PKPD awareness is vital if we are to attempt to relate preclinical results to the acute and long term consequences in humans. The debate on whether preclinical findings can be translation to the human usage is still engaging scientists across industry, academia and regulatory bodies.

Early integration of pharmacokinetic and dynamic reasoning is essential for optimal development of lead compounds: strategic considerations

Johan Gabrielsson[1], Hugues Dolgos[1], Per-Göran Gillberg[2], Ulf Bredberg[1], Bert Benthem[2] and Göran Duker[2]

[1] Discovery DMPK & BAC CVGI, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden
[2] Bioscience CVGI, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

The aims of this report are firstly to raise awareness among kineticists and pharmacologists as to why pharmacokinetic–pharmacodynamic (PKPD) integration is essential for target validation (TV), optimizing development of lead compounds (lead generation [LG] and lead optimization [LO]) and scaling these to human. A related aim is to demonstrate strategic examples of PKPD collaborations that have improved the planning, execution and evaluation of experiments in primary and safety pharmacology. Examples include design of TV studies, design and data 'pruning' of PKPD studies in LO, analysis of data with marginal and substantial temporal (time) differences between exposure and response, design of safety pharmacology studies, assessment of safety margin and assessment of uncertainties in predictions of first dose in human.

DR JOHAN GABRIELSSON
Dr Johan Gabrielsson is a Senior Principal Scientist at AstraZeneca R&D Mölndal. He is author of the book 'Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications' 4th ed. (2006). He is academically affiliated with the Department of Pharmacology, Gothenburg University, Sweden. His research focuses on modeling different aspects of endogenous turnover, such as functional tolerance and rebound phenomena by means of feedback, physiological limits and target-mediated drug disposition in collaboration with Professor Lambertus A. Peletier at Department of Mathematics at Leiden University, the Netherlands. He has conducted numerous workshops on biological (PK/PD) data analysis within and outside the pharmaceutical industry.

Introduction

Pharmacokinetics (PK) is the science of the time course of drugs in the organism – that is, it investigates what the body does to the drug. Pharmacodynamics (PD) concerns the study of the time course of biological effects of drugs, the relationship to drug exposure, and drug mechanisms of action – that is, it examines what the drug does to the body. We believe that a better understanding of the inter-relations between PK and PD and sometimes the lack of concordance between the two, might shed light on situations where one or other needs to be optimized in drug discovery and development. It is, therefore, our contention that *in vivo* pharmacology and *in vivo* PK must play a greater and more intelligent role in drug discovery if their combined value is to be fully utilized. In recent years, several articles have highlighted the need for drug discovery that is driven by creativity rather than by process or technology (for review see [1,2]). For example, there has been a tendency to focus narrowly on the target and to underestimate the complexity of the physiological role of the target in the intact organism [3]. Binding of a molecule may activate, inactivate or modulate a target's function, and further complications arise if a drug has to simultaneously interact with more than one target to express its therapeutic effect [2]. There are many examples of how process-driven and automated the Drug Metabolism and Pharmacokinetics (DMPK) screen has become, increasing the risk of falling into the trap of ascribing a crucial

Corresponding author: Gabrielsson, J. (Johan.Gabrielsson@Astrazeneca.com)

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and central role to a single parameter or just a few parameters (e.g. plasma protein binding, intrinsic hepatic clearance and transcellular flux). Often, for example, industrial scientists state that high plasma protein binding increases the dose size, that the average unbound plasma concentration should be greater than a multiple of three of EC_{50}, or that a short plasma half-life limits the potential for convenient daily dosing in human. Not long ago it was believed by many pharmacokineticists, pharmacologists and physicians that there was no relationship between drug concentration in plasma and the time course of drug action, and that one reason for this was that the pharmacological response often lagged behind the plasma concentration [4–6]. This apparent time lag between response and plasma concentration was first rationalized by [7] and further investigated by [8]. More recently, physiological turnover models have offered better solutions for drugs whose mechanism of action consists of either inhibition or stimulation of a physiological process involved in the clinical expression of drug action [5,9,10]. Mechanism-based modeling can therefore provide a truely effective link between *in vivo* pharmacology, DMPK and safety sciences.

The aims of this paper are primarily to explain the rationale behind pharmacokinetic–pharmacodynamic (i.e. quantitative pharmacology, PKPD) reasoning and to raise awareness among pharmacokineticists and pharmacologists of why PKPD integration is essential for target validation (TV), optimizing development of lead compounds (lead generation [LG] and lead optimization [LO]) and scaling these to human. Finally, we provide examples of PKPD collaborations that have improved the design, execution and evaluation of experiments.

What is PKPD reasoning?

Kinetic–dynamic reasoning should, whenever possible, be based on *in vitro* and *in vivo* concentration–time, response–time and concentration–response relationships (Fig. 1), with an underlying ambition to couple this to the disease state. In other words, PKPD attempts to describe the biological responses produced by drugs and to define the underlying mechanisms by which the responses are generated. Related to this is the need to address key issues such as temporal differences, exposure–effect relationships, plasma protein binding differences, safety/efficacy margin(s), forerunner information, dose–effect–time relationships and the rationale for clinical dose settings. We interpret *PKPD* as being synonymous with quantitative pharmacology.

Consequences of ignoring an integrated PKPD approach

What can happen if we fail to use an integrated PKPD approach to experimental design? As we will show, studies may be designed incorrectly, thereby either overestimating or underestimating the risk and/or efficacy of a compound. For example, protein binding across two or more species may differ substantially and even blur information about efficacious concentrations or safety margins. Thus, data should be on the basis of unbound concentrations to avoid significant errors in estimating risk, efficacy, or the steepness of the concentration–response relationship. Temporal (time) differences between drug concentrations in plasma and the time course of drug action may confound interpretation of the relationship between drug concentration and the intensity of a pharmacological effect. Drug-induced sensitization, tolerance and rebound are phenomena in which the magnitude of a pharmacological effect can be altered by repeated administration. This can often be tackled with appropriate PKPD analyses [11]. Consideration of active and interactive metabolites, stereo-specific assays and attention to turnover of endogenous ligands, are also needed [4]. Finally, ignoring the joint effects of *in vivo* pharmacology (e.g. potency) and *in vivo* DMPK (e.g. scaled clearance) may lead to erroneous decisions and, thus, halt early development. It is therefore important to remember that there are numerous examples of drugs with suboptimal PK characteristics but excellent PD (potent, efficacious and slow turnover of effect), including felodipine (Plendil), omeprazole (Prilosec) and quetiapine (Seroquel) (Table 1).

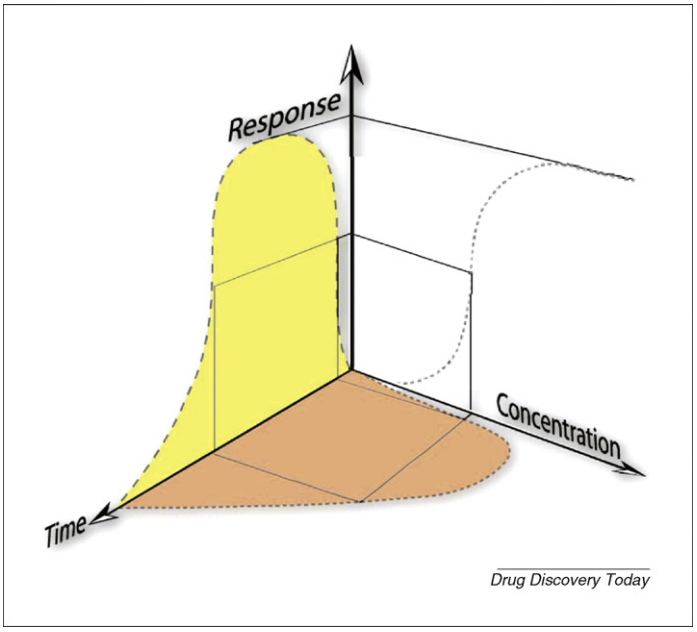

FIGURE 1

Schematic illustration of the concentration–response (right), concentration–time (bottom) and response–time (left) relationships that are part of pharmacokinetic–pharmacodynamic characterization [11].

PKPD integration during target validation and lead generation

Over the past two decades, the focus on *in vitro* high capacity assays and a 'process-led' way of working has resulted in a less stringent interdisciplinary approach to assessing a compound's combined PKPD characteristics, and more of a 'tick the box' approach. As early as TV and LG some compounds prove to be efficacious *in vivo*, in spite of the fact that their *in vitro* or *in vivo* PK properties are not optimal (e.g. high intrinsic clearance, low bioavailability, and/or short half-life). This means that we must consider how to evaluate the consistency between *in vitro* and *in vivo* PD properties, between *in vitro* and *in vivo* PK properties and between PD and PK properties if we are to improve the quality of selected compounds (combined value). At present we collect information about a compound's PK and PD properties, but we seldom confirm that the PK properties are adequate or sufficient *vis-à-vis* their *in vivo* pharmacology.

The importance of early PKPD integration is exemplified by compounds with a well-documented pharmacological effect in spite of high intrinsic clearance and low bioavailability (Table 1), or high

TABLE 1

Pharmacokinetic parameters in animals and human of felodipine, omeprazole and quetiapine

Compound	Species	f_u (%)	*F* (%)	CL (mL min^{-1})	$t_{1/2}$ (hours)	Comment	Human dose (mg)
Felodipine	Rat	1.5		108[a]			
	Dog			1875[a]			
	Human	<1	<15	5000[a]	5[b]	EC_{u50} <10 nM	2–10
Omeprazole	Rat		<5	25	0.12		
	Dog		15	150	1		
	Human	3	60	4900	<1	$t_{1/2resp}$ 15–20 hours	20–40
Quetiapine	Rat	10	<10	41	0.35		
	Dog	10	<10	450	1.5		
	Human	17		1890	3.6	$t_{1/2resp}$ one to two weeks	75–150[c]

[a] Oral clearance.
[b] Effective half-life.
[c] *b.i.d.*

in vitro intrinsic clearance but low clearance *in vivo*. Such a combination of characteristics has implications for evaluating the lead series and setting the criteria for a candidate drug target profile (CDTP).

PKPD integration during and after lead optimization

PKPD updated CDTP criteria will impact on the LO phase. It is obvious that a compound's PK characteristics are important and that these are intimately coupled with its *in vivo* pharmacology and safety margin. It is, therefore, very surprising that emphasis is placed on optimizing PK characteristics *in vitro* and *in vivo* and on optimizing target effects *in vitro*, but not on coupling these to the compound's *in vivo* properties via a holistic approach (Fig. 1). Optimization of a single property at a time such as metabolic stability (intrinsic clearance) may lead to high bioavailability but lack of efficacy, or vice versa. Too generic a filtering process may have detrimental consequences, which in the past could have resulted in drugs like omeprazole, felodipine and quetiapine being discarded.

It has become apparent that there is a need for substantial improvement in the processes (e.g. during LO) whereby the understanding of how the characteristics of a compound's target action (PD) and safety pharmacology and toxicology (SPT) profile are related to its exposure (PK) profile. It is, therefore, necessary to ask oneself during LO, 'What PD properties need to be improved, and what PK profile is needed to support PD?'. This is particularly challenging because most of our present day ways of achieving meaningful data include the use of animal studies. Hence, there is a need to raise awareness of the value of PKPD, commencing during TV and extending throughout LO. In an effort to do so, we highlight certain topics of interest below.

For an overview of the value afforded by a quantitative pharmacological approach during drug discovery and development, see Fig. 2. It can be seen that PKPD provides the integrative instrument for the entire discovery-development process. Successful kinetic–dynamic reasoning is on the basis of concentration–time, response–time and concentration–response relationships.

Plasma protein binding and unbound concentrations

General implications

The principal concern with plasma protein binding is related to its variability within and across different species, strains and disease models *in vivo*. If we disregard uptake of drug into blood cells, drug in plasma is circulating either as unbound compound C_u or as compound bound C_b to, for example, plasma proteins such as albumin and/or α1-acid-glycoprotein.

The equilibrium processes between unbound and protein bound plasma concentrations and unbound and bound tissue concentrations are depicted schematically in Fig. 3.

The equilibrium between unbound and bound drug for a low molecular weight compound in plasma is determined by the unbound concentration C_u, the concentration of the binding protein $[P_T]$, the number of binding sites n per protein molecule and the affinity between drug and protein K_a. The unbound concentration C_u is determined by the (oral) dosing rate and unbound clearance CL_u (Eqn (1)).

$$C_u = \frac{\text{dose rate}}{CL_u} \quad (1)$$

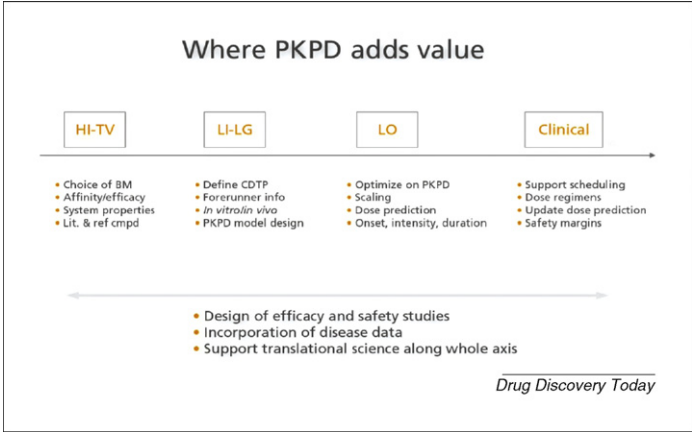

FIGURE 2

Overview of value added by quantitative pharmacology (PKPD) during drug discovery and development. The choice of a relevant biomarker (BM), information of affinity/efficacy, system properties (turnover of biomarker and impact of disease) and information regarding reference compounds guide the process of Hit Identification to Target Validation (HI–TV). During the Lead Identification–Lead Generation (LI–LG) phase, defining the Candidate Drug Target Profile (CDTP), using forerunner information and applying *in vitro/in vivo* relationships improves the characteristics of the lead compounds, and the PK and/or PD parameters are further optimized during lead optimization (LO). Kinetic–dynamic reasoning also guides the scaling of PK and PD properties including dose predictions to human. During the clinical phases, a quantitative pharmacological approach supports study design, dose scheduling, updating of dose predictions and definition of safety margins. PKPD thus provides the integrative instrument for the entire discovery-development process [11].

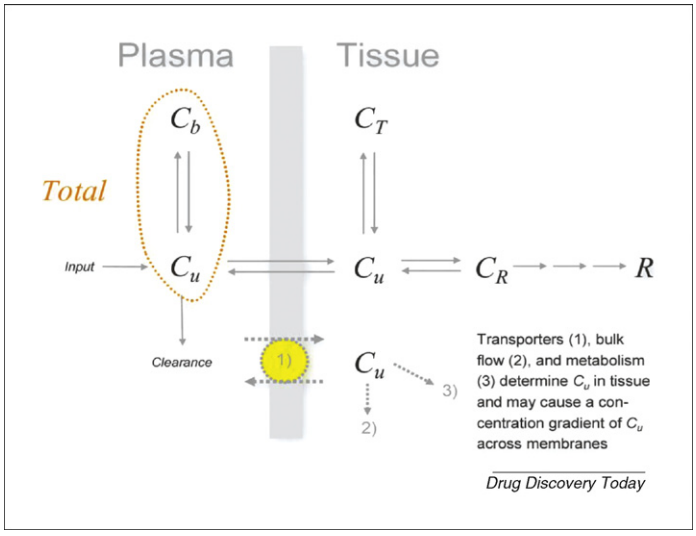

FIGURE 3

Schematic illustration of the equilibrium processes between unbound and bound drug in plasma and tissue. Deviations from a C_u plasma to C_u tissue ratio equal to unity occur when transporters (1), metabolism (2) and bulk flow (3, e.g. cerebrospinal fluid flow) act on unbound tissue concentrations. In tissue, drug is unbound (C_u), nonspecifically bound (C_T), or bound specifically to receptors (C_R). *R* denotes the pharmacological response [11].

Consequently, we believe that two parameters need to be optimized in LO to define an appropriate dose rate in human – the PD property of effective (i.e. unbound) concentration (C_u) and the PK property of unbound clearance (CL_u) (Eqn (2), optimize on CL_u C_u).

$$\text{dose} = \tau\, CL_u\, C_u \qquad (2)$$

where τ is the dosing interval. The total plasma concentration C may be viewed as the ratio of unbound concentration-to-free fraction.

$$C = \frac{C_u}{f_u} \qquad (3)$$

where f_u is the proportionality factor between the unbound C_u and total C plasma concentrations. Eqn (3) is unfortunately one of the most misinterpreted and misunderstood relationships in PK (for review see [12,13]). Therefore, when conceptualizing dependency and functionality, this equation should not be rearranged [13]. The total plasma concentration C is a consequence of C_u and f_u. Experimentally, the free fraction f_u is derived by measuring the unbound C_u and total C concentrations, for example, by means of equilibrium dialysis, at several concentrations.

The *free fraction* f_u, is generally constant at pharmacological concentrations, but becomes nonlinear in its nature with respect to unbound plasma concentrations approaching the concentrations of binding sites on the plasma protein. There are three regions of particular interest. At low free concentrations, binding is constant and f_u is independent of C_u. At higher unbound concentrations f_u starts to increase as more binding sites are occupied. At high unbound concentrations, the binding proteins become saturated, f_u approaches unity, and total and unbound concentrations approach each other asymptotically. Depending on the binding plasma protein, this nonlinearity starts to occur at different plasma concentrations, at concentrations as low as 10% of the binding protein's molar concentration. For albumin, this is seldom an issue because albumin occurs in plasma at about 500–700 μM. However, for α1-acid-glycoprotein, a protein with a low and highly variable plasma concentration (9–22 μM in human), the risk for nonlinear binding begins in the 1–3 μM concentration range. For many compounds f_u varies across species and strains depending on, for example, the plasma protein concentration (Table 2).

TABLE 2

Albumin concentration data in various species and strains

Species	Strain	Mean[a] (g L^{-1})	Mean (μmol L^{-1})
Mouse	CD-1	30–34	455–515
Rat	Sprague–Dawley	38–44	576–667
	Wistar	29–33[b]	439–500
Guinea pig	Hartley	24–27	364–409
Monkey	Rhesus	43–44	652–667
Dog	Beagle	29–34[b]	439–515
Minipig	Göttingen	29–31	439–470
Human	Caucasian	40–51	606–773

[a] Excluding data marked b, taken from [14].
[b] Internal AstraZeneca data.

The *in vitro* measurement of plasma protein binding comprises part of the early (LG) characterization of the PK properties of a compound. Most often, however, plasma protein binding is limited to a crude qualitative score of low (green), intermediate (yellow) and high (red) binding. This is not quantitative data that can be used for an improved or informed assessment of unbound concentrations, PK parameters, PD parameters (unbound potency and steepness) or safety margins. Therefore, we would suggest the use of quantitative *ex vivo* plasma protein binding, obtained at a minimum of two presumed concentrations (low and high), as an integral part of the *in vivo* PD protocol. This would enable us to draw conclusions about the impact of binding on EC_{50} and the steepness of the concentration–response relationship.

Species-dependent exposure–effect relationships

Measures of plasma protein binding for the correction of species-dependent differences were done *ex vivo* in project A for the conversion of total to unbound concentrations. Often, potency measures like EC_{20}, EC_{50} or EC_{80} are derived from some species (e.g. rat and gerbil) and safety measures like QT_{10} from others (e.g. guinea pig or dog). Because these species may exhibit different protein binding properties, it is necessary to make the comparisons on the basis of a protein binding independent scale (e.g. unbound concentrations such as EC_{u20} and C_{u10}). For example, because the same unbound concentration (e.g. 100 nM) of test compound A will, for the rat (free fraction f_u 12%), gerbil (f_u 14%), guinea pig (f_u 18%), dog (f_u 3%) and human (f_u 3.7%), give a respective total plasma concentration of 800, 700, 500, 3300, and 2700 nM, comparisons across species are difficult unless based on unbound concentrations. The estimated unbound concentration giving a 20% change from baseline of the primary effect (C_{u20}) may, however, be compared with unbound concentrations giving a 10% change from baseline of the secondary effect (e.g. C_{u10} for the QT-response). This enables a comparison between efficacy and a safety measure.

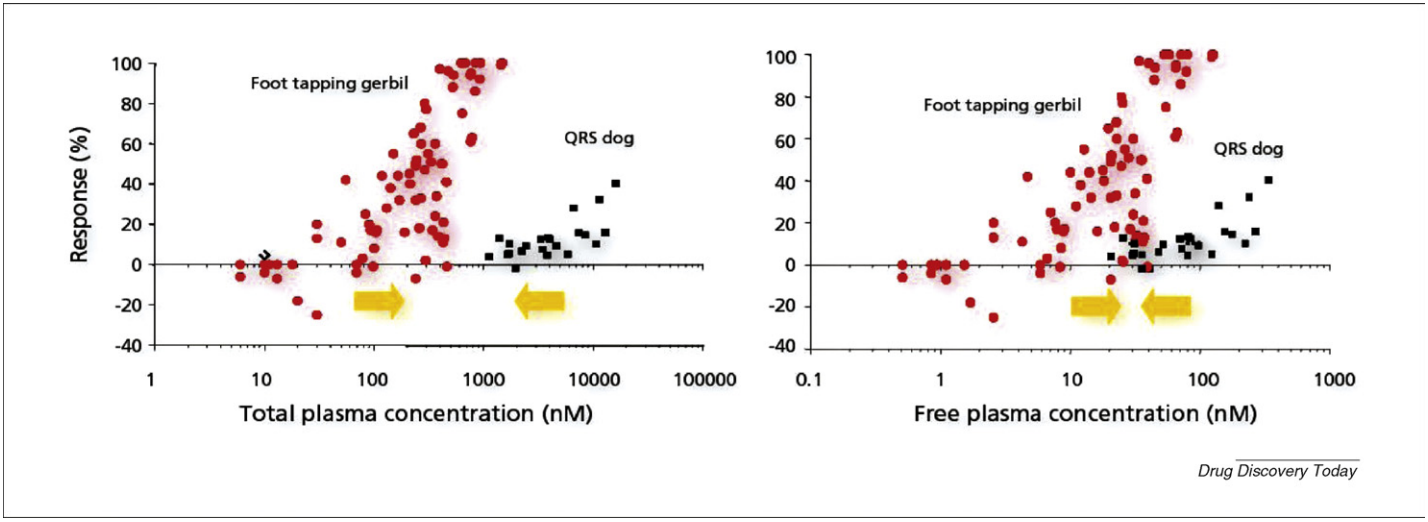

FIGURE 4

Efficacy and safety biomarkers for test compound A. In the gerbil model (foot tapping) and in the dog model (cardiovascular (QRS) effects) are plotted against total plasma concentration (left) and unbound plasma concentration (right). The yellow horizontal arrows illustrate schematically how the apparent safety margin diminishes when plasma protein binding differences in f_u are incorporated. The free fraction f_u is greater in gerbils than in dogs.

Fig. 4 shows how the apparent safety margin diminishes when a species-independent unbound concentration scale is used for a test compound with a greater free fraction in the primary effect (gerbil) than the secondary effect (dog) model. Of course, the opposite could occur if the free fraction of drug in the primary model species was less than the free fraction obtained from the safety species.

The aspects of concentration-dependent plasma protein binding and intra-assay (pooled plasma for *in vitro* measurement compared to concentration-dependent *ex vivo* measurements) variability in plasma protein binding are summarized in Fig. 5 for test compound B.

Using a constant *in vitro* value of free fraction (0.45%) in Fig. 5 (test compound B), will in this example result not only in erroneous predicted human clearance (>4.5 L min^{-1} in contrast to 0.9 L min^{-1} when a concentration-dependent f_u of 2–20% is used), but also in bias estimates of potency (EC_{u50}) and the safety margin. By contrast, a high free fraction of about 50% in rat, guinea pig and human further suggests species-dependent differences, and the necessity for this to be factored in when assessing kinetics, efficacy and safety. The consequence of linear versus nonlinear plasma protein binding on the concentration–response relationship is shown in Fig. 6. Note the steep concentration–response relationships for total (black) and linear (blue) unbound concentrations, in comparison to the unbound concentration–response relationship in the case of a nonlinear (red) free fraction. The two examples (Figs 5,6) demonstrate that plasma protein binding impacts on both PK and PD when assessment is on the basis of either total concentrations or erroneous constant binding. A steep concentration–response relationship may in some cases substantially limit

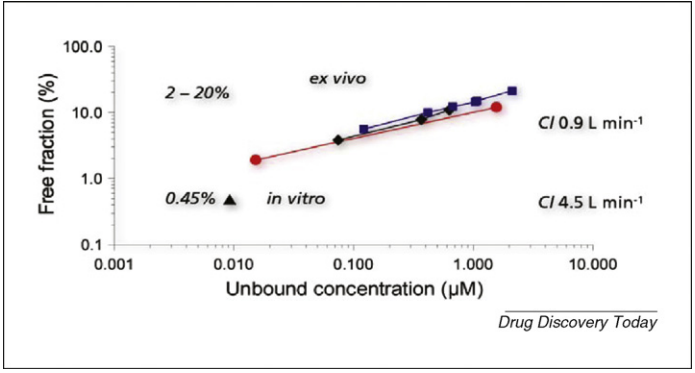

FIGURE 5

Free fraction (%) versus unbound concentration *ex vivo* (blue squares dog 1, black diamonds dog 2, red circles dog 3). Also included is the *in vitro* free fraction (0.45%) at a total plasma concentration of 2 μM corresponding to an unbound concentration <0.01 μM. All data from test compound B.

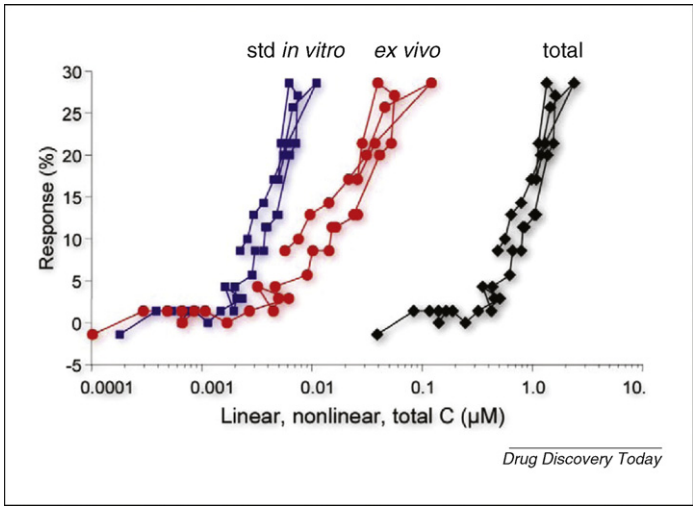

FIGURE 6

Plot of total (black), and unbound (using a constant free fraction, blue, and nonlinear free fraction, red) concentration–response relationship of test compound B. Note that the steepness of the red curve is less than the other curves. For the standard *in vitro* data pooled plasma was used and a constant free fraction of 0.45% was used to convert total concentrations to unbound concentrations. *Ex vivo* protein binding measurements were done on individual plasma samples obtained from the *in vivo* experiment.

the practical use of a compound, particularly when the response is a risk factor.

Confounding factors known to affect plasma protein binding are for example, species and strain differences, concomitant use of other drugs, anesthetics and surgical stress. Therefore, we strongly advocate *ex vivo* measurement of plasma protein binding (i.e. measurement from individual rather than pooled samples) in pharmacological or pharmacokinetic *in vivo* model experiments, to avoid quantitative (concentration-dependencies) and qualitative (the commercial source of proteins) differences such as those shown in Figs 5,6.

Temporal differences between concentration and response

Different classes of target may have very different temporal relationships between concentration–time and response–time profiles. The anti-thrombotic effect represents rapid equilibria ($t_{1/2}$ equilibrium less than seconds or minutes, Fig. 7, left) in contrast to the delayed antipsychotic effect of neuroleptics ($t_{1/2}$ response a few weeks, Fig. 7, right). Thus, the PK requirements for an optimal dynamic effect may vary substantially, depending on the type of target and the target's position in the biochemical pathway eliciting the effect. Because a generic way of working is therefore not possible, an integrated PKPD strategy has to be adopted using a case-by-case approach.

Not so long ago it was believed by many scientists that there was no relationship between drug concentrations in blood or plasma and the time course of drug action. An important reason for this was that the pharmacological effects of many drugs lag behind their drug concentration in plasma – *hysteresis* (Fig. 7, right).

This apparent dissociation between drug concentration and effect can be rationalized if not explained by the use of an effect compartment to account for the lag (Fig. 8A).

The effect compartment concept (Fig. 8A) linked to for example, the Hill equation (E_{max} model, Eqn (4)) may be used for describing such a relationship between drug concentration and intensity of pharmacological effect.

$$\text{effect} = E_0 + \frac{E_{max}\, C_e^n}{EC_{50}^n + C_e^n} \quad (4)$$

Here, E_0, E_{max}, EC_{50}, C_e and n denote the baseline effect, efficacy, potency, effect compartment concentration and slope factor, respectively. The E_{max} model is a mathematical representation of the solid gray *S-shaped* lines in Fig. 7. The analysis of rapid effects such as atrial effective refractory period prolongation *AERP*. Case Study 1 was carried out by means of effect compartment modeling using a multiple intravenous infusion protocol.

Although many investigators have used this approach without adopting a rigorous experimental verification procedure, such a 'hit and run' approach is often on the basis of a single dose experiment, with estimates of E_{max} (efficacy) and EC_{50} (potency) being reported in the literature. The effect compartment model assumes that the lag in pharmacological effect is attributable to distribution (seconds and minutes) of drug from plasma to biophase. The model is, however, unsuitable for a fairly large number of different pharmacological effects that are not rate-limited as a result of distribution; in such cases, the response occurs over hours, days or weeks. Nor does the effect compartment model give an opportunity to focus on specific processes leading to the expression of a pharmacological effect, as it cannot discriminate between drug (EC_{50} and E_{max}) and system (turnover, disease, etc.) properties. This is the primary reason why mechanism-based turnover models (Fig. 8B) have rejuvenated the field, permitting identification of the physiological components of drug action that are affected by numerous variables, including disease, other drugs, gender, age and species differences. These models also permit

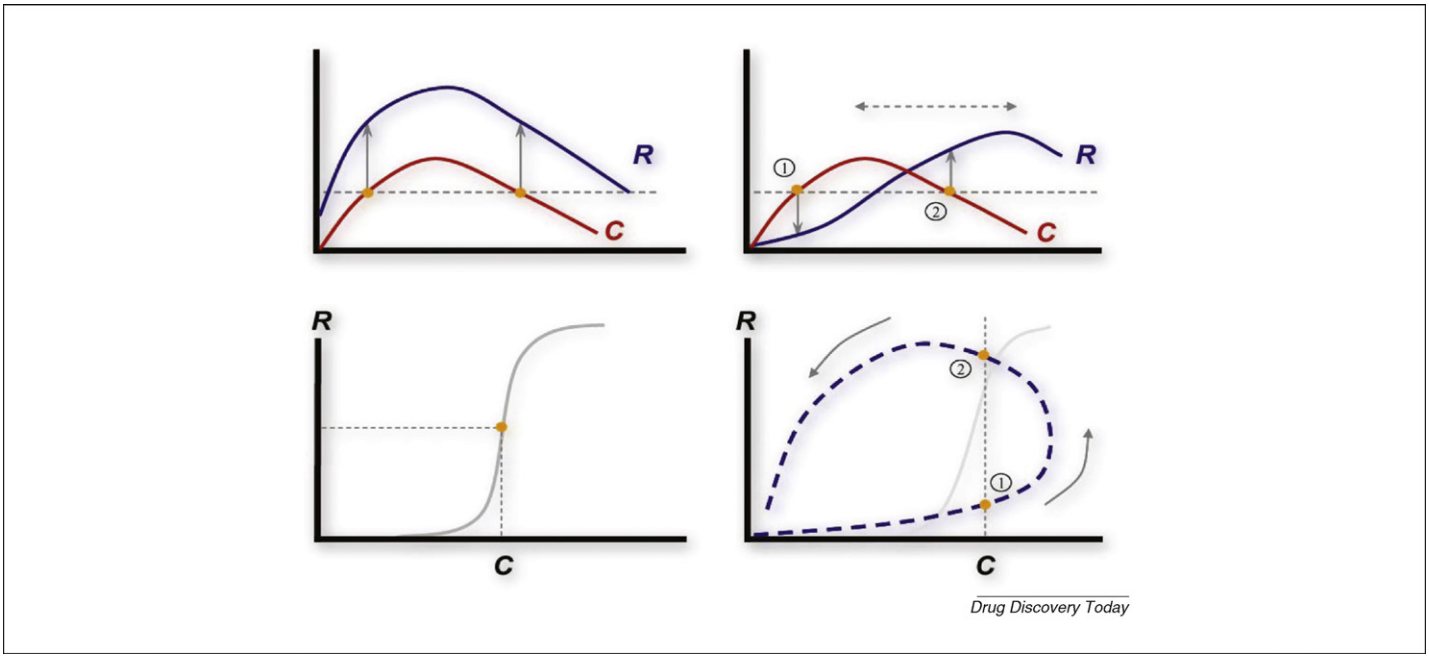

FIGURE 7

Schematic illustration of rapid (direct) equilibrium between concentration (*C*) and effect (*R*) (upper and lower left figures). Delayed concentration–effect relationship (hysteresis, upper and lower right figures). The gray S-shaped lines in the figure are the true concentration–response relationship at equilibrium.

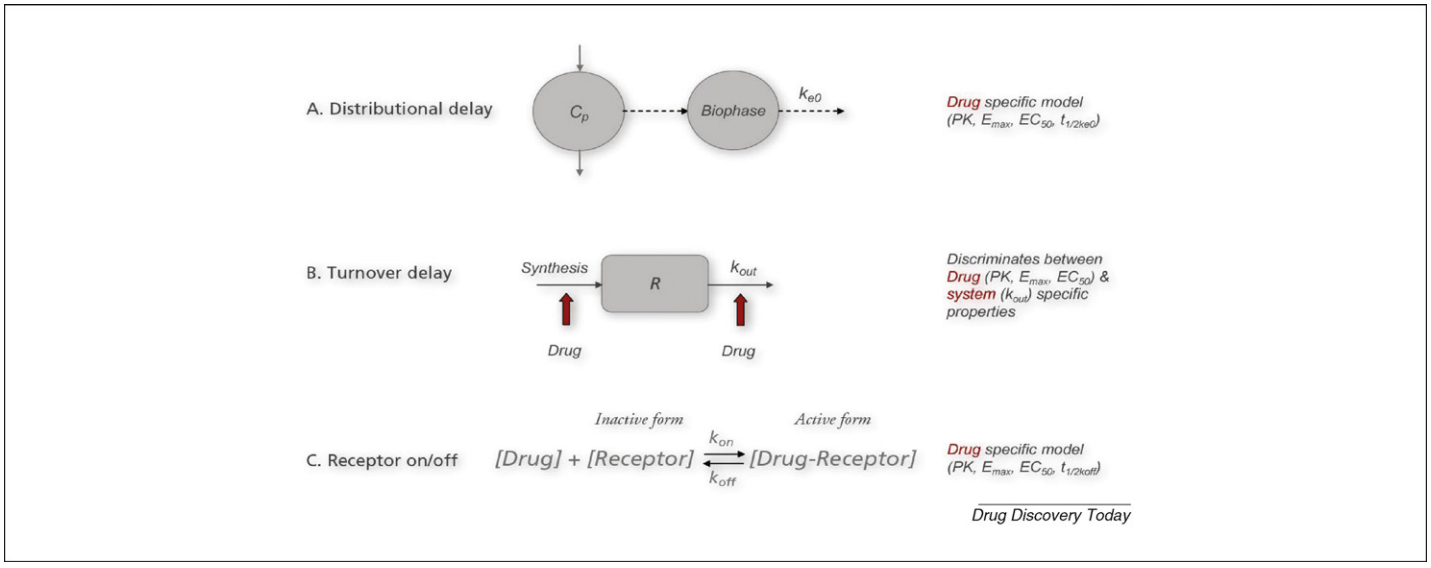

FIGURE 8

Schematic illustration of distributional models (**(A)** effect compartment models), turnover models **(B)** and receptor on/off models **(C)**). Note that distributional and receptor kinetic models do not discriminate between drug and system parameters. In turnover models there is a distinct differentiation between drug parameters (E_{max} and EC_{50}) and system properties (k_{in}, k_{out}, etc.).

separation of drug parameters (EC_{50} and E_{max}) from systems parameters (turnover rate and half-life of effect) (see also Case Study 3).

The third class of model that captures temporal differences between plasma concentration and response is the receptor binding kinetics model (Fig. 8C) [15,16]. To appreciate fully the *in vitro* estimate of, for example, potency and the impact of *Target Residence Time* for the assessment of potency and effect duration via its drug dissociation constant K_d, one also needs information about the first-order rate constant k_{off}. Figure 9 shows a simulation of the pharmacological response of two compounds having the same dissociation constants (K_d, i.e. ratios of k_{off}/k_{on} are the same) but a ten-fold difference in the k_{off} values, and the consequences on effect duration.

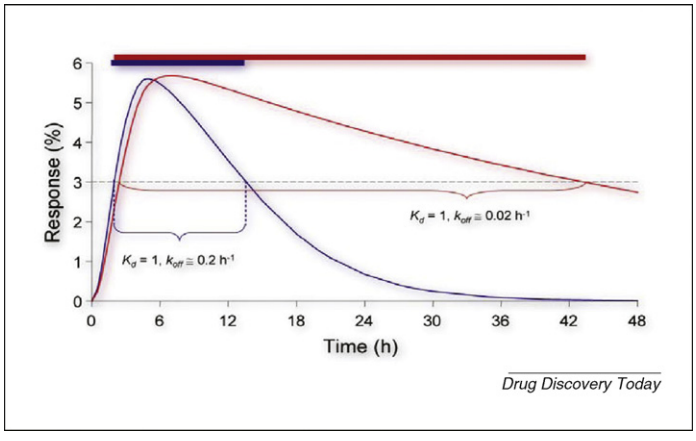

FIGURE 9

Schematic simulation of two response–time courses with the same dissociation constants K_d, but with a tenfold difference in the individual first-order off-rate constant k_{off}. Note the longer duration (red bar) time course with the smaller k_{off} (0.02 h^{-1}) rate constant.

We support Copeland *et al.* [16], who proposed that *in vitro* measurement of the dissociative half-life is a crucial metric of compound optimization, and could be a key indicator of *in vivo* effect duration and efficacy.

Safety/efficacy margins

Because the focus on 'safety margin thinking' increases (e.g. handling the hERG/*QT* issue), it is of utmost importance that complex calculations be based on good science. Essential questions regarding a compound's potency and effective concentration range are connected with determinations of how much of the compound and what duration of exposure are required to produce its desired and undesired effects (onset and intensity) *in vivo*.

Again, this can be exemplified by test compound A. If the safety margin is estimated from direct concentration–effect *QT* guinea pig data, the safety margin is overestimated because of the apparent hysteresis – that is there is a lag time between the measured unwanted effect and the measured plasma level (Fig. 10, Case A). Figure 10 (Case B) shows how the safety margin is increased (see also Case Study 1) when the temporal difference between plasma concentration and effect for both the primary effect (efficacy) and secondary effect (safety) is taken into account. Using the guinea pig model, a direct comparison was made between the potency of test compound A in prolonging MAPD of test compound A applying either a repeated rapid injection protocol (inoptimal protocol) or a multiple constant rate intravenous infusion + washout protocol (optimized protocol). The former protocol resulted in a C_{e10} (the plasma concentration measured at a 10% (ms) change in MAPD from baseline) of 6 μM, whereas the optimized protocol taking *hysteresis* into account resulted in a C_{e10} of 24 μM. This is an important consideration when designing protocols for safety pharmacology (see also Case Study 2).

Uncertainty in the target level of efficacy needed for a clinical effect (e.g. 20, 50 or 80% of maximal effect) and the corresponding plasma concentration range (EC_{20}, EC_{50} or EC_{80}) also impacts on

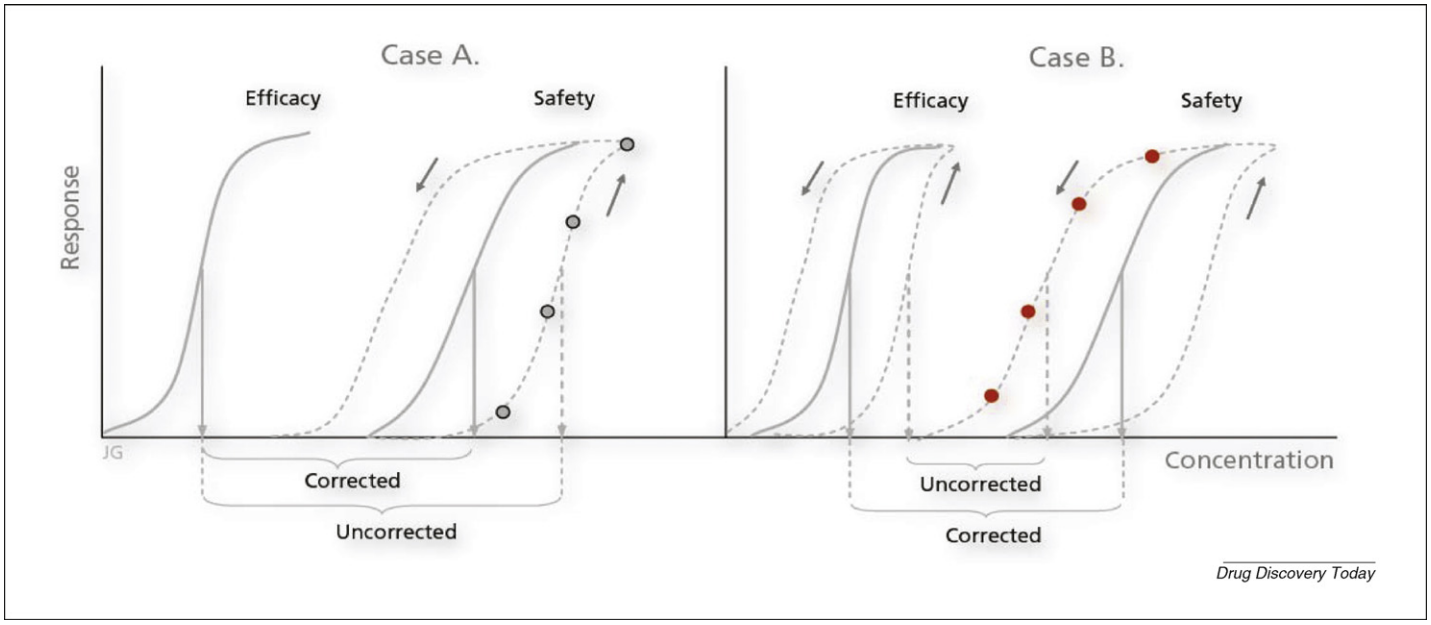

FIGURE 10

Schematic illustration of safety margin for Case A where the primary effect (efficacy) is compared to the secondary effect (safety) after an infusion + washout regimen of test compound A. Data were obtained only during the infusion. Unless the hysteresis is taken into account, the safety margin is overestimated. Case B shows a scenario with hysteresis in both the primary and secondary effect data. Safety data based on a rapid injection regimen where data are obtained only from the washout phase. Using this protocol, the safety margin would be underestimated unless both time lags were taken into account.

the assessment of a safety margin (e.g. see Fig. 4). It becomes even more difficult when comparing efficacy/toxicity on an *in vitro/in vivo* basis, particularly when one encounters bioanalytical problems for highly bound compounds, large species-dependent differences in binding, or concentration-dependent binding.

Forerunner information

Clinical (proof of principle, PoP) and preclinical (dog electrophysiological effects) data from test compound B showed that the desired effect was achieved at approximately the same unbound plasma concentrations. Unbound concentrations were, therefore, used as guidance for the effective concentration range of test compound B (Fig. 11). Ideally, one should use the *in vivo* dog concentration–effect profile to target effective concentrations enabling predictions of human doses.

Clinical information (PoP) has also been utilized in Case Study 1 of test compound B to bridge preclinical *in vitro* and *in vivo* data.

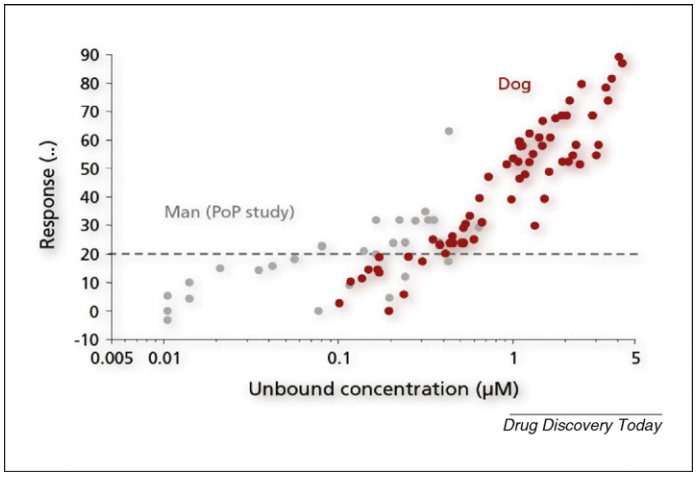

FIGURE 11

Response versus unbound concentrations of test compound B in humans (PoP study) and dogs. Note how well data superimpose in the 0.1–1 μM concentration range.

Dose–effect–time analysis

The most common approach to *in vivo* PKPD modeling involves sequential analysis of plasma concentration versus time data and effect versus time data, such that the plasma kinetics drives the dynamics. Under certain circumstances, such as for local drug delivery, systemic exposure to drug may not be needed. Hence, systemic concentrations are not measured; nor do they add value. In such a situation, effect versus time data inherently contain useful information about the turnover characteristics of the effect (half-life of response), the drug's biophase kinetics and the PD characteristics (potency). This situation is of a particular relevance in early drug discovery when a validated bioanalytical assay may not be available, *in vivo* PD data are good, and some knowledge of the inter-relationship between the drug's kinetics and dynamics would be advantageous. Examples where such analysis has been/would be beneficial include situations where local drug delivery is required (budesonide), negligible systemic concentrations are obtained (Xalatan), active (omeprazole) or reactive metabolites are produced, the drugs are endogenous substances, and there is a need to predict clinical doses [17,18].

Case Study 1: preclinical study design and estimation of clinical doses

Study design

Leading into LO studies for test compound B, the plan was to estimate potency for the primary effect (AERP prolongation) and safety margins against unwanted cardiovascular effects in the anaesthetized dog model by repeated rapid intravenous injections

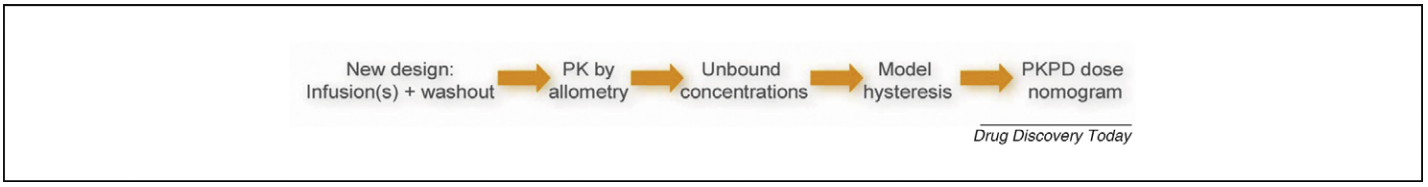

FIGURE 12

Scheme of the PKPD (quantitative pharmacology) strategy during LO for test compound B. It was decided to use a new design strategy, predictions of PK parameters in human by means of allometry. Comparisons across compounds and species were based on unbound plasma concentrations. Temporal differences were taken into account and a dose nomogram combining both PK and PD uncertainties was constructed for human doses.

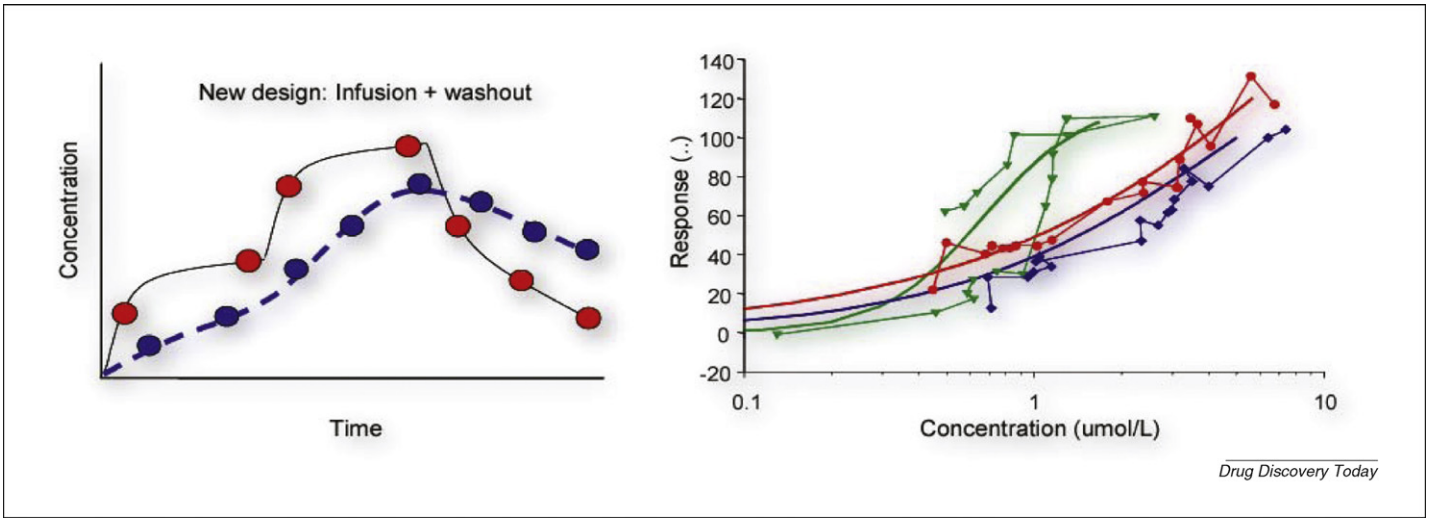

FIGURE 13

Schematic illustration of the proposed multiple infusions regimen with washout (left, red symbols denote plasma concentrations and blue the pharmacological effect). The right hand panel shows hysteresis loops of observed concentration–effect data (symbols) and the fitted concentration–effect relationship at equilibrium (smooth lines). Note that concentration and response measurements do not have to occur at the same time points. An appropriate PKPD model will take temporal sampling differences into account.

of preCD candidates. As a result of close interaction between pharmacology and DMPK, the original protocol was thoroughly revised (Fig. 12).

Major changes included replacement of rapid intravenous injections with a multiple constant drug infusion protocol followed by a washout period with continuous recordings (Fig. 13, left). Thus, temporal differences between concentration and effect could be used (hysteresis, Fig. 13 right) and adjustments made for plasma protein binding. Plasma protein binding was obtained *ex vivo* for the prediction of unbound concentrations.

Clinical dose predictions

In collaboration with DMPK it was decided that the Candidate Drug Target Profile (*CDTP)* should *not* include specific screening criteria on caco-2 permeability, oral bioavailability, *in vivo* clearance and intrinsic clearance in microsomes. Rather, the *CDTP* should be based on the combined utility of such criteria (i.e. the estimated clinical dose), which was set as 1/4 of the clinical dose of the forerunner. Therefore, key variables for dose prediction were *in vivo* effective unbound concentration for 20% prolongation of AERP (C_{u20} dog) and predicted human clearance (on the basis of rat, dog and monkey). A dose nomogram was constructed for the range of clinical doses, incorporating the experimental uncertainty in target concentration (C_u) and uncertainty of predicted human clearance (Table 3). Differences in plasma protein binding across compounds and species/strains were also incorporated. The data were then used for clinical dose predictions (Table 3) and for a more accurate comparison of cardiovascular safety margin across compounds.

Factors to be considered for the human starting dose need to include the novelty of the agent, its *in vivo* potency and receptor occupancy in humans, mechanism of action, knowledge of species-specificity of receptors, *in vitro* cell concentration–response curves of biological effects in animals and human, quantitative methods of PKPD reasoning and safety margin on the basis of unbound concentrations.

In summary, an integrated approach involving quantitative pharmacology (PKPD) better categorizes candidate drug profiles and thus improves LO. A key achievement in this project was the

TABLE 3

Predicted dose (shaded area) as a fraction of forerunner dose

CL/F	Effective concentration range		
	1/6	**1/3**	**1/2**
1/4	1/24	1/12	1/8
1/2	1/12	1/6	1/4
3/4	1/8	1/4	~1/3

The estimated target concentration test compound was 1/3 and the predicted oral clearance (CL/F) was 1/2 of forerunner, giving a daily dose, which is about 1/6 (1/3 × 1/2 = 1/6) of that of forerunner. The red area (~1/3) denotes goal not fulfilled.

thorough redesign of the *in vivo* pharmacological model, which meant abandoning the multiple rapid injection regimen in favor of the controlled intravenous infusions regimen followed by washout. By combining the data so obtained with predicted clinical PK it is then possible to compare more accurately the safety margin of different compounds and to make more informed predictions about potential clinical doses. The collection of both rising and declining response–time and concentration–time data, is, from an experimental design point of view, the optimal approach.

Case Study 2: PKPD redesign of in-house safety pharmacology prenomination studies

Safety margins are essential information for different project transitions. A useful example is the safety margins for hERG/*QT* effects. Generally, a safety margin between the therapeutic (steady-state) plasma level C_{ss} and *QT* prolongation (e.g. in the dog) of a factor of 100 was considered to be necessary to minimize the risk for *QT*-related arrhythmias in human. As C_{ss} is often very difficult to predict (depending, e.g. on target class and compound of interest), such calculated safety margins are frequently totally misleading in decision-making. Therefore, in collaboration with safety pharmacology, the protocols and design of all in-house prenomination SPT studies were scrutinized. The most important change was to replace the repeated bolus injection regimen with a multiple continuous infusion regimen followed by washout. The exact design, including the length of the infusions and the length of the washout period, has to be based on prior pharmacokinetic information of each specific test compound. In an ongoing program these modified protocols are being tested in the different SPT models that have been used. It seems probable that these tests will show that calculations of safety margins can be greatly improved. If so, the modifications will be implemented and communicated to other AZ sites where that particular SPT model is being used (e.g. guinea pig monophasic action potential duration (MAPD) method). Table 4 displays a schematic diagram for the assessment of safety margins.

TABLE 4

Safety margins (shaded area) based on unbound clinical target concentration tabulated against unbound concentration for QT_{10}

Unbound QT_{10} concentration (nM)	Unbound target concentration (nM)		
	10	100	1000
100	10:1	1:1	<1
1,000	100:1	10:1	1:1
10,000	1000:1	100:1	10:1

The red area denotes no safety margin.

There are still advocates of multiple rapid intravenous injections for the assessment of potential cardiovascular risk factors [19]. This is because there is a substantial risk of over- or underpredicting risk, simply because of the inoptimal design. Figure 14 demonstrates this situation by means of a simulation of a study protocol. The pharmacodynamic responses (dashed vertical lines, left) are measured before the actual exposure measurement (solid red circles). When plotting the concentration–response relationship (Fig. 14, right) there is a substantial possibility of overestimating the risk, because the measured response reflects the prior exposure history rather than the actual (subsequently determined) exposure.

The blue line (Fig. 14, right) represents the concentration–response relationship at steady-state. This relationship is difficult to obtain from the multiple injection (bolus) regimen which lacks information about the upswing/downswing of both concentration and response over time. This is why we advocate a safer and more scientifically attractive multiple infusions + washout protocol.

Ideally, the key message from a design point of view is that we collect response and concentration measurements on both the up- and down-swing of their respective time curves. The more optimal study design then leads to improved quality of the data and better assessment of the safety margin.

In summary, the decision-making that underpins the design of SPT studies is enhanced by a PKPD approach, enabling safer and more appropriate models to be employed and, therefore, better assessment of safety margins.

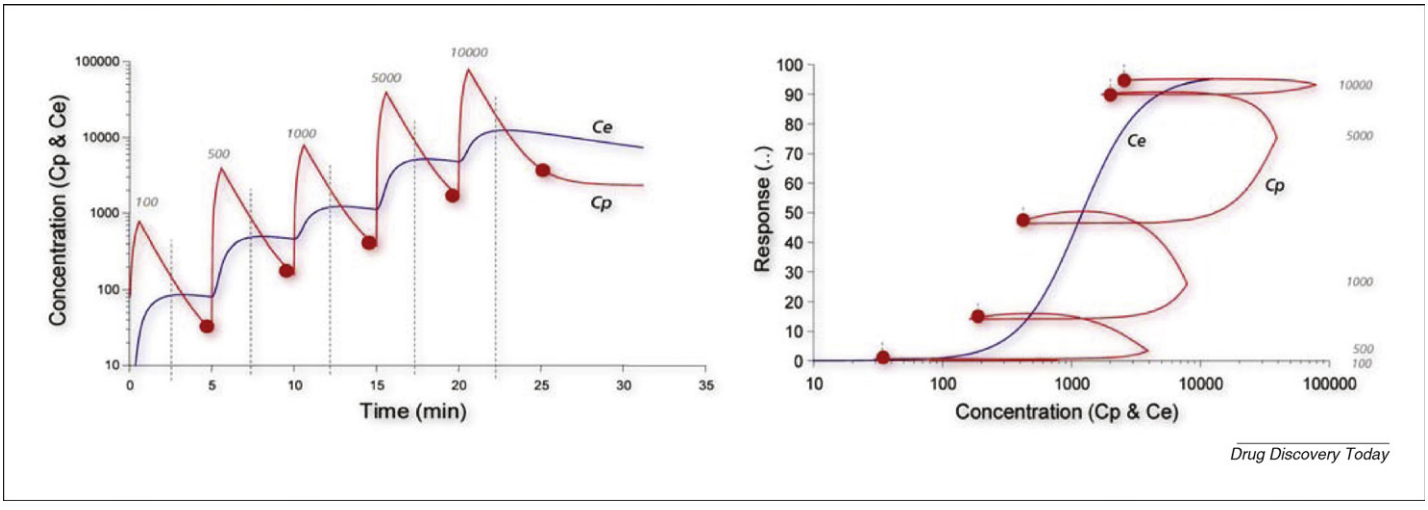

FIGURE 14

The left hand plot shows the concentration–time relationship of plasma concentration (C_p, solid red line) and the biophase concentration (C_e, solid blue line). The dashed vertical lines indicate the time of response measurement and solid red circles the times of plasma exposure measurements. The right hand plot shows the plasma and steady-state concentration–response relationship. Note that the impression of overestimating the risk (e.g. QT-prolongation, MAPD) is pronounced with this protocol.

Case Study 3: PKPD strategy and dose predictions

This case study exemplifies a situation with large temporal differences between plasma concentration of test compound and the pharmacological response. The LO program for test compound C had an integrated approach to *in vivo* PK and PD. The parameters governing LO were the *in vivo* kinetic parameters (CL, bioavailability) and *in vivo* potency IC_{50}. Test compound C is active at very low total plasma concentrations ($f_u < 0.1\%$, high potency), enabling low systemic exposure and a high safety margin. A low predicted human dose was achieved with the combination of IC_{50} and CL. The PKPD strategy was to model exposure from repeated oral dosing (gavage) and body weight turnover (PD) in mice on a high caloric café diet, to estimate potency and half-life of effect. These potency values also served together with safety parameters as a basis for the safety margin (Fig. 15).

The PKPD strategy was to model the concentration–effect data from repeated oral dosing. Using data on scaled clearance (CL/*F*) and a target concentration range (including IC_{50} obtained from modeling), a dose nomogram with tentative clinical doses was constructed for compound C (Table 5).

Improvements in the model included physiological limits and a complete response time course for the washout period including the return to baseline and beyond. The PKPD mouse (disease) model was then scaled allometrically to human applying the primary model parameter k_{out} (half-life of response), and the *IC_{50} parameter* was corrected for plasma protein binding differences between animals and human (for the selection of effective target concentrations).

In summary, the key components of this analysis are the model-based estimation of effective plasma concentration range C_e and the turnover half-life of the biomarker. The latter may have a bearing on the duration of the first repeated dose human study. An integrated PKPD approach in animals followed by allometric scaling to human provides a basis, not only for SPT studies, but also for predictions of drug clearance and dose size in human.

TABLE 5

Dose nomogram of the predicted dose size (mg) for test compound C based on *in vivo* effective unbound plasma concentration range (nM) and predicted human clearance values (L min^{-1})

Clearance (L min^{-1})	Effective concentration range (nM)		
	15	**100**	**150**
0.1	1 mg	10	15
0.17	2	15	20
0.34	5	30	50

Case Study 4: estimating dose of compound D in LO by calibration with forerunner information

The LO program for a back-up to compound D (inhibitor) has combined PK and PD knowledge from preclinical and clinical studies on compound D to identify which back-up compounds have the greatest potential for convenient daily dosing in human. Plasma concentrations of compound D at a known effective dose were simulated based on its known PK. A theoretical relationship between preclinical effect and clinical plasma concentration was then established by coupling the human effective plasma concentration to the effect observed at these concentrations in the rat PD model. Drug was infused to different steady-state levels in different groups of rats and the change in biomarker determined at equilibrium, providing full

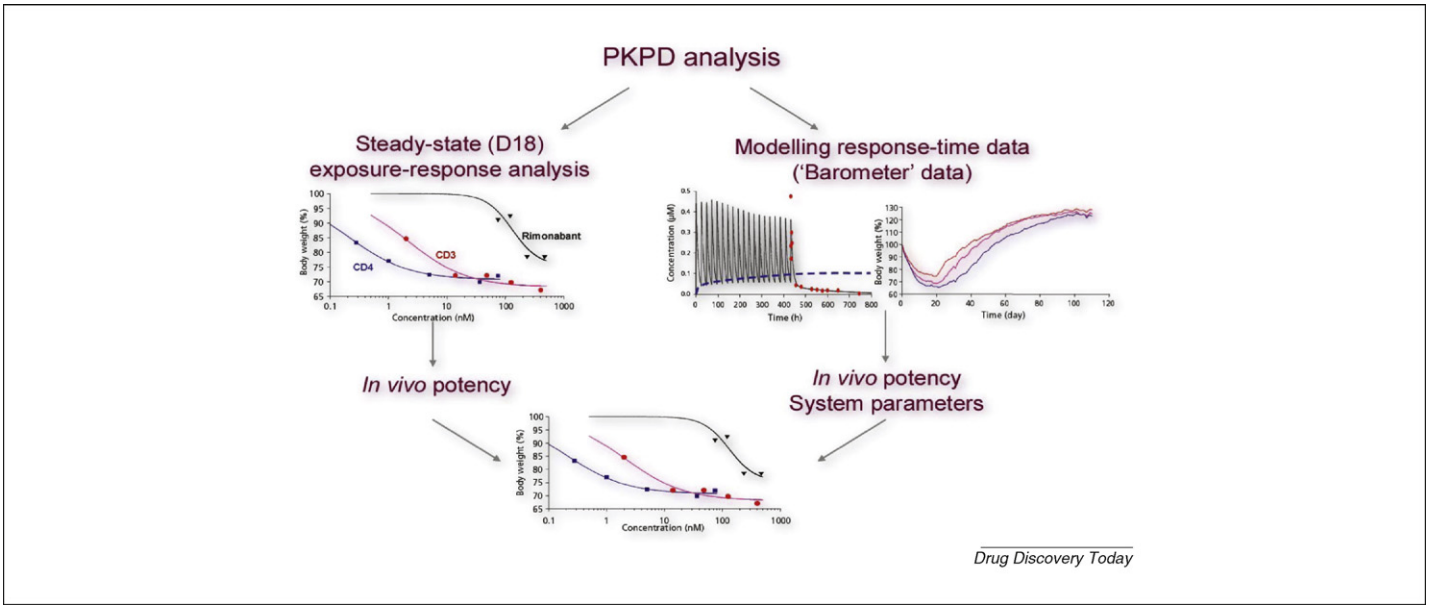

FIGURE 15

Conceptual illustration of two modeling approaches for the analysis of body weight reduction as a function of exposure and time. The left hand approach uses body weight reduction Day 18 of treatment as a function of the steady-state exposure level for the estimation of potencies of test compound C. The right hand approach simultaneously fits a PKPD model to the complete response–time courses of three dose (response) levels. The latter analysis arrived at similar potency values for the compounds as the steady-state approach. A PKPD model (right hand approach) can then be used for predictions of the response–time courses at other dose levels as well as for scaling of PK and PD properties to human. The key properties in the PKPD model (right) are the half-life of response and potency of test compound. Both approaches gave the concentration–response relationship at the bottom of the figure. Both the steady-state approach (left) and the PKPD modeling approach (right) should lead to the bottom plot including an effective concentration range.

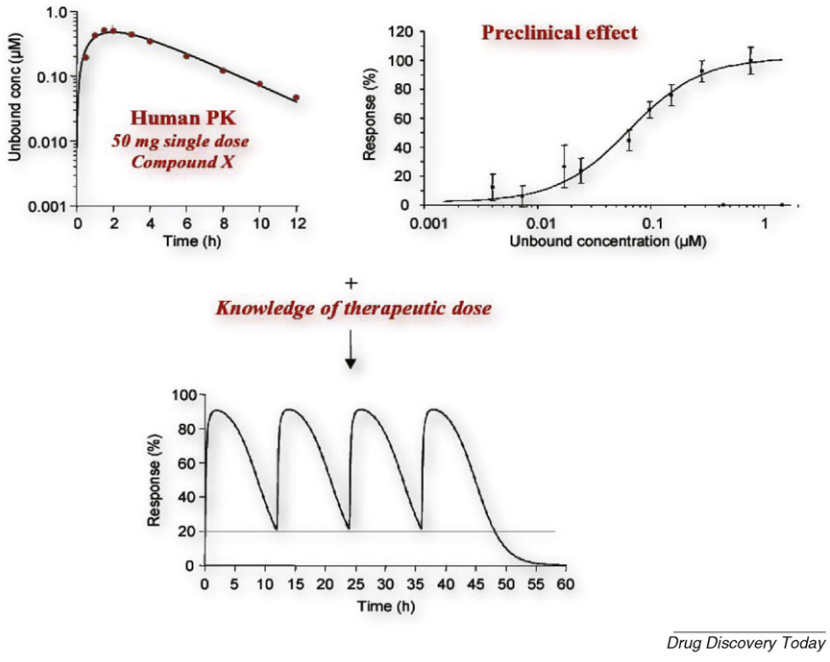

FIGURE 16

Assessing a relationship between human PKPD and preclinical PD for reference compound D. Upper left plot shows on semi-logarithmic scale a concentration–time course after 50 mg of compound D in humans. Upper right hand plot shows the concentration–response relationship in rats. The bottom plot is a simulation combining the upper two relationships for repeated dosing. The horizontal line at 20% response is the lowest effective response level.

concentration–effect data for selected compounds. The resultant relationship was simulated graphically by relating the effect in the preclinical model to time after giving a therapeutically effective dose of test compound D (Fig. 16).

Because clinical data suggested that the effect was correlated with the duration above C_{min} rather than the steady-state concentration *per se*, it was assumed that the minimum effect at any time (20%, Fig. 16) was the crucial parameter.

Human PK of the back-up compounds was then predicted, on the basis of allometry from one or more species. Predictions were updated because more data became available. The compounds were investigated by full concentration–effect curves in the rat *in vivo* model. The relationship established for the forerunner was then applied to the back-up compound, and the predicted effective dose set at a level resulting in a >20% effect at any time. Corrections were made for species-dependent differences in plasma protein binding because it was assumed that effect was driven by the free concentration.

In summary, the knowledge of the human PK of a compound together with knowledge of its therapeutic dose can be used to predict a relationship between human PKPD and preclinical effect. This relationship can then be used to determine the potentially effective dose of back-up compounds.

Case Study 5: modeling of functional adaptation and rebound for the assessment of the impact of slower plasma kinetics

The nicotinic receptor agonist project battles with two fundamental challenges with respect to flushing (adverse effect) and tolerance/rebound of the primary effect (antilipolytic effect, reduction of the plasma nonesterified free fatty acid [NEFA] levels). Nicotinic acid has a low volume of distribution (0.3 L kg^{-1}) and systemic clearance (0.28 L h^{-1} kg^{-1}), and the bioavailability is good (>80%) in human. It is metabolized to nicotinuric acid (conjugation with glycine; low affinity, high capacity and saturable) and nicotine amide (high affinity and low capacity). Nicotinic acid exhibits rapid turnover in plasma of laboratory animals (rat, guinea pig) and human, with a half-life of 1–5 and 30–60 min, respectively. Drug–drug interactions have been shown with salicylic acid, which also uses the conjugation route with glycine.

The rapid rise in the plasma concentration after a dose of nicotinic acid has been documented to cause flushing which lasts for about 30–60 min in human. The same has been shown in the guinea pig. The lowering of NEFA (mM) also has a rapid turnover with a rapid onset, steep concentration–response relationship, tolerance development, and a dramatic rebound effect upon cessation of the nicotinic acid dosing. The mean antilipolytic effect during a multiple constant rate infusion regimen (followed by washout) of nicotinic acid to four rats is shown in Fig. 17 together with the model-predicted response. Figure 17 also shows a superimposed simulation of the same infusion regimen, but with an exponential decay of the infusion rate at 110 min. This shows that the rebound phenomenon could be avoided provided there was slower plasma kinetics of the test compound.

The primary response (antilipolysis) and flushing occur within the same nicotinic concentration range (0.7–1.5 μM) in plasma (Fig. 18). With clinical doses of 1–4 g nicotinic acid per day, this range is easily reached with a peak plasma concentration of 100–250 μM, although the exposure does not last more than a couple of hours owing to the rapid plasma kinetics. Extended release (ER)

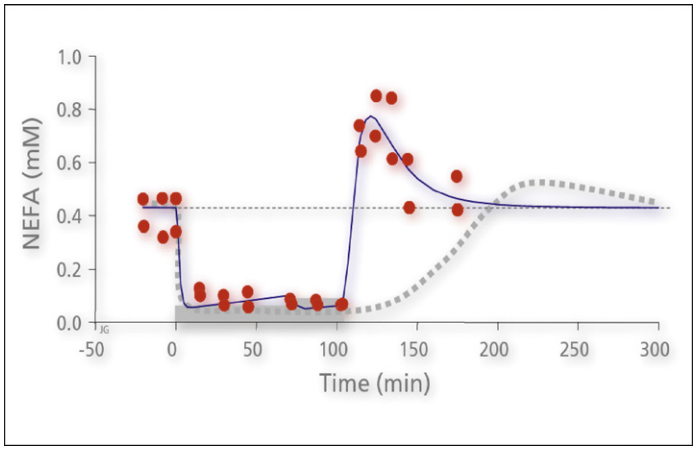

FIGURE 17

Observed (symbols) and predicted (solid line) response (reduction in plasma concentration of non-esterified fatty acids (NEFA)-time data following a multiple infusion regimen of nicotinic acid to rats. The solid grey bars show schematically the increasing infusion rates and their duration. The red dashed line indicates the baseline and the dashed grey line a simulation of the NEFA time course following exposure to a test compound with an extended half-life.

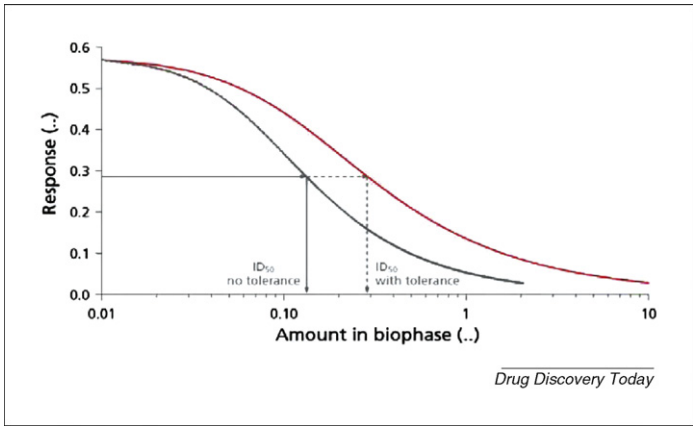

FIGURE 19

Schematic plot of NEFA response versus amount (μg) of nicotinic acid in the biophase at equilibrium. Curves are normalized to the same baseline value. The gray curve denotes a nontolerant system and the red curve a system with feedback. The same dose gives less effect in a tolerant system as compared to a nontolerant.

formulations, with a slower absorption rate profile of nicotinic acid partly prevents flushing but there is a risk of hepatotoxicity (nicotine amide route of metabolism) and gastrointestinal adverse reactions.

Because flushing may seem to be driven by the rate of change in the nicotinic acid concentration rather than the steady-state concentration *per se*, and because it is also subject to tolerance development, a PKPD model could simulate different input scenarios and thereby elucidate optimal absorption profiles (shape of the rise of nicotinic acid plasma concentration) of nicotinic acid. As shown in Fig. 17, one may hypothesize that slower plasma kinetics (longer half-life) may ameliorate the effects of rebound. The test compound would be 'on board' longer than nicotinic acid, to counteract the rebound, which PKPD modeling could cast light on.

Figure 19 may be viewed as the *real value* of this analysis. Going from a rather complicated biological behavior as a result of a multiple consecutive intravenous infusion regimen followed by washout, we have by means of a modeling approach condensed the complexity into a relatively simple dose–response relationship of Fig. 19. A tolerant system requires more drug to give the same response at equilibrium. This is displayed in the right-ward shift of the red dose–response curve. In this case, one needs more than double the dose at the 50% response level.

In summary, PKPD modeling enables important predictions to be made, such as the effects of changes in drug delivery and kinetics. The goals of such an analysis are to condense complexity (Fig. 17) to simplicity (Fig. 19). In other words, the impact of pharmacodynamic complexities such as tolerance and rebound

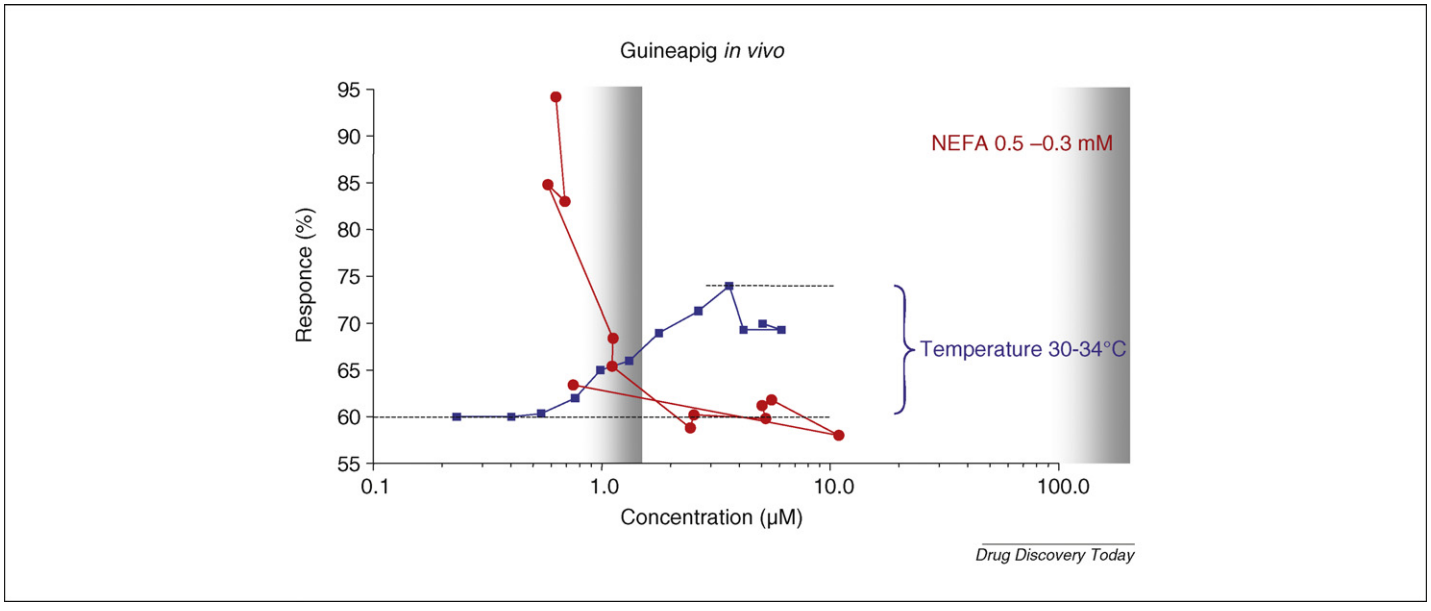

FIGURE 18

Observed response (NEFA filled red circles, temperature filled blue squares) versus total plasma concentration of nicotinic acid in the guinea pig after a constant intravenous infusion followed by washout. Note the steep concentration–response relationship of NEFA and also how the primary effect (NEFA) coincides with the adverse effect (temperature increase) at similar plasma concentrations.

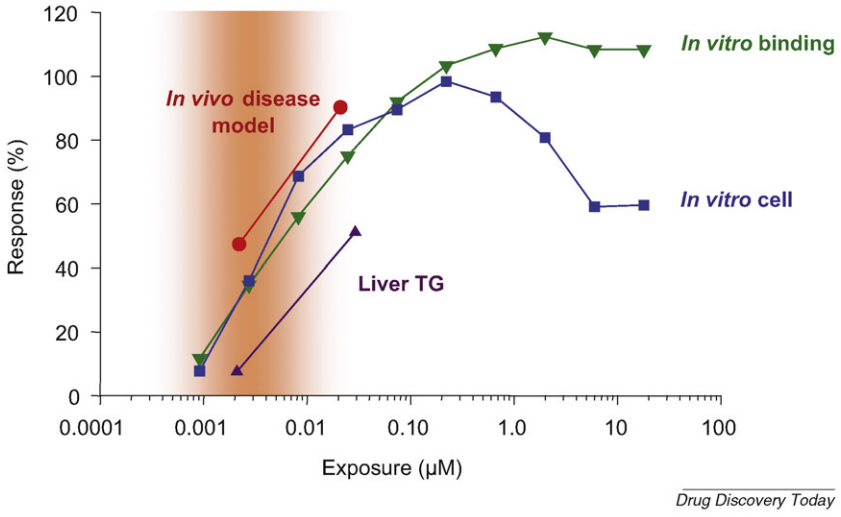

FIGURE 20

Pooled data of *in vivo* curing rate (disease model, red symbols), *in vitro* receptor binding (filled green triangles), *in vitro* gene expression (filled blue squares) and *in vivo* liver triglycerides (TG filled purple triangles) plotted against plasma concentration of test compound E.

can be tackled by quantitative models. Utilizing this information it is possible to target changes necessary for improving a compound's profile.

Case Study 6: linking preclinical disease model information to *in vitro* screening binding and gene expression data

Compound E was carried through a pivotal chronic *in vivo* study over a couple of months to establish its disease-curing effect in animals. The outcome was exceptional in that a 45 and 90% disease curing effect was seen at approximately 2 and 20 nM plasma concentrations at steady-state (Fig. 20). Screening data of *in vitro* binding and gene expression were collated and plotted in the same diagram to show the relationship between *in vivo* and *in vitro* characteristics. It is encouraging to note that *in vitro* binding falls in the same ballpark exposure-wise as the positive outcome in the *in vivo* disease model.

It is also striking that exposure to test compound giving a 40–50% curing rate in the *in vivo* disease model results in a very low (high specificity) triggering of liver triglycerides. The latter used as a biomarker of untoward effects. On the contrary, aiming for a 90% or higher curing rate *in vivo* results in a higher level of liver triglycerides owing to higher test compound exposure (lower specificity).

In summary, the integrated PKPD approach employed in this case study, illustrates the predictive advantage gained when data from *in vivo* disease models are interpreted and integrated with high throughput *in vitro* or *in vivo* biomarker data. This has important implications for TV, *in vitro*/*in vivo* correlation and first time in human studies.

Perspectives

Quantitative pharmacological reasoning (PKPD) focuses on concentration–response and response–time relationships with special emphasis on the impact of drugs on disease. The aims of this report are firstly to raise awareness among kineticists and pharmacologists (regardless of their area of therapeutic interest) as to why PKPD integration is essential for TV, optimizing development of lead compounds (LG and LO) and scaling these to human. A related aim is to demonstrate examples of PKPD collaborations that have improved the planning, execution and evaluation of experiments in primary and safety pharmacology. The examples discussed include design of TV studies, design and data 'pruning' of PKPD studies in LO, focus on the combined impact of unbound effective concentrations C_u and unbound clearance CL_u in maximizing the combined PK and PD value, analysis of data with marginal and substantial temporal differences between exposure and response, design of safety pharmacology studies, assessment of safety margin and assessment of uncertainties in prediction of first dose to human. We have not, however, discussed the importance of active or reactive metabolites which may be necessary to factor in at certain project stages, although we think this topic needs a more in-depth discussion in a separate forum.

A primary requirement for increasing awareness of the value of PKPD integration is establishing the inter-relationships between *in vitro* and *in vivo* PK and PD properties. This PKPD strategy should start during TV and continue throughout LG-optimization. One should strive for a kinetic–dynamic strategy based on concentration–time, response–time and response–concentration relationships. Related to this is the need to address key issues such as experimental design, temporal differences, exposure–response relationships, plasma protein binding differences, safety/efficacy margin(s), forerunner information, dose–effect–time relationships and the rationale for clinical dose settings during LO.

One way of enhancing awareness of the value of a PKPD approach is to collect and demonstrate good examples for each therapeutic area where this strategy has not just helped clarify data but has even changed the course of drug design and/or development. Another is to create an open and inviting collaborative environment for scientists in pharmacology, DMPK and safety. A

third, is by providing a generous continuing education package to give scientists at all levels an incentive not only to come to your company but also to stay and prosper within the PKPD area. In an effort to do this AstraZeneca has liaised with Gothenburg University on a Master's program in quantitative pharmacology (Integrated Quantitative Pharmacology, http://www.pharmguse.net/advanced/iqp-main.html). In addition, we run PKPD appreciation courses for medicinal chemistry and bioscience, and for project leaders about the advantage and requirements of quantitative thinking.

To benefit fully from PKPD reasoning throughout the drug discovery/development process, an all-level appreciation of organizatorial (resources) and scientific hurdles needs to be established.

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