying equation $K_d = k_{off}/k_{on}$. In some instances, this may be the only way to get a reasonable estimate of the three parameters for large numbers of compounds, but researchers need to be aware of the caveats of such calculations for conformationally dynamic targets and should make these caveats explicitly clear in their published works.

3. Evolution and refinement of the drug-target residence time model

There has been a remarkable response to the drug-target residence time model, with over 1200 articles published to

date that cite the original description of this model [7]. Table 1 provides some examples of compounds that display long drug-target residence times. From these varied studies, it is clear that a prolonged drug-target residence time can lead to potent and durable intracellular and in vivo PD effects (reviewed previously in [14] and references therein). Several refinements and challenges have been raised with respect to the original formulation of this model [19–22]. These can generally be categorized as follows: (1) drug-target residence time needs to be considered in the context of the PK half-life of compounds; (2) the original model does not adequately account for the impact of target saturation on target occupancy; and (3) the original model largely ignores the importance of k_{on} in determining intracellular duration of target occupancy. All of these points have merit and have helped to refine the details of the model.

3.1. Durable pharmacodynamic effects in cells

The drug-target residence time model anticipates that the durability of cellular PD and phenotypic effects, as measured by washout experiments, would be correlated with the value of τ_R measured for the cell-free drug-target binary complex [7,14]. Indeed, this expectation has been realized for a broad range of compounds and targets, but a general trend of longer intracellular residence time than expected (from the cell-free value of τ_R) has also been routinely observed.

Table 1. Some examples of compounds displaying long residence times.

Compound	Target	Residence Time (h.)
Amlodipine	L-type calcium channel	0.6
Triclosan	*F. tularensis* enoyl-ACP reductase	0.67
Oseltamivir (Tamiflu)	Viral neuroaminidase	0.8
Telmisartan	Angiotensin II receptor 1	1.3
Roniciclib	Phospho-CDK2/cyclinA	2.1
Candesartan	Angiotensin II receptor 1	1–3
DADMe-Imm-G	Human PNP	2–3
Efavirenz	HIV-1 reverse transcriptase	4.1
Geldanamycin	HSP90	4.6
4-hydroxytamoxifen	Estrogen receptor	4.7
Allopurinol	Xanthine oxidase	5.0
Saxagliptin	Dipeptidyl-peptidase IV	5.1
Desloratidine	Histamine H1 receptor	>6.0
Lapatinib	EEBB2/EGFR	>7.0
GSK1070916A	Aurora B/C kinases	>8.0
Rofecoxib	Cyclooxygenase 2	9.0
Maraviroc	CCR5 receptor	13.9
Tiotropium	Muscarinic M3 receptor	34.7
Finasteride	Steroid 5a-reductase	>40
Deoxyconformycin	Adenosine deaminase	40
Rivastigmine	Acetylcholinesterase	>48
Ispinesib	Kinesin spindle protein	>50

For example, Wood et al. [23] were among the first to demonstrate a clear correlation between off-rate (the reciprocal of τ_R) and duration of intracellular PD effect (recovery of receptor phosphorylation after washout) among inhibitors of the epidermal growth factor receptor tyrosine kinases (EGFR). As summarized in Table 2, these workers compared three now-FDA approved EGFR inhibitors that are used in the treatment of cancer patients: lapatinib, gefitinib and erlotinib. Subsequently, Willemsen-Seegers et al. [24] studied the association and dissociation of these three drug-EGFR complexes by surface plasmon resonance (SPR). Although all three inhibitors bind EGFR with similar affinity (Table 2), their residence times differ dramatically, and this latter parameter correlated well with the duration of intracellular PD (Table 2). One very clear observation from the data in Table 2 is that the intracellular residence time for all three EGFR inhibitors is considerably longer than one might expect on the basis of the values of τ_R measured in cell-free biochemical or biophysical assays. This trend has been observed across a broad range of compounds and targets, and it is likely related to the influence of compound rebinding, driven by the value of k_{on}, on the overall drug-target residence time within cells.

Although the original drug-target residence time model focused almost exclusively on the dissociation rate constant, k_{off}, it has become increasingly clear that the intracellular residence time of a drug-target complex can be greatly extended by a repetitive cycle of slow complex dissociation and rapid compound rebinding (Figure 2(C)). The importance of compound rebinding in extending intracellular drug-target residence time has been championed by Georges Vauquelin and colleagues (e.g. [25,26],).

One of the clearest demonstrations of the role of drug rebinding in extending intracellular residence time comes from study of the transition state analogue DADMe-Immucillin-H (DADMe-ImmH) inhibiting purine nucleoside phosphorylase (PNP) activity in human erythrocytes [27]. PNP occurs as a homotrimer with strong anti-cooperative inhibitor binding among the three active sites, such that binding of inhibitor to one subunit reduces the affinity of the inhibitor for binding to the active sites of the second and third subunits. In vitro, binding of DADMe-ImmH to PNP and then jump-dilution at 37°C results in only partial restoration of enzymatic activity, presumably due to dissociation of inhibitor from the two weaker-binding subunits, with a residence time of about 12 minutes ($t_{1/2}$ = 8.3 minutes). Despite this modest residence time, one observes prolonged inhibition of PNP within erythrocytes upon washout. Gebre et al. [27] observed a biphasic release of ^{14}C-labeled DADMe-ImmH from human erythrocytes

Table 2. Biochemical, biophysical and cellular data for three FDA-approved EGFR tyrosine kinase inhibitors. Data from references [23] and [24].

Drug	$K_i^{apparent}$ (nM)[a]	K_d (nM)[b]	k_{on} ($M^{-1}s^{-1}$)[b]	k_{off} (s^{-1})[b]	τ_R (min.)[b]	Time to 50% Recovery of Cellular PD[a, c]
Erlotinib	0.70	0.70	4.74×10^6	3.31×10^{-3}	2.2	<1 day
Gefitinib	0.40	1.40	5.46×10^6	7.63×10^{-3}	5.0	~2.6 days
Lapatinib	3.00	0.42	1.11×10^5	4.70×10^{-5}	354.0	≫ 4 days

[a]Data from Wood et al. [23].
[b]Data from Willemsen-Seegers et al. [24].
[c]HN-5 cells (squamous cell carcinoma cell line) were treated with 1 μM of each drug for 4 hours prior to washout of drug. PD refers to the recovery of EGFR tyrosine phosphorylation. Time to 50% recovery of vehicle (DMSO) treated cell EGFR tyrosine phosphorylation levels was estimated by interpolation from the data graphs presented in [23]. For lapatinib, <20% recovery of PD was observed over the entire 4 day period of post-washout evaluation.

upon washout, with half-lives of 48 minutes and 84 hours (Figure 3). They inferred that the rapid phase of radioligand release corresponded to drug dissociation from the two weaker-binding subunits of the PNP homotrimer and that the slower phase represented drug dissociation from the tighter-binding subunit. However, considering the rapid phase of radioligand release, this represents a ca. 4-fold longer duration of residence time relative to what was observed in the cell-free enzyme assay. These researchers then repeated the erythrocyte washout experiment in the presence of a large excess of unlabeled DADMe-ImmH (300 μM) to prevent radiolabeled drug from rebinding to PNP after dissociation. In the presence of the excess unlabeled drug, the release of ^{14}C-DADMe-ImmH was again biphasic, but now with half-lives of 9.1 minutes and 1.65 hours for the fast and slow phases of release, respectively (Figure 3). In the absence of rebinding (due to the excess label-free compound), the half-life for the fast phase of intracellular drug-target dissociation matched well with the value obtained in the cell-free enzyme assay (9.1 vs. 8.3 minutes). The prolonged intracellular residence time of the DADMe-ImmH:PNP complex – due to repetitive cycles of slow dissociation and rapid rebinding – translated into a significantly prolonged period of PD when compared to the PK of drug concentration in systemic circulation [27]. Gebre et al. [27] showed plasma levels of DADMe-ImmH and inhibition of erythrocyte PNP activity as functions of time after dosing of healthy human volunteers at a range of doses from 0.25 to 3 mg/kg. At all doses, the drug concentration reached C_{max} at about 4 hours after dosing and was significantly diminished within 24 hours. In contrast, the inhibition of PNP activity was sustained for up to 21 days after dosing ([27] and Figure 3). As illustrated by the data from Gebre et al. [27], even a relatively modest in vitro residence time (e.g. ca. 10 minutes or longer) can translate into quite prolonged target modulation within cells and, by extension, within the human body. Of course, factors in addition to k_{on} and k_{off} can influence the temporal duration of target occupancy; these additional factors may include rates of compound permeation into cells, passive and/or active efflux of compounds out of the cell, intracellular compound metabolism and the like. For example, in the case of the immunicillins, Gebre et al. [27] showed that intracellular target occupancy was limited by the slow uptake of compounds into cells, mediated by the nucleoside transporters ENT1 and ENT2.

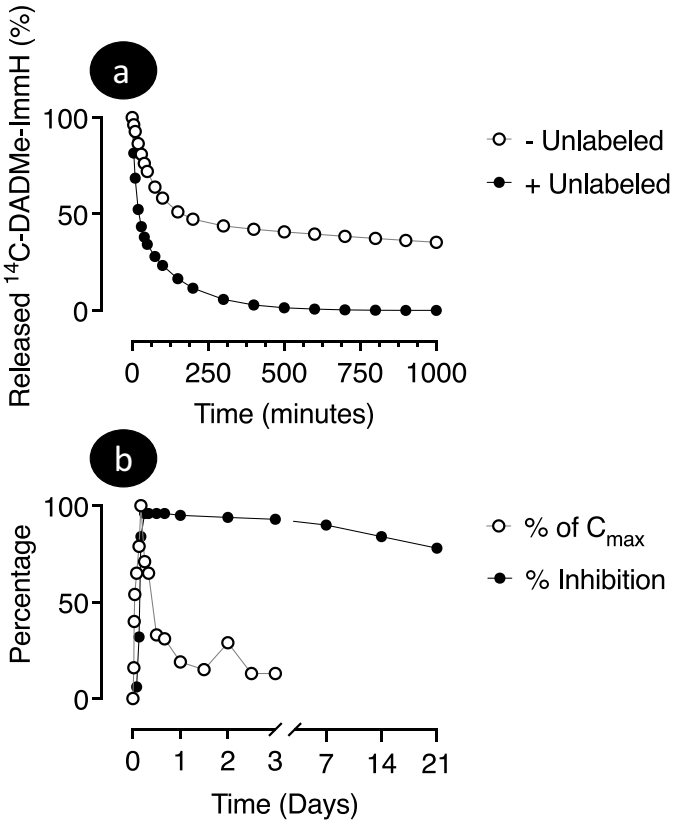

Figure 3. The effect of drug-target rebinding on the intracellular residence time of the drug-target complex and its impact on the PK/PD relationship for the drug in vivo. **A**. Release of ^{14}C-DADMe-ImmH from human erythrocytes in the absence (open circles) and presence (black circles) of unlabeled drug. This graph represents idealized data points based on the dissociation rate constant (or $t_{1/2}$) reported in [27]. **B**. PK/PD relationship for DADMe-ImmH in healthy human volunteers. The open circles represent the systemic concentration of drug as a function of time after a single dose, as a percentage of the C_{max} value. The black circles represent the percentage inhibition of the target enzyme purine nucleoside phosphorylase (PNP) as a function of time after a single dose. Data points in this graph were estimated by visual inspection of data presented in [27]. As such, the graph is not intended to be quantitatively precise, but rather functions to illustrate the general trends in the reported data.

Our understanding of the impact of k_{on} on both equilibrium binding affinity (K_d) and intracellular PD has also evolved since the initial formulation of the drug-target residence time model. While early studies of the relationship of k_{on} and k_{off} suggested that for many high affinity interactions, k_{off} was the predominant determinant of K_d, more recent and more comprehensive surveys of inhibitors of protein kinases provide a different perspective. For example, Willemsen-Seegers et al. [24] studied a total of 49 protein kinase inhibitors that targeted a broad range of enzymes. These researchers found, in contrast to earlier studies, the k_{on} varied significantly – by as much as 4–5 orders of magnitude – across the surveyed inhibitor-kinase pairs. No systematic variation of k_{on} as a function of K_d was seen among this data set [24]. Among the same data set a similar range of 4–5 orders of magnitude was observed for k_{off} values, which did show a clear trend of increasing value (faster values of τ_R) with increasing value of K_d (decreasing affinity). Very different results were obtained by Georgi et al. [28] in a survey of binding kinetics for 270 protein kinase inhibitors targeting 40 clinically relevant enzymes. These workers found better agreement between k_{on} and K_d ($r = 0.80$) than between k_{off} and K_d ($r = 0.10$). Most interestingly, these researchers also found that this pattern was 'gradually inverted with increasing inhibitor potency.' They went on to show that k_{on} values were nearly unchanged between preclinical and FDA-approved drugs while, in stark contrast, k_{off} values shifted toward longer residence time for FDA-approved drugs. They thus concluded that the data point to an over-abundance of compounds with longer residence times among clinically efficacious kinase inhibitors [28].

3.2. Kinetic competition with endogenous ligands

It is well known that orthosteric ligands must compete with endogenous levels of the natural ligand(s) (e.g. substrates for

enzymes, agonists for receptors) for a target, within the context of the cellular environment. The inhibitory effects of drug binding that compete with a natural ligand is said to be surmountable by high concentrations of the endogenous ligand. In cell-free biochemical assays, it is common to observe a shift from competitive (i.e. surmountable) inhibition to non-competitive (i.e. insurmountable) inhibition for an orthosteric pharmacophore series with increasing residence time of the compounds [6]. In a number of studies (previously reviewed in [7] and [14]) researchers noted that not only did the duration of cellular PD increase with residence time, but the cellular potency of compounds itself increased with drug-target residence time. This effect was not anticipated in the original formulation of the model, but it may be the result of the increasing inability of endogenous substrate/agonist to effectively compete with drug for target binding, as the drug-target residence time increases. This hypothesis was experimentally confirmed for antagonists of the angiotensin II receptor 1 (AT_1) [29]. As illustrated in Figure 4, one observes a clear, hyperbolic relationship between the drug-target residence time and the degree of cellular insurmountability of antagonism for a collection of sartan antagonists of AT_1. This relationship may be reflected in the greater and more sustained blood pressure lowering seen with candesartan relative to losartan. For example, Lacourciere et al. [30] compared blood pressure lowering for 50–100 mg losartan and 8–16 mg candesartan over a 36-hour period after dosing. The mean change in systolic blood pressure for losartan was about −9 mm Hg within 1 hour of dosing and diminished over time to −2 mm Hg by 36 hours post-dosing. In contrast, the much lower dose of candesartan resulted in a ca. −13 mm Hg change in systolic blood pressure within 1 hour of dosing and was sustained to −12.5 mm Hg at 36 hours after dosing.

Similarly, Nederpelt et al. [31] demonstrated that the insurmountability of NK1 receptor antagonists was dependent on their drug-target residence time, but was also influenced by the agonist-target residence time of the competing endogenous agonists of this receptor. For example, antagonistic efficacy was greatest for the slowly dissociating antagonist aprepitant when competing with the slowly associating agonist NKA and least efficacious for the combination of the rapidly dissociating antagonist DFA with the rapidly associating agonist SP. This important study adds a temporal dimension to the concept of intracellular competition between drugs and endogenous substrates/agonists of their intended target proteins.

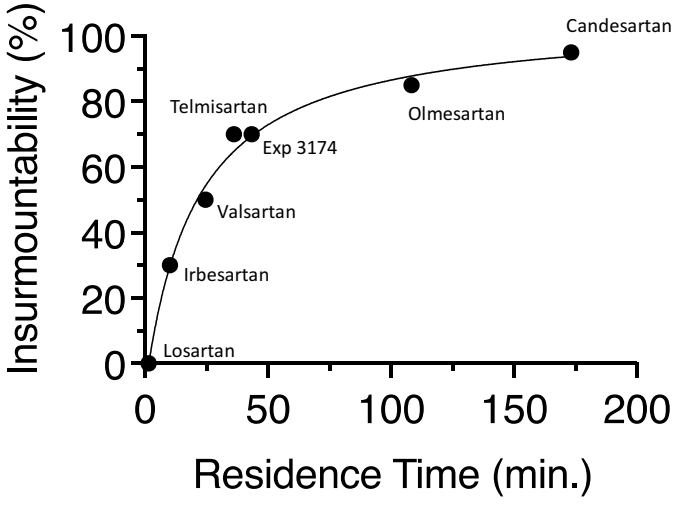

Figure 4. Relationship between drug-target residence time and the percentage of insurmountable antagonism displayed by different sartan class drugs, targeting the AT_1 receptor. Data used to construct this figure were taken from [29].

3.3. Relating drug-target residence time to cellular and in vivo pharmacology

A broad range of studies have demonstrated a clear relationship between drug-target kinetics or residence time and intracellular and/or in vivo pharmacological efficacy. Many of these studies have been summarized in previous reviews [7–14]. These studies demonstrate an important role of drug-target binding kinetics for intracellular and in vivo pharmacological action across a spectrum of target types, including protein kinases [23,24,28,32–34], protein methyltransferases [35–37], chaperone proteins [38,39], G-protein coupled receptors [15,31,40–42] and many more.

3.4. Integration of pharmacokinetics and drug-target residence time

There has been broad recognition that the impact of drug-target residence time on durable PD is interdependent with the pharmacokinetics of the drug. Hence, several attempts to integrate PK and residence time have appeared in the recent literature [19,20,43,44]. Of particular note, the Tonge and Fisher laboratories have integrated compound PK rate constants with those for drug-target association and dissociation into a single kinetic equation that successfully predicts the sustained PD observed after significant diminution of systemic drug levels for antibiotics (the post-antibiotic effect) [43] and targeted cancer therapeutics [44]. More recently, Georgi et al. [28] have performed a comprehensive survey of binding kinetics (BK) across the drugged kinome and have combined these data for select compounds with available PK data, to simulate temporal changes in in vivo target occupancy (TO) by two different models: one that treats target binding by the K_d value alone (the PK-K_d model) and one that treats drug-target interactions in terms of association and dissociation rates (the PK-BK model). They find that the integration of BK data in general was more predictive of TO changes with time after dosing and better accounted for the temporal target selectivity of kinase inhibitors. They conclude from these data that slow dissociation rates play a pivotal role in the clinical success of kinase inhibitors, and they go on to speculate that ‘fast plasma clearance can be – theoretically – a desirable feature when combined with differentiated BK profiles for primary and secondary targets.’

The impact of residence time for sustained in vivo TO is generally determined indirectly by measuring the target-associated PD effects of compound over time after dosing. Recently, Lee et al. [34] reported a novel approach to measure in vivo target occupancy directly and related this to drug-target residence time. Using a library of inhibitors of soluble

epoxide hydrolase as a model system, they developed an approach in which a long residence time compound of interest (inhibitor A) is administered orally to rodents and the PK profile of the compound in systemic circulation is monitored by standard methods. The PK profile shows the typical C_{max}, then exponential decay of systemic drug concentration typical of enteral administration of drugs. Once systemic compound concentration has returned to a minimum level, a high dose of a second inhibitor of the target enzyme (inhibitor B) is administered. The high concentration of inhibitor B displaces residual inhibitor A from the tissue-associated target and this residual amount of inhibitor A returns to the blood stream, causing a secondary peak of inhibitor A concentration in the PK profile (Figure 5(A)). Lee et al. [45] demonstrated that the area under the curve (AUC) of this secondary peak can be directly related to the drug-target residence time of the compound (Figure 5(B)).

3.5. Structural and thermodynamic determinants of drug-target residence time

A number of studies have been reported that attempt to understand the structural determinants of drug-target residence time with the goal of 'tuning' the residence time to meet the in vivo needs of a particular therapeutic effect (see, for example [46],). Other studies have attempted to understand the contributions of ground state and transition state enthalpic and entropic components to residence time (see, for example [47],). Still other studies attempt to use computational molecular dynamics methods to divine the escape trajectory of drug dissociation from a target protein, again with the aim of defining the structural elements of rate-limiting steps in drug escape (see, for example [48],). Space does not permit a comprehensive review of these important studies, but many of these have been covered in recent reviews [49,50].

4. Expert opinion

The importance of drug-target kinetics and residence time, in particular, in shaping the temporal profile of in vivo PD has continued to crystallize as more data are reported in the scientific literature. Based on anecdotal comments from colleagues at a number of large pharmaceutical companies, it is likely that a trove of additional data, using the drug-target residence time model in preclinical compound optimization, have been accumulated but are not available to the public. It is my expectation that the use of the drug-target residence time model for preclinical compound evaluation will continue to expand within the biotechnology/pharmaceutical industry and will also grow among government and academic researchers as well.

The utility of this model will be further enhanced by continued exploration around four key points. First, more systematic study of intracellular residence time would be helpful in understanding in what biological situations a longer residence time may be favorable and the point at which further extension of the drug-target residence time may provide only incremental improvements in intracellular pharmacological impact. Almost every drug discovery program incorporates intracellular assays of compound impact on some biochemical or biological readout of target function. These provide a quantitative basis for measuring intracellular residence time through washout experiments (which, as described above, may be quite a bit longer than $1/k_{off}$ as measured in cell-free biochemical or biophysical assays). In much of the literature to date, such cellular washout experiments have been reserved for one or a few advanced compounds. However, systematic studies of the kinetics of functional rebound after washout, for compounds of varying affinity (IC_{50} or K_d) and drug-target residence time (τ_R from cell-free assays), could be quite informative in understanding the extent to which τ_R impacts the amplitude and duration of pharmacological effect. What the optimal value of τ_R is will surely vary from target to target and perhaps among cell types for a common drug-target pair. The relative contributions of

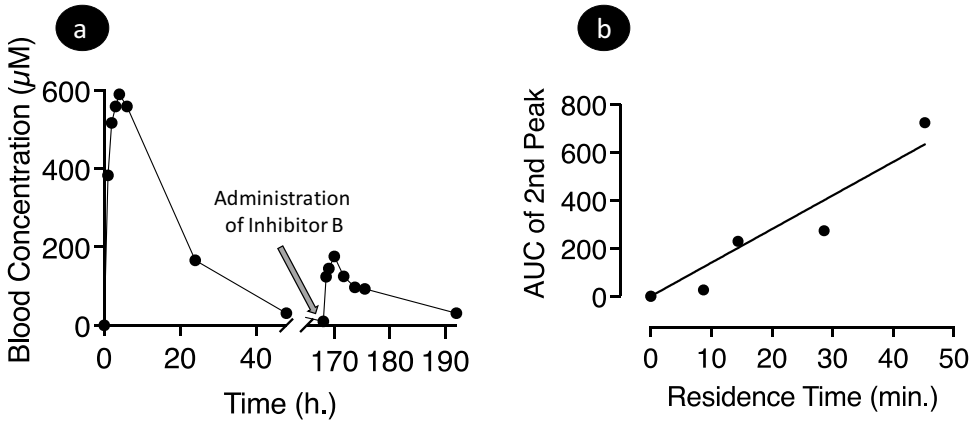

Figure 5. **A**. Systemic concentration of inhibitor A as a function of time after a single dose administered by oral gavage to mice. When the drug concentration in systemic circulation has reached a minimum, a large dose of inhibitor B is administered, as indicated by the gray arrow in this graph. Inhibitor B displaces any residual target-bound inhibitor A in the tissue, resulting in a second peak of inhibitor A concentration in circulation. Data points for this graph were estimated by visual inspection from a graph in [45]. **B**. Relationship between the area under the curve (AUC) for the second peak (from **A**) for different inhibitors of the enzyme soluble epoxide hydrolase. Data points for this graph were estimated by visual inspection from a graph in [45]. As such, the graphs in this figure are not intended to be quantitatively precise, but rather function to illustrate the general trends in the reported data.

k_{on} and k_{off} to intracellular residence time also need to be more systematically studied. As reviewed in this article, there is increasing evidence that intracellular drug-target rebinding – driven mainly by k_{on} is an important contributor to overall intracellular residence time. There are also cases for which k_{on}, rather than k_{off}, may be the primary driver of changes in drug potency, as was reported for the sulfonamide inhibitors of carbonic anhydrase [51]. Hence, building up an empirical database of such information can help drive better resource utilization within drug discovery organizations.

A second key need is a better understanding of the structural determinants of longer residence time. Some efforts toward this end have been reported in the recent literature [46,52], but more data is clearly needed. Like compound affinity, the structural determinants of residence time will also likely vary from target to target. Nevertheless, more comprehensive studies across different members of common target classes may reveal general trends that may be exploited by medicinal chemists. In particular, structural studies (e.g. crystallography, NMR and cryo-electron microscopy) comparing structurally related members of a common pharmacophore series that represent short and long residence times could be quite informative (see, for example [53],). Hypotheses generated in this manner with regards to the importance of specific recognition elements can then be tested with new compound synthesis coupled with site-directed mutagenesis. Ultimately, drug escape trajectories could be posited and tested in this manner. A related third key point is the need to more systematically account for the role of conformational dynamics in drug-target interactions [54] and especially in mapping the escape trajectory (or trajectories) that define the drug-target residence time. In this last regard, continued computational studies of residence time via molecular dynamics [48] may help to better refine common strategies for slowing drug 'unbinding' from their target proteins.

Finally, a better understanding of the dynamic interplay between drug-target residence time, drug distribution and drug pharmacokinetics is critical to derive the greatest value from the drug-target residence time model. Several studies have been published in which the temporal relationship between in vivo PK and PD have been studied for pairs of compounds of similar structure, similar K_d similar PK half-lives, but dramatically different τ_R (e.g. [53],), but additional data is clearly needed. Again, the interplay between PK and τ_R is likely to vary among targets and among pharmacophores, but some generalities may nevertheless emerge from more systematic studies of this type [28,43,44].

Ultimately, all attempts to predict the amplitude and duration of the pharmacodynamic effects of a drug in humans are limited by the reductionist nature of preclinical research. Whether based on IC_{50}, τ_R, multi-compartmental PK models, or other preclinical measures, one must always bear in mind that human physiology is complex; hence, preclinical drug optimization must always be multifactorial, and no one measurement will suffice for this purpose. Nevertheless, the drug-target residence time model, as one component of multiparametric drug discovery, has clearly had considerable impact on how compound optimization is assessed by drug discovery researchers, as indicated by the large number of scientific articles that have utilize this model. As additional studies are carried out and published, greater refinements and improvements of the drug-target residence time model will continue to evolve.

Declaration of interest

The author is an employee and shareholder of Accent Therapeutics, Inc. He has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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