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Pharmacokinetic–Pharmacodynamic Modelling: History and Perspectives

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A major goal in clinical pharmacology is the quantitative prediction of drug effects. The field of pharmacokinetic–pharmacodynamic (PK/PD) modelling has made many advances from the basic concept of the dose–response relationship to extended mechanism-based models. The purpose of this article is to review, from a historical perspective, the progression of the modelling of the concentration–response relationship from the first classic models developed in the mid-1960s to some of the more sophisticated current approaches. The emphasis is on general models describing key PD relationships, such as: simple models relating drug dose or concentration in plasma to effect, biophase distribution models and in particular effect compartment models, models for indirect mechanism of action that involve primarily the modulation of endogenous factors, models for cell trafficking and transduction systems. We show the evolution of tolerance and time-variant models, non- and semi-parametric models, and briefly discuss population PK/PD modelling, together with some example of more recent and complex pharmacodynamic models for control system and nonlinear HIV-1 dynamics. We also discuss some future possible directions for PK/PD modelling, report equations for general classes of novel semi-parametric models, as well as describing two new classes, additive or set-point, of regulatory, additive feedback models in their direct and indirect action variants.

KEY WORDS: pharmacokinetics; pharmacodynamics; modelling; review; history.

INTRODUCTION

Over the last 40 years, pharmacokinetics–pharmacodynamics (PK/PD) has evolved from the basic concept of the dose–response relationship to sophisticated models enabling the understanding of the underlying mechanism of drug action. This shift has primarily resulted from improved

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analytical methodologies, advances in computer hardware and software, increased regulatory and academic interest and the continuous refinement of pharmacodynamic models based on physiological mechanisms. In this article, we review, from a historical perspective, the progression of the understanding of the concentration–response relationship from the pioneering works in the 1960s to PK/PD modelling, established nowadays as a well-known scientific discipline. This review is not exhaustive, but rather traces models that symbolize key pharmacodynamic relationships or illustrate important features in use today. We only consider non-steady-state PK/PD experiments, thus models devised to describe the dynamics of the effects in response to changing drug concentrations (see also the recent minireview by (1)).

We first discuss a general modelling framework describing PK/PD models, and in particular so-called direct and indirect response. We will then chronologically examine the succession of important pharmacodynamic models from the 1960s until now (year 2004).

In particular, we will focus on direct concentration–response relationships describing: linear models and nonlinear models relating drug dose or concentration in plasma to effect, biophase distribution models and in particular effect compartment models, and early models describing irreversible interaction of chemotherapeutic agents with target cells. In the next section, we review models for indirect mechanism of action that involve primarily the modulation of endogenous factors and in a more general context and models for cell trafficking. Transduction systems will be then described. In the next section we will navigate through the 1980s and early 1990s, developmental years, which saw the emergence of tolerance, time-variant, non- and semi-parametric models and population PK/PD modelling. we will then give some examples of more recent and complex pharmacodynamic models, in particular control system and nonlinear HIV-1 dynamics and set-point models for oscillatory behaviour. The last part of this article will briefly discuss some future directions of pharmacodynamic modelling, such as computing, semi-parametric modelling and regulatory feedback modelling. To limit the size of the review, we will only briefly mention models for multiple drugs administration. In general all the models described below extends naturally to the case of multiple drugs once their respective pharmacokinetics are taken into account, and models for additivity, synergism or antagonism (2) are introduced to describe their interactions.

For purposes of clarity, efforts were made to keep equations as general as possible and the number of symbols to a minimum, for this reason the equations reported in the original references were changed to make notation uniform.

GENERAL FRAMEWORK

In the following we will take the point of view that the input to a PK/PD model is drug concentration in an observed compartment. We will assume that either parametric (e.g. multi-exponentials) or non-parametric functions (e.g. interpolants or regression splines[3]) adequately represent the time course of drug concentration in an observed site. This separation of pharmacokinetics from pharmacodynamics is of course appropriate for the case that the drug's dynamics do not influence its kinetics. Any PK/PD model so defined can be viewed as a model for the response (effect) of a system to an input (drug concentration). A classification of different kinds of PK/PD models has been proposed and discussed in Refs. (3–5).

We now give somewhat formal description of PK/PD systems. The response (effect, E) to an input in an observed site is functionally related to time (t) relative to the time of input of drug ($t = 0$), and observed drug concentration C, by means of a function (G):

$$E(t) = G[t, C(-\infty, t)] \tag{1}$$

where $C(-\infty, t)$ represents drug concentration in the observed site from time $-\infty$ to t. A system so defined depends on the input applied before and at time t: such as system is called causal or non-anticipatory. We will not consider anticipatory systems here, which depend on future inputs or expectations (as in mind/body interaction in, e.g., an addictive drug).

First, a system can be static or dynamic. For a static system:

$$E(t) = G[t, C(t)] \tag{2}$$

i.e. the effect depends only on the current time and concentrations of drug, but not on past concentrations. Such a system is also called memory-less. Equation (1) represents the more general dynamic or memory system, where the response depends on present and past inputs. In the case that

$$G[t, 0] = 0, \quad -\infty < t < \infty \tag{3}$$

the system is said to be relaxed or at rest, i.e., its response is excited by drug concentration, but zero otherwise. Many physiological responses

[3]Splines are flexible functions, which are basically piecewise polynomials that join at certain locations called breakpoints and match their derivatives up to the degree minus one of the polynomials. For example a linear spline matches only the values of the polynomials at the breakpoints to give the somewhat familiar "broken line".

can be considered relaxed once a constant baseline value is subtracted out. More complicated unrelaxed physiological responses are ones exhibiting circadian variation and/or non-constant production of endogenous substances.

Second, the response of a relaxed system to an input can be linear or nonlinear with respect to the input. For a linear system, the following holds:

$$G\,[t, \alpha C_1(-\infty, t] + \beta C_2(-\infty, t]] = \alpha G\,[t, C_1(-\infty, t]] + \beta G[t, C_2(-\infty, t]] \tag{4}$$

i.e., the response to the sum of two inputs equals the sum of the responses to each one, whereas in a nonlinear system, this is not true.

Finally, the system can be time-invariant or time-variant. For a time-invariant system, the response does not change irrespective of the time that an input is applied, while this does not hold for a time-variant one.

The convolution between two functions, h and I, represents a linear, time-invariant dynamic system at rest, i.e.

$$\int_0^t h(\tau) I\,(t - \tau)\,\mathrm{d}t \stackrel{\text{def}}{=} h^* I \tag{5}$$

can represent a PK system when, e.g., $h(t)$ is a disposition function, unit–impulse response, for a site of interest, and $I\,(t)$ an input function (6).

Most PK/PD models can be built by composing cascades of dynamic linear and static nonlinear sub-systems (7). Nonlinear dynamic systems (i.e., most interesting PK/PD systems) can be built up by combining dynamic and static functions, obtaining so-called cascade structured models. In such models, the output of one function is the input to another.

The so-called "effect compartment" model (8) is an example of what we call a direct-response model to contrast it with indirect action models (4) (see below). Both direct-response models and indirect-response models are composed of two sub-system cascades, one nonlinear static and one linear dynamic sub-model, but the order of the sub-models differs as described above.

DIRECT ACTION MODELS

In a direct-response model, a linear-dynamic model is followed by a static non-linear model. From a physiological point of view, such a model has the interpretation of a kinetic (often called the link) PK system followed by a memory-less interaction of drug with the body that describes the effect.

Linear E vs. Dose/C Relationships

In the early days of pharmacodynamics, it was recognized that the intensity of many pharmacological effects is linearly related to the logarithm of the amount of drug (A) (9,10):

$$E = s \log A + e \tag{6}$$

where s and e are the slope and the intercept terms. Levy (9,10) and Levy and Nelson (11) published a number of mathematical expressions, which related the time course of pharmacological activity of a drug to its first-order elimination:

$$\log A = \log A_0 - \frac{k}{2.3}t \tag{7}$$

where A_0 is an extrapolated intercept at time 0 and k is the first-order elimination rate constant. Substituting $\log A$ and $\log A_0$ in Eq. (6) from Eq. (5) and rearranging yields

$$\frac{E - e}{s} = \frac{E_0 - e}{s} - \frac{k}{2.3}t \tag{8}$$

which can be simplified and rearranged to

$$E = E_0 - \frac{ks}{2.3}t \tag{9}$$

where E_0 is a theoretical intercept. This relationship (Eq. (9)) was supported by studies of drugs that showed plasma concentrations decreasing exponentially and the effect decreasing linearly with time (9,10). Although limited by the assumptions of monoexponential drug elimination and linear dose–response relationship, these simple models provided pharmacodynamic parameters (slope values) that could be easily calculated graphically by simple linear regression. They provided quantitative measurements of the intensity of the pharmacological effect and were used to compare different drugs or combination of drugs. Concepts of duration of the response and effect after repeated drug administration (9,12) also evolved based on these simple relationships.

Nonlinear E vs. Dose/C Relationships

The apparent linear relationship between concentration and pharmacological response reflected the fact that effects were analysed over only a very limited concentration range. Moreover, linear models were recognized to be only valid when the effect was either less than 20% (linear) or within 20–80% (log-linear). Owing to these limitations, Wagner (13) proposed the use of the Hill equation to describe the concentration–response relationship. The rationale for this approach was based on the law of mass action and classical receptor occupancy theory (14). The rate of change of the drug–receptor complex (RC) is given by the following equation:

$$\frac{d\mathrm{RC}}{dt} = k_{\mathrm{on}}(R_{\mathrm{M}} - \mathrm{RC})C - k_{\mathrm{off}}\mathrm{RC} \tag{10}$$

where R_{M} is the maximum receptor density (therefore, $R_{\mathrm{M}} - \mathrm{RC} = R$, the concentration of free receptor), C is the drug concentration at the site of action, k_{on} is the association rate constant and k_{off} is a dissociation rate constant. At equilibrium, Eq. (10) can be rearranged to yield

$$\mathrm{RC} = \frac{R_{\mathrm{M}}C}{K_{\mathrm{d}} + C} \tag{11}$$

where K_{d} is the equilibrium dissociation constant $k_{\mathrm{off}}/k_{\mathrm{on}}$. What is now needed is a "transduction" function, $z(\mathrm{RC})$, which relates occupancy at the receptor level with pharmacological effect.

$$E = z(\mathrm{RC}) = z\left(\frac{R_{\mathrm{M}}C}{K_{\mathrm{d}} + C}\right) \tag{12}$$

Assuming that the response produced by the concentration of drug C is simply proportional to the fraction of occupied receptors σ, that is $E \propto \sigma\mathrm{RC}$, the effect is as follow (13):

$$E = \frac{E_{\max}C}{\mathrm{EC}_{50} + C} \tag{13}$$

where $E_{\max}$ is the maximal effect (proportional to R_{M}) and EC_{50} is the concentration of drug producing 50% of $E_{\max}$. We note that an alternative approach is proposed by (15), in which the simple model $z(\mathrm{RC}) = \sigma(\mathrm{RC})$ is criticized because, among other reasons, it would predict $\mathrm{EC}_{50} + C = K_{\mathrm{d}}$. The transduction function proposed by (15) is itself a hyperbolic (Michaelis–Menten) relationship, under which

$$E = z(\mathrm{RC}) = \frac{E_{\max}\mathrm{RC}}{K_{\mathrm{E}} + \mathrm{RC}} \tag{14}$$

where K_E is the concentration of RC producing 50% of E_{max}. Combining Eq. (11) with the previous equation yields

$$E = \frac{E_{max} R_M C}{K_d K_E + (R_M + K_E) C} \tag{15}$$

This approach allows differentiating between occupancy at the receptor level and K_E, which might prove important in *in-vitro/in-vivo* PK/PD correlation studies (16,17). Because Eqs. (13) and (15) collapse to the same model when no information about K_d is available, in the following we will only refer to model (13) and derive models according to the corresponding representation, it is understood that model (15) could be substituted in any place where model (13) will be encountered in the following.

Borrowing again from receptor theory, one can change Eq. (13) (as well as Eq. (15), see (15) to take into account for multiple receptor, and obtain the familiar Hill equation:

$$E = E_0 + \frac{E_{max} C^\gamma}{EC_{50}^\gamma + C^\gamma} \tag{16}$$

where we add E_0 to indicate baseline effect and when integer, γ represents the number of molecules bound to the receptor. These relationships are curvilinear and allow a more general description of the entire dose–response relationship.

A feature common to all models discussed above is that maximum effects are predicted to occur simultaneously with peak drug concentrations, since obviously if C^* is the maximum observed drug concentration, the maximum observed effect, E^*, is

$$E^* = E_0 + \frac{E^* C^{*\gamma}}{EC_{50}^\gamma + C^{*\gamma}} \tag{17}$$

However, it was soon observed that for many responses E^* lags behind or ahead of C^*, because of temporal displacements caused by kinetics, physiological or pharmacological mechanisms. In these situations a plot of E vs. C shows an hysteresis loop (C^* is before E^*, see e.g. bottom panel of Figs. 1 and 11) or proteresis (C^* is after E^*, see Fig. 13). A hysteresis or proteresis loop can in general be justified assuming a time-variant model (e.g. tolerance, see below), but hysteresis or proteresis cannot be justified by any model in which E is related to C by a time-invariant function, since if $C(t_1) = C(t_2)$ than $G[C(t_1)] = G[C(t_2)]$ for any t_1, t_2.

Biophase Distribution Models

In 1968, Segre (8) first introduced the concept of a biophase compartment (the term can be found back in Furchgott (18) in 1955, and (19), where "it indicates a space containing the receptors, or to be interposed between the receptors and ... extracellular fluid". This is the first model that explicitly introduces the concept of an hypothetical compartment which is driven by plasma concentrations and that is directly related to the effect, and it was used to explain the fact that the pressor effects of norepinephrine lagged appreciably behind its concentration profile in blood. Taking advantage of the emergence of sophisticated computing programs (in particular early versions of the SAAM program (20) currently distributed, in evolved form derived from CONSAAM (21) by the University of Washington (22)), he derived a model capable of describing the time delay between plasma concentrations and effect time curves. This model was further refined later in (23) and it will be described in detail below.

Around the same time, the awareness of delayed pharmacological effect relative to drug concentrations in plasma raised the alternative, and ultimately incorrect, hypothesis that the site of drug action might be associated with one of the compartments used to characterize the kinetics of drug disposition (for example the peripheral compartment in a two-compartment model). Wagner in 1968 (13) suggested that either plasma concentrations or another compartment could be associated to the effect. Shortly afterwards, Levy *et al.* (24) reported that the pharmacological effect of LSD was correlated to the drug level in the slowly equilibrating compartment. The same idea was still used years later, with the study of Sheiner and co-workers. (25) who reported that the prolongation of the QT interval more closely paralleled the saliva concentration curve of procainamide than the plasma concentration curve. Kramer *et al.* (26) also reported a closer relationship between the time course of inotropic effects and estimated digoxin concentrations in the slowly equilibrating peripheral compartment. Although, in some cases, it was thought plausible to associate a physiological compartment to the site of drug action, most authors emphasized correctly that a pharmacological response associated with a peripheral compartment of a disposition function model was probably a result of coincidence without any obvious physiologic interpretation, and that often observed compartments which were not of relevance for drug disposition appeared to be more, but imperfectly so, associated to drug effect.

In 1978, Dahlstrom *et al.*'s study (23), characterizing the relationship between morphine concentrations in rat plasma and whole brain and drug effects (vocalisation, and vocalisation after discharge) introduce a model that could solve the problem. In their study the time delay between the

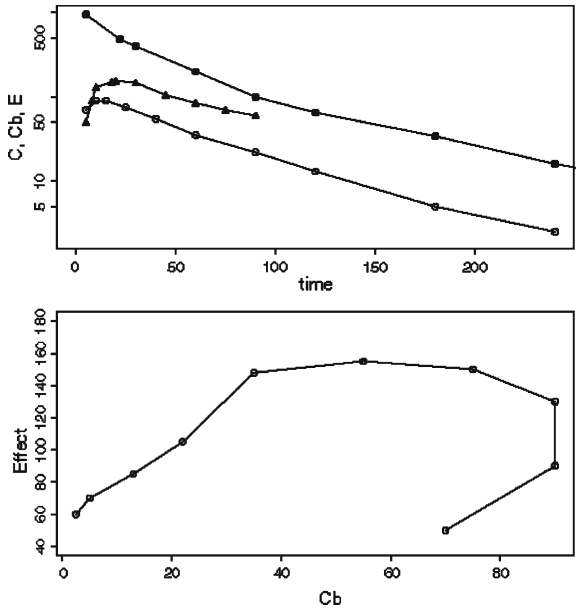


Fig. 1. Morphine plasma, brain concentrations and effect obtained graphically from (23). The delay between plasma concentrations C (circles), brain concentrations C_b (open circles) and response E (diamonds) (upper panel) gives rise to a counterclockwise hysteresis loop in the $C_b - E$ relationship (lower panel).

concentrations in either of the observed central or brain compartments and the response gives rise to a counterclockwise loop (hysteresis) in the C_b vs. E relationship (Fig. 1). Thus, even sampling a likely candidate for pharmacological effect (the brain) did not show a direct relationship between pharmacological effect and observed drug concentrations.

The model proposed in (23) was based on the effect compartment model presented in (8) and re-introduced an important concept for characterizing observed response in respect to PK. The model clearly decoupled drug disposition in plasma or in a peripheral compartment (the brain) from drug transport to an hypothetical effect site and ultimately drug effects.

Their model used two hypothetical effect compartments to adjust for the temporal dynamics of drug effect. A schematic representation of model is presented in Fig. 2. The distribution of drug between the two effect compartments is described as follows:

$$\begin{aligned} \frac{dC_{e_1}}{dt} &= k_b C_b - k_{12} C_{e_1} \\ \frac{dC_{e_2}}{dt} &= k_{12} C_{e_1} - k_{e_0} C_{e_2} \end{aligned} \tag{18}$$

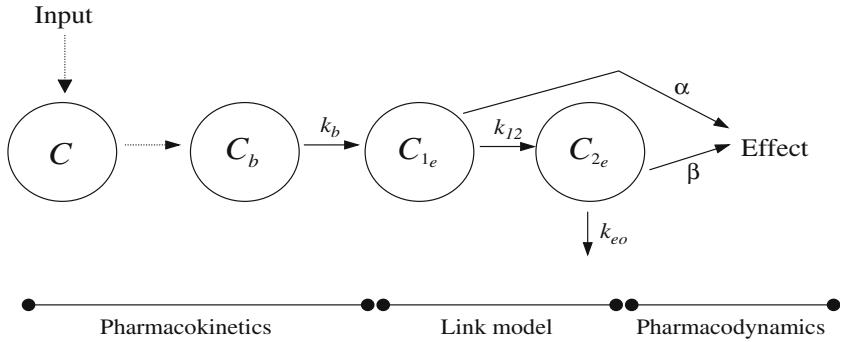


Fig. 2. Schematic representation of the model proposed by Segre (8,23), linking pharmacokinetics and pharmacodynamics. The brain concentrations C_b are linked to a first and then to a second biophase compartment (with concentrationsand C_{e_1} and C_{e_2}) by first-order rate constants (k_b and k_{12}), k_{eo} characterizes the rate of loss from C_{e_2}. Effect is a weighed sum of C_{e_1} and C_{e_2}: $E = \alpha C_{e_1} + \beta C_{e_2}$ (see text).

where C_b is concentration in brain, C_{e_1} and C_{e_2} are the concentrations in hypothetical effect compartments 1 and 2, respectively, k_b is the transfer rate constant from the brain compartment to the first effect compartment, k_{12} is the transfer rate constant from the first to the second effect compartment and k_{eo} (k_{21} in the original manuscript) is the elimination rate constant from the second effect compartment. The responses, vocalisation and vocalisation after discharge, were represented using a linear combination of the concentrations in the effect compartments (using summer functions in SAAM), that is,

$$E = \alpha C_{e_1} + \beta C_{e_2} \tag{19}$$

where α, β were coefficients to be estimated. Their model was used to simultaneously fit the pharmacokinetic data from plasma and (whole) brain, and the kinetics of the analgesic response. Since C_{e_1} and C_{e_2} are unobserved, the rate constant from the brain compartment to the first effect compartment was fixed, with no loss of generality, to an arbitrary unit value, and the kinetic of loss to the effect compartment was set up so that it did not influence the pharmacokinetics of the drug in brain.[4] The model recognized not only that an hypothetical effect compartment must be decoupled from PK disposition using a kinetic "link" model, but that often a complex link is necessary to represent effect data (a feature that was to be rediscovered almost 20 years later by, e.g. (27)).

[4]Using SAAM, this was simply achieved by adding a positive flux ($k_b C_b$) in the equation for the brain compartment.

The same approach was used by Sheiner *et al.* (28) by incorporating an effect compartment as a kinetic link between the plasma concentrations of a neuromuscular blocking drug and the skeletal muscle paralysing effect. As proposed by Segre, the amount in the effect compartment was linked to the plasma compartment by a first-order process:

$$\frac{dC_e}{dt} = k_{1e}C - k_{e0}C_e \tag{20}$$

where C_e is the drug concentration in the effect compartment, C is the plasma drug concentration, k_{1e} is first-order rate constant from central to effect compartments and k_{e0} the first-order rate constant of drug elimination from the effect compartment, characterizing the temporal aspects of drug equilibration with the site of action. The rate constant k_{1e} was set to a negligible value to avoid influencing the pharmacokinetics of the drug. The effect was then modeled by directly incorporating C_e into the Hill equation:

$$E = \frac{C_e^{\gamma}}{C_e^{\gamma} + EC_{50}^{\gamma}} \tag{21}$$

This, as it become to be known, effect compartment model, or link model became very popular and was used to explain the hysteresis in the $C-E$ plot of many drugs. In applications following (28), k_{1e} has been arbitrarily set equal to k_{e0}, as opposed to, e.g. the unit value of Segre's model. This choice adds the convenience that at steady state the concentration in the hypothetical compartment, C_e^{ss}, directly equals the steady-state concentration in plasma C^{ss}, instead of $C_e^{ss} = C^{ss}k_{1e}/k_{e0}$ as for model (20). In general the value of k_{1e} is irrelevant for the purposes of the PK/PD analysis.

Irreversible Effects

The use of log-linear or Michaelis–Menten models was justified by the assumption of reversible and fast, relative to PK, interaction between drug and receptor. However, the reversibility aspect of this mechanism precluded application to therapy with certain antibiotics, antimetabolites and alkylating agents, which usually depend on the irreversible or covalent incorporation of drug into cell metabolic sites or pathways.

Jusko (29) described a pharmacodynamic modelling approach for cell-cycle non-specific chemotherapeutic drugs (Fig. 3a). It introduced a compartment for chemotherapeutic effect, defined as separate from the central compartment and linked to it by first-order rate constants. Considering the

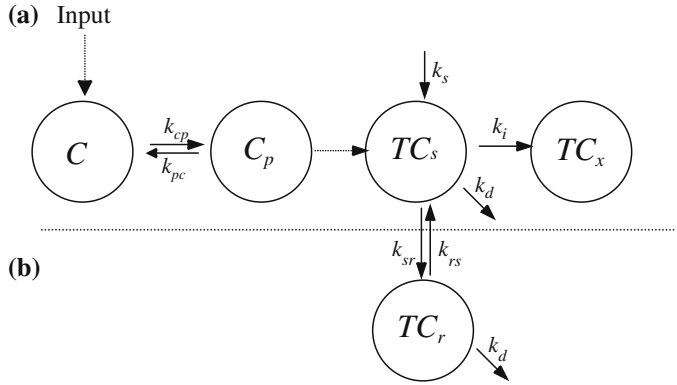


Fig. 3. (a) The site of chemotherapeutic effect C_p is a homogenous compartment in equilibrium with the central compartment C with first-order links (k_{cp} and k_{pc}). A small portion of the dose is involved in an irreversible reaction with the receptor on the target cells (TC = TCs for the cell-cycle specific inhibition model see below), which ultimately produces mitotic arrest of these cells (TC_x). It is assumed that viable cells increase in number (k_s) and are subject to physiologic degradation (k_d) (29). (b) Extension of the model to cell-cycle specific inactivation. The pool of targeted cells was divided into the sensitive cells, T_s and the insensitive cells T_r to the drug and is inter-convertible. Cells in each compartment are interconversible with rate constants k_{rs} and k_{sr} (30).

molecular interaction of drug and cell receptor and the natural cell turnover, the rate of change in quantity of target cells (TC) with time was written as

$$\frac{d\mathrm{TC}}{dt} = (k_s - k_d)\mathrm{TC} - k_i TC A_p \tag{22}$$

where k_s and k_d are rate constants for increase in cells number at their natural mitotic rate and physiologic degradation, respectively, k_i the rate constant for the irreversible reaction leading to the mitotic arrest of target cells, and A_p is the amount in the peripheral chemotherapeutic compartment (receptor site).

If F is the fraction of surviving cells ($F = \mathrm{TC}/\mathrm{TC}_0$ where ($\mathrm{TC}_0 = TC(0)$), after all potentially effective drug was eliminated at time t, it can be derived by integration of Eq. (22) to yield

$$\log(F) = (k_s - k_d)t/2.3 - k_i \int_0^t A_p(t)\,dt \tag{23}$$

Under the assumption that only a negligible fraction of A_p is involved in an irreversible reaction with the receptors (and therefore the distribution

of the drug to the tissue site could be approximated by a standard two-compartment model), it can be shown that after all the drug is eliminated, the relationship (23) becomes

$$\log(F) \approx (k_s - k_d)/2.3t - k_{12}/k_{10}k_i \,\text{dose} = \alpha - K \;\text{dose} \tag{24}$$

which predicts a log-linear relationship between the fraction of surviving cells and the dose of the drug. The parameter K was used to quantify the chemotherapeutic efficacy of different drugs.

Under the main assumption of the model, negligible fraction of drug lost to interaction, the right side of Eq. (24) is basically independent on the distribution pharmacokinetics of the drug. As pointed out in the original reference, the model could be used to follow the dynamics of cell survival and the kinetics of drug, but in this case the distribution part of the model (which "disappears" in the asymptotic relationship with dose was derived) would need elaboration, while still retaining the assumption of receptors contained in a homogeneous compartment which does not alter drug kinetics. Partial incorporation of drug kinetics together with a further refinement to cycle-specific irreversible inactivation was described a couple of years later (30). This model was based on a two-compartment cell system where the pool of targeted cells was divided into the sensitive cells, TC_s and the insensitive cells TC_r to the drug (Fig. 3a, b). Cells in each compartment are inter-convertible, with transformation rate constants k_{rs} and k_{sr}. Equation (22) was rewritten as

$$\begin{aligned} \frac{dTC_s}{dt} &= k_{rs}TC_r + (k_s - k_{sr})TC_s - k_i TC_s A_p \\ \frac{dTC_r}{dt} &= k_{sr}TC_s - k_{rs})TC_r - k_d TC_r \end{aligned} \tag{25}$$

where drug only interacts with the sensitive cells. The dynamics of cell survival were analysed by simplifying the model using the negligible fraction of drug lost to interaction assumption. Thus, model (25) becomes

$$\begin{aligned} \frac{dTC_s}{dt} &= k_{rs}TC_r + (k_s - k_{sr})TC_s - TC_s K \;\text{dose}\; N \\ \frac{dTC_r}{dt} &= k_{sr}TC_s - k_{rs}TC_r - k_d TC_r \end{aligned} \tag{26}$$

where N is the total number of given doses. Equation (26) can be seen immediately as describing a bi-exponential (decay) function, and the solution to (26) was fit to the cell-survival data (Fig. 4 corresponding to Fig. 4 in the original manuscript). The incorporation of multiple doses

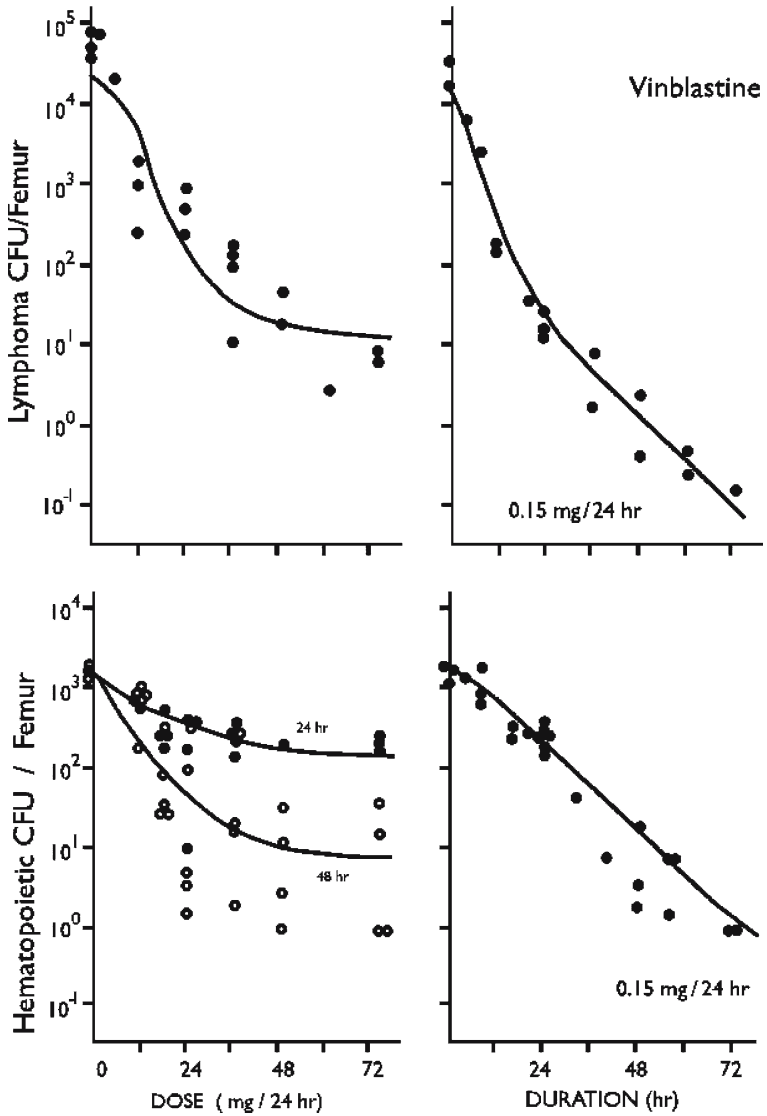


Fig. 4. Dose–time–survival curves for the effects of vinblastine on hematopoietic and lymphoma cells in the mouse femur. The circles are experimental data the lines represent the nonlinear, least-squares computer fit of the data to the model. Reproduced from (30) with kind permission from Springer.

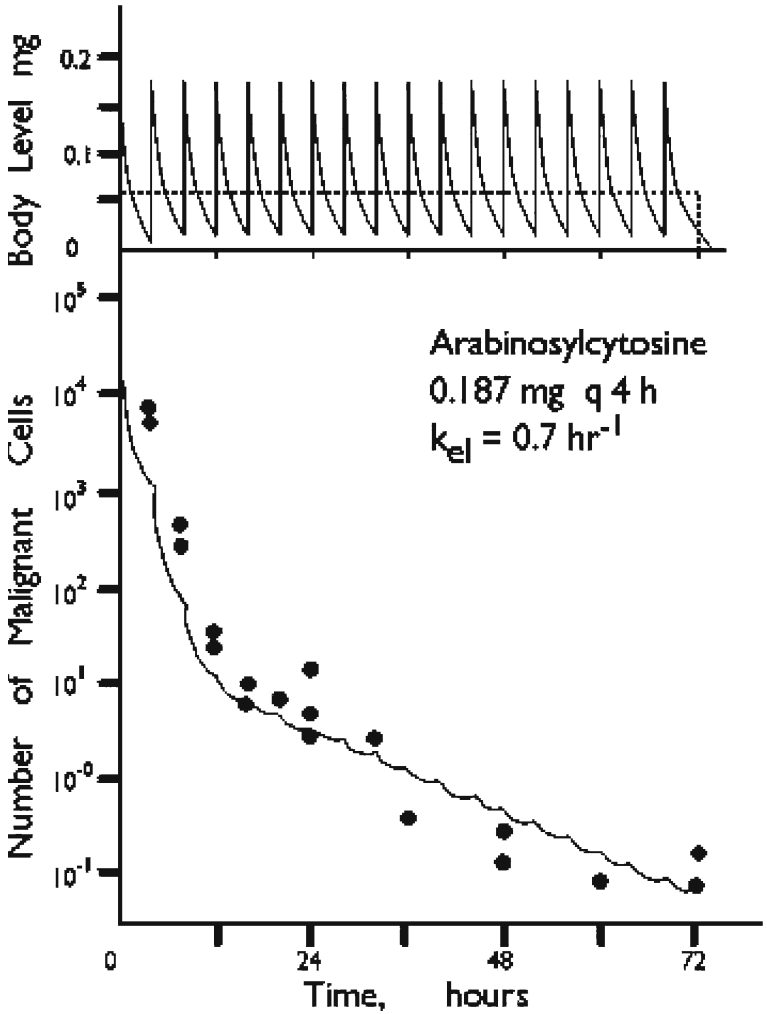


Fig. 5. Cell-survival vs. time curve for the effects of multiple doses of arabinosylcytosine on lymphoma cells in the mouse femur. The circles are experimental data and the theoretical cell-survival curve (solid line) was generated by numerical integration. Reproduced from (30) with kind permission from Springer.

drug kinetics in the model could only be achieved using simulation (since the software available to the author did not allow to fit a model expressed as a set of differential equations) (see Fig. 5, corresponding to Fig. 6 in the original manuscript).

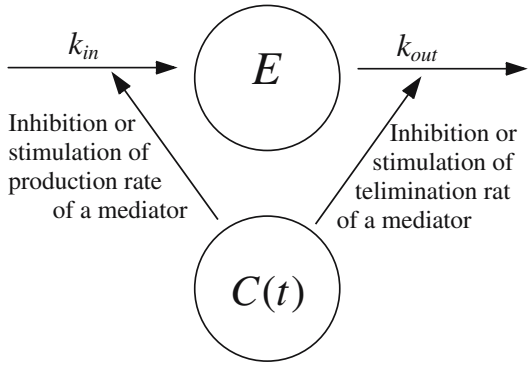


Fig. 6. Basic concept of indirect pharmacodynamic response models (4). Effects are mediated by an endogenous substance (mediator). Indirect acting drugs modulate these effects (E) by either stimulating or inhibiting the production rate (k_{in}) or the elimination rate (k_{out}) of the mediator.

INDIRECT ACTION MODELS

Two basic conceptual approaches have been developed for analysing hysteresis between drug plasma levels and pharmacodynamic response: in the previous sections, we discussed direct models, in which the pharmacological effect was considered a direct consequence of drug action and the delay in response was thought to reflect the time required for the drug to reach its site of pharmacological action. Alternatively, the drug receptor interaction initiates a series of downstream biochemical events that account for the observed time lag. Indirect mechanisms of action involve primarily the modulation of endogenous factors that mediate the drug effect. In a more general context, we present in this section other types of indirect models, namely models for cell trafficking.

In indirect dynamic models, a dynamic linear sub-system follows a static nonlinear one. The dynamic model describes the formation and loss of an endogenous variable. From a physiological point of view, such a model has the interpretation of a memory-less interaction of drug with body structure, followed by an endogenous (linear) kinetic system directly related to the effect.

Modulation of Endogenous Factors

The earliest description of drugs acting through indirect mechanisms came from Ariens (14). He explained how drugs induced their effects not because of their interactions directly linked to an observable effect with receptors, but because the interaction affected the fate of endogenous

compounds and effects that were mediated by those substances. In 1968, Nagashima *et al.* (31) proposed the first outline of an indirect action model, capturing prothrombin complex activity (P) in the blood of normal volunteers who received oral dose of warfarin. They treated the complicated processes of blood coagulation by introducing two processes that describe prothrombin synthesis and degradation.

The net change of prothrombin complex activity (P_{net}) was modeled as

$$P_{net} = P_{syn} + P_{deg} \tag{27}$$

where P_{syn} is prothrombin synthesis rate, P_{deg} the prothrombin degradation rate. Nagashima *et al.* showed that the prothrombinopenic response to the anticoagulant drug, which appears 2–4 days after the administration of a single dose, could be related linearly to P_{syn} according to

$$P_{syn} = P_{syn}^0 - s \log C/C_{min} \tag{28}$$

where C_{min} is the minimum extrapolated warfarin concentration at P_{syn}^0, s is a slope and $P_{syn}(0)$ the level of prothrombin before drug administration. Based on the relationships described above and assuming first-order elimination of warfarin (according to Eq. (7)), he derived a mathematical expression that permitted the determination of the time course of P:

$$\frac{dP}{dt} = k_{deg}P^0 - s \log \frac{C^0}{C_{min}} + \frac{sk}{2.3}t - k_{deg}P \tag{29}$$

where $k_{deg}P^0 = P_{syn}^0$ with k_{deg} the degradation rate constant and C^0 is the extrapolated concentration a time 0. His study demonstrated that the time course of P following warfarin administration could be adequately characterized on the basis of rate constants for the synthesis and the decline of this factor.

Shortly afterwards, Sheiner (32) described a computer program for warfarin dosage adjustment in patients. The program was designed to suggest dose adjustment based on previous prothrombin times, drug dosage and therapeutic goals and limits. The effect of warfarin was modeled assuming that the synthesis rate of the clotting factor was decreased by increasing drug levels up to some maximum level at which the clotting factor synthesis rate becomes zero. He used a linear model for the effect of drug with constraints on the fractional effect to lie between zero and one according to

$$\begin{aligned} \frac{dP}{dt} &= -k_{\text{deg}}P + (1 - xC)k_{\text{syn}} \quad \text{for } 1 - xC > 0, \quad P < 1 \\ \frac{dP}{dt} &= -k_{\text{deg}}P \qquad\qquad\qquad\qquad \text{for } 1 - xC < 0 \text{ or } P = 1 \end{aligned} \tag{30}$$

where k_{syn} is the maximal synthesis rate of the clotting factor P and x is a "sensitivity factor" relating a given drug level to its effect on the synthesis rate. The model was fitted to the available patient-response data and then used for dosage individualization.

Theophanus (33) used the same PK model for warfarin elimination, but modelled the effect of warfarin on the prothrombin complex activity as

$$\frac{dP}{dt} = -k_{\text{syn}} \ln \frac{C}{C_{\max}} - k_{\text{deg}}P \tag{31}$$

where $C_{\max}$ is a concentration of warfarin which produced total inhibition of prothrombin complex synthesis.

In the years 1981–1982, Abbrecht and O'Leary (34,35) underlined the limitations of the previous models: that a Michaelis–Menten type of relationship between drug and enzyme was not accounted for, and that the assumption of a warfarin dose that completely inhibits prothrombin complex synthesis was inappropriate in absence of a Michaelis–Menten type model. They presented a more physiologic model for warfarin pharmacodynamics. The time-dependent prothrombin activity was described according to

$$\frac{dP}{dt} = k_{\text{syn}} \left(1 - \frac{C}{C + \text{EC}_{50}}\right) - k_{\text{deg}}P \tag{32}$$

where in this case $C = C_{\text{u}}$, the free fraction of warfarin in plasma.

This model was the first example of a more systematic description of different types of indirect responses that followed in the early 1990s (4). Dyneka *et al.* proposed four indirect response models that are based on drug effects that either stimulate or inhibit the production or loss of a mediator that modifies the kinetics or a response variable (Fig. 6). The two general equations for the rate of change of the effect response were written as follows:

$$\begin{aligned} \frac{dE}{dt} &= k_{\text{in}}[1 \pm g(C)] - k_{\text{out}}E \\ \frac{dE}{dt} &= k_{\text{in}} - k_{\text{out}}[1 \pm g(C)]E \end{aligned} \tag{33}$$

where k_{in} or k_{out} indicate the synthesis or degradation of a mediator, respectively, that can be either inhibited (−) or stimulated (+) and $g(C)$ represents the classical inhibitory (I) or stimulation functions (S) relating concentrations to the response:

$$\begin{aligned} I(C) &= \frac{I_{max}C}{C + EC_{50}} \\ S(C) &= \frac{E_{max}C}{C + EC_{50}} \end{aligned} \tag{34}$$

We remark that any monotonic function can be used in (33) provided that $g(0) = 0$, and $0 \leq I(C) \leq 1$.

These equations provided a mechanistically reasonable description for a number of pharmacokinetic–pharmacodynamic relationships and served as bases for further generalization and elaboration of models incorporating drugs (drug combinations, metabolite activity) and PD system complexities (tolerance, time-variant).

Indirect action models represent an active area of investigation: extension to include a precursor to describe tolerance and rebound effect (36) and drug interactions (37) have been recently proposed. Many other factors, such as presence of active metabolites, binding to multiple receptors and disease progression have also been incorporated in indirect action models in the past years.

Models for Cell Trafficking

Wald (38) proposed to apply the general framework of indirect action models to characterize the effects of methylprednisolone on basophil cells trafficking between two pools, the blood and the extravascular sites. Since basophils are major contributors to the development of allergic and anaphylactic reaction, this model was developed to more accurately define and extrapolate corticosteroids-induced alterations in the distribution of circulating cells and provide parameters for steroid sensitivity following corticoid administration. This model was consistent with the view that exposure to corticosteroids produces a concentration-dependant decrease in the recirculation of these cells from peripheral compartments, resulting in a net decrease in the amount of basophils in the blood. Equations describing the above patterns were as follows:

$$\begin{aligned} \frac{dB_B}{dt} &= +I(t)k_{in}B_E - k_{out}B_B \\ \frac{dB_E}{dt} &= -I(t)k_{in}B_E - k_{out}B_B \end{aligned} \tag{35}$$

where B_B and B_E are, respectively, the amount of basophil cells in the blood and the extravascular sites, $B_B(0) = B_B^0$, and $B_E(0) = B_E^0$ and

$$I(t) = 1 - \frac{C}{C + EC_{50}} \tag{36}$$

The rate constants k_{out} and k_{in} characterize the movement from the blood to the extravascular sites. Since the amount of basophil cells in the extravascular sites is large in respect to basophils in blood and remains essentially constant (at a level B_E^{ss}) model (35) collapses to

$$\frac{dB_B}{dt} = +I(t)k_{in}^0 - k_{out}B_B \tag{37}$$

where $k_{in}^0 = k_{in}B_E^{ss}$.

This model, with the incorporation of the monoexponential PK model for methylpredisolone, was fitted to blood histamine concentrations, which directly parallel the movement of circulating basophils (39). Figure 7; corresponding to Fig. 3 in the original manuscript, shows the result of the fit of the model to the data.

As stated in the original article "Because many other leukocytes exhibit similar response to corticosteroids, this model is potentially applicable to a variety of perturbations in cell recirculation phenomena". Extension of this work to incorporate circadian pattern of the cells as well as application to other cell types was also reported at that time (40).

Transduction Models

The pharmacological effect of many drugs and hormones are often mediated by time-dependant transduction, whereby the final response is a result of a signalling cascade controlled by secondary messengers. When these post-receptor events are rate limiting, drug effects can lag considerably behind plasma concentrations. These phenomena have been documented for steroids which exhibit a delay of several hours between maximum responses and peak steroid concentrations (41). This delay has been attributed to the induction of protein synthesis following steroid receptor binding (42). Free corticosteroids diffuse into cells and interact with cytosolic receptors, and activated drug–receptor complexes translocate into the nucleus, causing transcriptional modifications of the expression of secondary messengers, proteins or enzymes (43). Based of these properties, a first generation model was proposed to examine steroid response (activity of the hepatic enzyme tyrosine aminotransferase TAT) in relation to plasma prednisolone concentrations (44). Although this model

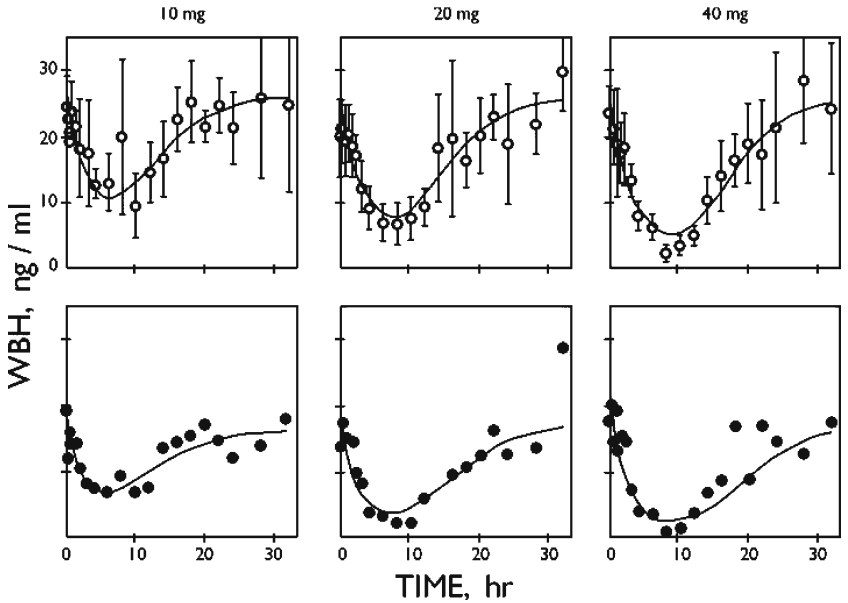


Fig. 7. Composite graph showing whole blood histamine (WBH) vs. time for 3 dosage levels of methylprednisolone with depiction of the average data (±SD) for 5 subjects (top) and one individual subject (bottom). Solid lines were generated by using nonlinear least-squares regression to fit the model to data from all dosage levels simultaneously. Reproduced from (38) with kind permission from Springer.

handled successfully the obligatory steps between receptor activation and induction of the enzyme by introducing a lag time, several restrictions and weaknesses limited its applicability. A few years later, a second-generation model was described for prednisolone pharmacokinetics and pharmacodynamics in rats (45). A schematic representation of the model is presented in Fig. 8. The relationship between pharmacokinetics and pharmacodynamics was analysed using the following equations:

$$\frac{d(R)}{dt} = -k_{on}\mathrm{RC} + k_{off}\mathrm{RC} + \mathrm{RC}^* \tag{38a}$$

$$\frac{d\mathrm{RC}}{dt} = -k_{on}\mathrm{RC} - (k_{off} + k_{DNA})\mathrm{RC} \tag{38b}$$

$$\frac{d\mathrm{RC}^*}{dt} = (k_{DNA}\mathrm{RC} - \mathrm{RC}^*)/\tau_1 \tag{38c}$$

$$\frac{dM_1}{dt} = \frac{\mathrm{RC}^* - M_1}{\tau_1} \tag{38d}$$

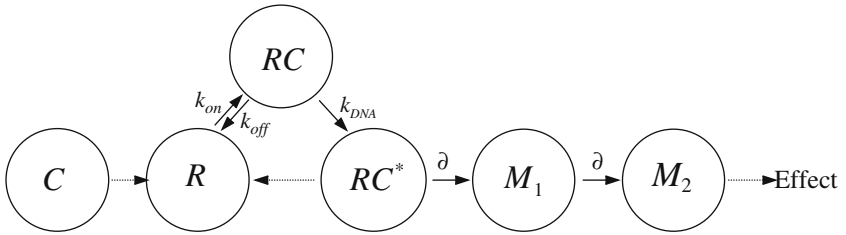


Fig. 8. Schematic representation of a transduction model for corticoid action. The drug concentration C binds to the hepatic cytosol receptor R to form a drug receptor complex RC at the association and dissociation rate constants k_{on} and k_{off}, respectively. The constant k_{DNA} defines the binding of activated complexes RC* in the nucleus with DNA. The period between receptor binding in the nucleus and the response is handled by inserting a series of transit compartments M_1 and M_2. The passage from one to the other is described by the transfer time τ. Effect is directly proportional to M_2 (see text).

$$\frac{dM_2}{dt} = \frac{M_1 - M_2}{\tau_1} \tag{38e}$$

$$\frac{dTAT}{dt} = \kappa M_2^{\gamma} - k_{\text{deg}}\text{TAT} \tag{38f}$$

where R, RC RC* are the concentrations of, respectively, free receptor in the hepatic cytosol, drug–receptor complexes and activated drug–receptor complex in the nucleus that has bound to DNA, k_{on} and k_{off} the rate constants for association and dissociation to and from the receptors and k_{DNA} the rate constant of binding of activated drug–complex receptor in the nucleus with DNA. In Eq. (38c) the term RC* indicates the rate of return of receptor to the nucleus. The period between receptor binding in the nucleus and the response was handled by inserting two transit compartments, M_1 and M_2 that represent secondary messengers (Eqs. (38d) and (38e)) and the passage from one compartment to the next was described by a transfer time τ_1. The response was modeled as directly proportional to M_2 (weighted by an efficiency factor χ and a power term γ) and was declining at a constant rate k_{deg}.

In the latter model, the number of transit compartments was determined empirically to be equal to 2. A more general transit equation model was suggested a few years later by Sun and Jusko (46), described by the following:

$$\frac{dM_i}{dt} = \frac{M_{i-1}}{\tau_{i-1}} - \frac{M_i}{\tau_i}, \quad i = 2, \ldots, n \tag{39}$$

where $n = 1, \ldots, n$, is the number of secondary messengers. To avoid the specific receptor dynamics required by Eqs. (38), RC could be replaced

with the E_{max} or Hill equations (Eqs. (13) and (16)). This approach was successfully applied to several substances exhibiting time delays caused by post-receptor events (i.e., β-adrenergic agonists (47, 48) and chemotherapeutic agents (49).

DEVELOPMENTAL YEARS

Tolerance Models

The phenomenon of decreased effect after repeated administration of a drug, or tolerance, was characterized in the mid-1990s. Several models were proposed that, when integrated with a pharmacokinetic model, allowed the quantitative characterization of the development of tolerance. A first model for tolerance used a time-series model (50) where the effect is a function of the recent history of plasma concentrations as in the following relationship:

$$E(t) = E_0 + s_0 C_0(t) + s_1 C(t-1) + s_2 C(t-2) + \cdots + s_m C(t-m) \quad (40)$$

where the s_i and $C_i(t-i), i = 1, \ldots, m$, are coefficients and drug concentration corresponding to m previous periods determined at equispaced time.

A semi-mechanistic model for tolerance with a more physiologic interpretation was described by Ekbad and Licko (51). The model proposed the transformation of a hypothetical substance R (which could be conceptualised as receptors) to a substance RC (which could be conceptualised by occupied receptors). The model assumed that the substance R is produced at a constant rate (re-generation of available receptors) and that it is eliminated at a first-order rate (degradation of receptors). RC is eliminated at another first-order rate constant (down-regulation of receptors). Assuming that the pharmacological effect is related to the amount of RC, the system responds transiently to an alteration of the concentration of the agonist. By modulating the different parameters of the model, this simple transformation scheme was able to characterized different phenomena's involved in the mechanism of tolerance.

To model the tolerance of caffeine on heart rate, Chow *et al.* (52) used a function that declined exponentially as a function of time. The effect was described by the following linear relationship:

$$E = E_0 + \alpha C_e(t) e^{-kt} \quad (41)$$

where α relates C_e to the effect, which is progressively attenuated at a rate constant k. This model implies that, if steady-state concentrations of

cocaine were maintained long enough, drug effect would eventually disappear entirely. Because it ignored agonist kinetics *per se* and assumed monotonically increasing tolerance, this model could not describe different doses or levels of exposure to agonist on development to tolerance and did not allow tolerance to decrease at some late time when agonist has disappeared.

A different model was proposed to describe the development of tolerance to furosemide-induced diuresis (53). The Hill model was used to describe the excretion–response relationship:

$$E = E_0 + \frac{E_{\max} U(t)^{\gamma}}{U(t)^{\gamma} + U(t)_{50}} \tag{42}$$

where $U(t)$ is the urinary excretion rate of furosemide and $U(t)_{50}$ the parameter reflecting the sensitivity of the system to the drug. To model tolerance, $U(t)_{50}$ was modified such as it decreased exponentially as a function of the cumulative excretion of furosemide:

$$U(t)_{50} = U_{0,50} e^{k \int_0^t U(t)\, dt} \tag{43}$$

where $U_{0,50}$ is the initial value of the excretion rate when no tolerance has developed and k is a coefficient.

A compartmental pharmacokinetic–pharmacodynamic model for nicotine tolerance was proposed by Porchet *et al.* (54). They postulated the generation of a hypothetical substance (C_{m}) in a "tolerance compartment" that acted as a non-competitive antagonist of the effects of nicotine (Fig. 9). The hypothetical antagonist was assumed to arise according to a first-order process with rate constant (k_{mo}), driven by the concentration of nicotine in the central compartment:

$$\frac{dC_{\mathrm{m}}}{dt} = k_{\mathrm{mo}}(C - C_{\mathrm{m}}), \quad C_{\mathrm{m}}(0) = 0 \tag{44}$$

The effect was modelled using a standard non-competitive antagonist model:

$$E = E_0 + E_{\max} \frac{C}{C + \mathrm{EC}_{50}(1 + C_{\mathrm{m}}/C_{\mathrm{m},50})} \tag{45}$$

where $C_{\mathrm{m},50}$ quantifies the degree of tolerance attainable for a given steady-state nicotine concentration. For nicotine, tolerance develops quickly, and

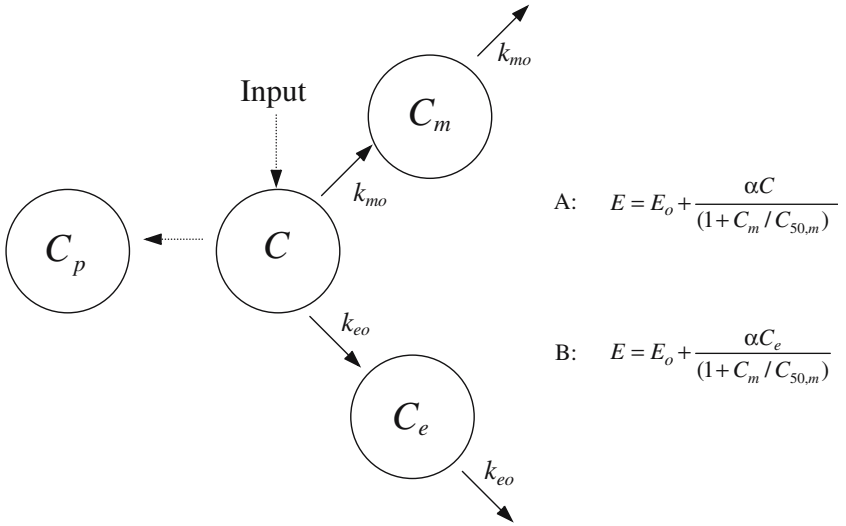


Fig. 9. Schematic representation of a compartmental pharmacokinetic–pharmacodynamic model for tolerance proposed by Porchet (54)(model A) and adapted by Shi (55)(model B). In both models, tolerance is modelled by introducing a hypothetical compartment whose concentrations (C_{m}) are driven by the concentrations in the central compartment (C). The rate constant k_{mo} describe the generation and loss of C_{m}, which acts as an antagonist of the effects of the agonist. As opposed to model A that directly relates the effect to C, model B introduces an effect compartment to characterize the time delay of drug action.

a maximal effect is not identifiable. Therefore Eq. (45) was simplified to a linear relationship yielding

$$E = E_0 + \frac{\alpha C}{C + C_{\mathrm{m}}/C_{\mathrm{m},50}} \tag{46}$$

where α is the slope governing the linear relationship of E to C.

An extension of this model was elaborated a few years later (55) to model the tolerance of caffeine to its cardiovascular effects. In that approach, the observed concentrations of caffeine in the central compartment (C) were linked to the positive agonist aspects of observed effect through a hypothetical effect-site concentration (C_{e}). Equation (46) became

$$E = E_0 + \frac{\alpha C_{\mathrm{e}}}{C + C_{\mathrm{m}}/C_{\mathrm{m},50}} \tag{47}$$

These models provided a descriptive empirical quantification of the time course of tolerance and its relationship to drug exposure to the drug (agonist), although a mechanism for tolerance was never included in the models.

More physiological models for tolerance (hyper-sensitization) typically involve some regulatory feedback mechanism which "dumps" (or "amplifies") the response as a function of current response level. An example of such model is reported in (56), where acute tolerance development of alfentanil electroencephalographic effects in the rat is described. The model takes the following form:

$$E = E_0 + E_{\max} \frac{C_e^{\gamma}}{C_e^{\gamma} + EC_e^{\gamma}} + \alpha X \tag{48}$$

where X (E_f in the original reference) indicates the feedback response, and $\alpha (\alpha \leq 0)$ is now the "gain" of the (negative) feedback subsystem. The feedback response is the convolution of the current effect with an exponential:

$$X = k_f E(t)^* k_j e^{-k_f t}$$

where k_f is the rate of feedback development. For an arbitrary steady-state level of $C_e (C_{e,ss})$ the model predicts (note that at steady state $X = E^{ss}$):

$$E^{ss} = \frac{E_0 + E_{\max} \frac{C_{e,ss}^{\gamma}}{C_{e,ss}^{\gamma} + EC_e^{\gamma}}}{1 - \alpha} \tag{49}$$

which shows the effect of the "gain" in dumping the direct action of $C_{e,ss}$ on the effect.

We remark that investigation of tolerance requires the use of sophisticated study protocols, often including the use of computer controlled infusion pumps (57–59).

Regulatory feedback models appear to be sometimes misunderstood in the literature. An example is (60) which uses a descriptive empirical model similar to the ones reported above to "model" the effect of nitroglycerin on vasodilation together with the associated counter-regulatory vasoconstriction. The model used by the authors failed to incorporate a feedback mechanism between current vascular size (or/and corresponding diastolic pressure) and vasoconstriction but it allowed handling counter-regulation in a simplistic way.

Time-Variant Models

Whereas basic response models assume a constant steady-state baseline value in the absence of a drug, some biomarkers may exhibit non-stationarity or a time-dependant baseline. This phenomenon is generally

observed for endocrine processes, e.g. where the spontaneous changes in the hormone plasma level throughout the day results from the fluctuation of hormone secretion. To account for this intrinsic variation, Francheteau *et al.* (61) developed a model that integrated the hormone secretion fluctuation for the description of the hormone-lowering effect of a drug. The model took into account the variation in response observed after administration of a placebo (since the intrinsic variation of the response over time in the absence of drug is generally documented by a placebo experiment) and the drug. It assumed that the change with time in the physiological response during the placebo periods resulted from fluctuations in the concentration of two auxiliary variables, which are analogous to the concentrations of two hypothetical endogenous molecules E_1 and E_2 with opposite action. Assuming competitive interaction between E_1 and E_2 for binding to receptors and a linear relationship with the effect, the measured response was subsequently given by

$$E(t) = E_{\mathrm{m}}(t)(1 + E_{\mathrm{max}_1} C_1(t) + E_{\mathrm{max}_2} C_2(t)) \tag{50}$$

where $E_{\mathrm{m}}(t)$ is the mean level of $E(t)$ over time after placebo administration and, respectively, E_{max_1} and E_{max_2} the maximal positive and negative effect elicited by the concentrations C_1 of E_1 or C_2 of E_2.

Assuming competitive interaction between the drug and endogenous molecules, the mathematical formulation of E after drug was given by

$$E(t) = E_{\mathrm{m}} \left(1 + \frac{E_{\mathrm{max}_1} C_1(t) + E_{\mathrm{max}_2} C_2(t) + E_{\mathrm{max}} C'_e(t)}{1 + C'_e(t)} \right) \tag{51}$$

with $C'_{\mathrm{e}} = (C_{\mathrm{e}}(t)/EC_{50})^{\gamma}$.

Integration of intrinsic fluctuation in the predicted pharmacodynamic response after drug administration was achieved by reversing Eq. (50) to yield

$$W(t) = E_{\mathrm{max}_1} C_1(t) + E_{\mathrm{max}_2} C_2(t) = \frac{E(t)}{E_{\mathrm{m}}} - 1 \tag{52}$$

The variable W was obtained by smoothing the time course of individual response after placebo to give a continuous profile. Previously estimated pharmacokinetic parameters and W both served in the PK/PD model for predicting the drug-related time course of E using classical pharmacodynamic models. The applicability of this joint description of drug-related and a placebo response was demonstrated with the example of a compound acting on prolactin, which exhibits marked circadian variation.

The time-course of endogenous cortisol concentrations, which follow a circadian rhythm, was characterized using an indirect response model with the production rate parameter, k_{in}, replaced with a time-dependant function (62) involving the use of a single cosine function:

$$k_{in} = I_m + I_a \cos[(t - t_z)2\pi/24] \tag{53}$$

where I_m is the mean input rate, I_a is the amplitude of the input rate, t_z is the peak time and $2\pi/24$.

The study of (63) on the chronopharmacokinetics of nicotine shows the modelling of the effects of both meals and circadian influences on the clearance of nicotine. Clearance was modelled as a function of time, $\mathrm{Cl}(t)$, and split in three components: a baseline value, the circadian (diurnal) variation, circadian(t) and the effect of meal, meal(t), which, in that application, is to increase clearance. The model for clearance was written as follows:

$$\mathrm{Cl}(t) = \mathrm{Cl}_0(1 + \mathrm{circadian}(t))(1 + \mathrm{meal}(t)) \tag{54}$$

In order to compare across two possible approaches, two different approaches a non-parametric and a parametric one were used. For the parametric one

$$\mathrm{circadian}(t) = A \sin[(t - \varphi)2\pi/\omega] \tag{55}$$

where ω is the period of the circadian component of clearance, fixed to 24 h, and A (amplitude, i.e. maximal variation) and φ (phase) were estimated from the data.

The effect of meal was given by the following:

$$\mathrm{meal}(t) = \sum_{k=1}^{m} \mathrm{Ind}(t - t_k \geq 0) \frac{\alpha\beta}{\beta - \chi} \left(e^{-\beta(t-t_k)} - e^{-\chi(t-t_k)} \right) \tag{56}$$

where t_k indicates the time of the kth of m meals and $\mathrm{Ind}(t - t_k \geq 0) = 1$ for $t - t_k \geq 0, 0$ otherwise.

For the non-parametric model cubic splines were use instead of the cosine and exponential functions. For the circadian component a periodic cubic spline was used: this matches all derivatives at boundaries of the period (in this case 0 and 24 h), in the same way of the cosine and sine functions shown above.

The meal effect took the form:

$$\text{meal}(t) = \sum_{k=1}^{m} \text{Ind}(t - t_k \geq 0) S(t - t_k) \tag{57}$$

where $S(t)$ indicates a natural cubic. To make $S(t)$ a meaningful model for the meal effect, it was constrained to be positive, and equal to zero at time zero (the time of meal) and at the last breakpoint. The position (in time) of the last breakpoint of the spline is one of the parameters to be estimated: it characterizes the duration of the meal effect because $S(t)$ is identically zero at this time and thereafter.[5]

Figure 10 shows the influence of time-varying clearance on simulated nicotine concentrations during intravenous infusion of nicotine corresponding to the estimates reported in (63).

Non-parametric and Semi-parametric Models

Beginning in the early 1980s, a number of "non-parametric" direct action models were proposed. The attempt was to provide tools useful in the exploratory stage of data analysis, to help decide which parametric model to use next or provide final analysis when no such model could be identified. As we discussed previously, since the concentrations in plasma rather than at the effect site are usually measured, the $C_e - E$ relationship is obscured by equilibration delays that intervene between the two concentrations, and the plot of E vs. C often shows hysteresis.

The model proposed by Segre (8,23) and then elaborated by Sheiner *et al.* (28) among others describes the pharmacokinetics, link and pharmacodynamic models using parametric (sub)-models. The parameters of the model are estimated by fitting the model to the observed values of C and E. A plot of the estimated concentration in the "effect site" vs. E will not show hysteresis.

A more non-parametric approach to correct hysteresis was adopted by Hull *et al.* (64). The effect site was linked to the plasma by a first-order process represented by a single rate constant (k_{eo}), but no assumption regarding the form of the pharmacodynamic model was required. Choosing two times (one during drug input as C was rising and one later as C was falling) when the drug gave 70% of its maximal effect, they adjusted k_{eo} to make C_e identical at

[5]The results obtained by the two approaches were similar in respect to size of meal effect and circadian variation. The non-parametric estimate however indicated that the circadian variation is possibly more complex than a simple sine function, obtaining a non-symmetric function showing a flat region in clearance between 6:00 PM and 3:00 AM.

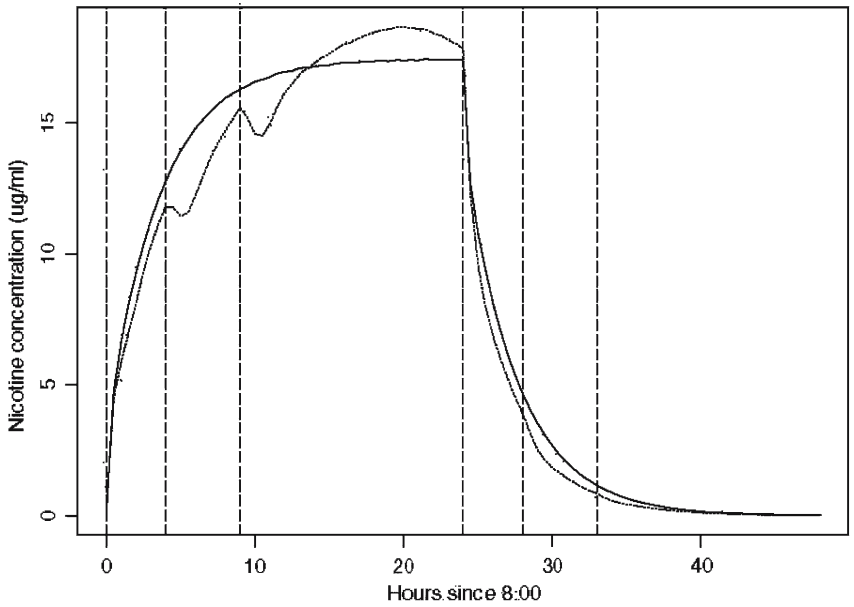


Fig. 10. Influence of time-varying clearance on simulated nicotine concentrations during intravenous infusion of nicotine. Nicotine is assumed to be dosed by constant infusion of 1.25 mg/h for 12 h. The solid line indicates the simulated nicotine concentration assuming constant clearance, the broken line the nicotine concentration assuming time-varying clearance. The vertical dashed lines are times of meal. (Circadian and meal related changes in nicotine clearance could influence cigarette smoking behaviour, smokers typically smoke after meals, and a decline in plasma nicotine concentration due to an increase in clearance could contribute to the urge to smoke. Some smokers smoke less in the evenings, and that behaviour might be contributed to by the lower clearance in the evening compared to the morning).

these two times. The criterion of superimposition was the "horizontal distance" (difference) between the values of C_e that produced a single identical degree of E on the ascending and descending limb (Fig. 11). With k_{eo} so estimated, they could predict C_e for all observation times to obtain a full $C_e - E$ relationship.

A subsequent model of Hull *et al.* approach was elaborated by Fuseau and Sheiner (65). The n observation pairs of observed effects (E_i) and predicted concentrations in the effect compartment (C_{e_i}) were used to estimate k_{eo}. The idea was to choose k_{eo} so that the two limbs of the C_e vs. E curve were collapsed, using as a criterion of superimposition a function of "vertical" distance (difference) between the two limbs of the curve (Fig. 11b). This was achieved by computing, for a trial value of k_{e0}, the C_{e_i} (i = 1,n) values; for each C_{e_i}, an approximate value corresponding to

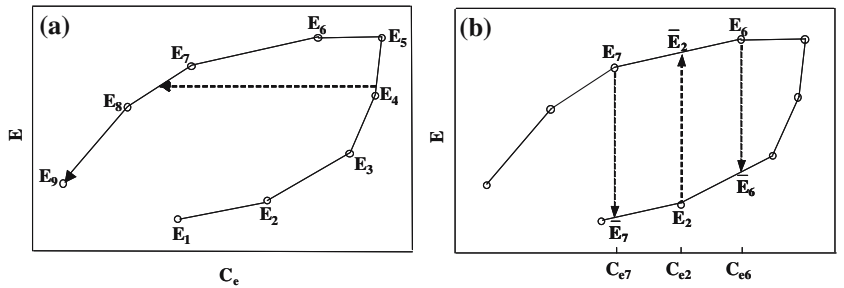


Fig. 11. Effect vs. predicted C_e. (a) The degree of superposition of the two limbs of the hysteresis is chosen as the horizontal distance at a particular chosen E. k_{eo} is adjusted to make C_e identical at these two times (64). (b) The distance between the two limbs of the curve is evaluated by the vertical difference between each pair of observed (E_i) and interpolated ($\overline{E}_i$) effect at the same C_{e_i}.k_{eo} is chosen to minimize the mean of those squared differences (65).

the effect ($\overline{E}_i$) on the opposite limb was obtained by linear interpolation between the two nearest observed E points on the same limb.

The estimated $k_{e0}(\hat{k}_{e0})$ is the value that minimizes the average of the squared differences between observed and interpolated E:

$$\hat{k}_{e0} = \min_{k_{e0}} O(k_{e0}) = \frac{1}{n}\sum_{i=1}^{n}(E_i - \bar{E}_1)^2 \tag{58}$$

A further elaboration was proposed by Unadkat *et al.* (66), which eliminated the requirement of parametric PK model in favour of a non-parametric representation of the concentrations using simple linear interpolating splines.

Verotta and Sheiner developed an extension of the algorithm described by Fuseau by allowing the relaxation of the requirement of a single $C_e - E$ hysteresis and allowing the simultaneous analysis of multiple data sets (67). Figure 12 depicts an example of a $C_e - E$ relationship exhibiting a double hysteresis loop. The pharmacokinetic model was approximated using interpolating linear splines. For each trial k_{eo} predicted effects were obtained for all overlapping limbs of the hysteresis curve over a grid of equispaced points over the range of overlapping limbs. The value of k_{eo} that gave the most collapsed hysteresis was chosen based on the average of all the squared vertical distance between pairwise combinations of predicted effects obtained for each point of the grid.

The effect compartment models of (23), (28) and its non-parametric variants (64–67) can only explain equilibrium delays in which the effect lags ahead of the PK observation compartment. However, it was recognised that equilibrium delays between the venous sampling site and the

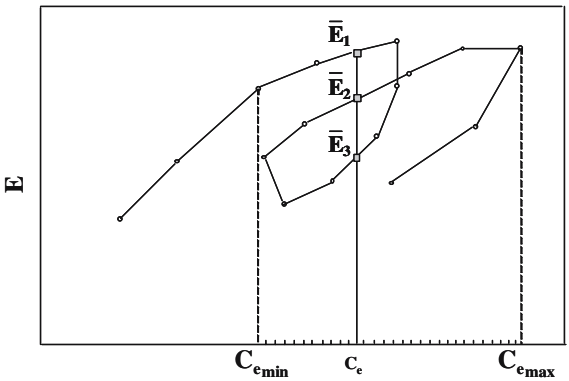


Fig. 12. Collapsing a complex hysteresis loop. (i) The range of overlap $[C_{e_{min}}, C_{e_{max}}]$ is determined, (ii) a grid of equispaced points is defined over the grid, (iii) for each point on the grid predictions are obtained for each limb and (iv) the average of the squared distances between all pairwise combinations of these predictions is obtained and summed for all points of the grid.

arterial concentrations might lead to proteresis (clockwise hysteresis) in the C–E curve (Fig. 13). Verotta *et al.* implemented a procedure to describe both proteresis and hysteresis) (68). As shown in Fig. 14, two first-order links were postulated that relate the concentrations in the venous sampling site (C_v) and (C_e) to a central (unobserved) arterial blood (C_a) compartment. The kinetic-link model was written in the form of differential equations as follows:

$$\begin{aligned} \frac{dC_a}{dt} &= \cdots + I(t) \\ \frac{dC_e}{dt} &= k_{ea}C_a - k_{e0}C_e \\ \frac{dC_v}{dt} &= k_{va}C_a - k_{v0}C_v \\ C_a(0) &= C_e(0) = C_v(0) = 0 \end{aligned} \tag{59}$$

where $I(t)$ is the input function to the arterial compartment and the ellipsis in the first equation indicates an unspecified portion of the expression for dC_a/dt describing the kinetics of distribution and elimination for the arterial compartment. The parameters k_{ea} and k_{va} quantify the rate of drug input from the arterial compartment to the two others, whereas the parameters k_{eo} and k_{vo} quantify the rate of equilibration of the latter. Since C_e and C_a are unobserved concentrations, their units are arbitrary and it was assumed that $k_{va} = k_{vo}$ and $k_{ea} = k_{eo}$; this is just a convenience since at steady-state $C_a = C_v = C_e$. The only effect of k_{ea} and k_{va}

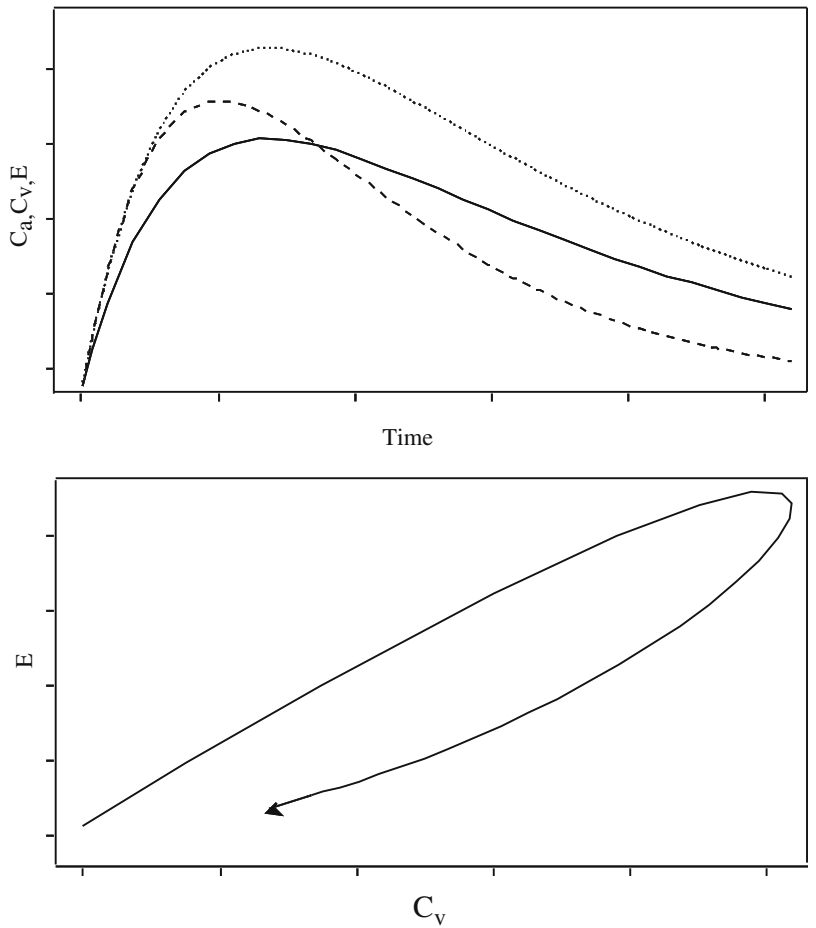


Fig. 13. (upper panel) The effect (solid line) peaks at the same time as the concentrations in the arterial compartment (C_a) (dotted line) but is delayed relative to the venous sampling compartment (C_v) (dashed line) leading to lower panel: a proteresis.

is to scale the C_e or C_a profile but does not modify the shape of the concentration-response curve $C_e - E$, but simply scales and such that. Under model (51), a plot of E vs. C_e describes a proteresis when k_{eo} is greater then k_{vo} and a hysteresis in the opposite case.

The estimates of C_e for an arbitrary choice of k_{eo} and k_{va} were obtained by means of numerical convolution and deconvolution. Indicating the convolution operator by *, the distribution of drug between arterial and venous blood under model (59) is

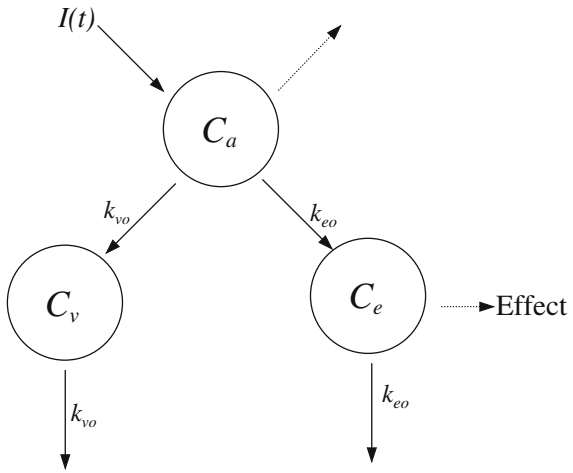


Fig. 14. The concentration in the venous sampling compartment (C_v) is deconvoluted to predict the unobserved concentrations in the arterial compartment (C_a). Predicted concentrations in the effect compartment (C_e) are obtained by numerical convolution of C_a (see text). k_{vo} is the first-order loss rate from C_v.$I(t)$ is the input function.

$$C_v = k_{vo} e^{-k_{vo} t} * H_a * I(t) \tag{60}$$

where H_a is the disposition function of the arterial compartment. Constrained deconvolution (69) was used to yield an estimate of a monotonically decreasing $H_a(\hat{H}_a)$ conditional on k_{vo} and $I(t)$. Second, predicted C_e were obtained by convolution:

$$\hat{C}_e = k_{eo}(e^{-k_{eo} t}) * \hat{H}_a * I(t) \tag{61}$$

The parameters k_{eo} and k_{vo} were estimated using the algorithm presented above (67) searching for the values of both rate constants yielding the most collapsed hysteresis E vs. C_e. Using the k_{eo} and k_{vo} so estimated, the associated plot of E vs. C_e yielded the steady-state pharmacokinetic–pharmacodynamic relationship.

Elaboration of the non-parametric effect compartment model was presented by Veng-Pedersen *et al.* (70) and Mandema *et al.* (27). Their program (COLAPS) modelled the equilibration kinetics between C and C_e relaxing the requirement of a monoexponential link model. The model enabled a more general drug transport to the effect compartment. The drug level curve $C(t)$ is transformed into the biophase level $C_e(t)$ by convolution with a biophase conductance function $L(t)$:

$$C_e(t) = L(t) * C(t) \tag{62}$$

When $L(t)$ is a monoexponential we have the model of (64). Veng-Pederson, *et al.* (70) used a biexponential, in principle any monotonic non-increasing function $L(0) = 1$ could be used.

Further investigations regarding the use and the performance of the traditional effect compartment model or the so-called extended-effect compartment model handling equilibration delay between arterial an venous concentrations and those relaxing the assumption of first order links between plasma and effect compartments were described in the late 1990s (71,72).

In (73) the non-parametric effect compartment model approach was brought to a somewhat logical conclusion: since PD could be related to an observed PK site by means of a link with an hypothetical compartment, in principle PD could be related as well to dose linked to PD by a transfer function representing an hypothetical compartmental structure linking dose to the effect site avoiding PK measurements all together.[6]

The analysis was based on a model describing the time dependence of drug effect on (unobserved) drug concentration in an hypothetical effect compartment. The model consists of

(1) a known representation for the input rate of drug $I(t)$,
(2) a parametric model relating to an unobserved variable C_e,
(3) a non-parametric model relating C_e to E.

Ref. (73) used

$$C_e(t) = L(t, \alpha) * I(t) \tag{63}$$

where

$$L(t, \alpha) = \frac{e^{\alpha_1 t} * \sum_{k=1}^{m} \alpha_{2k} e^{\alpha_{2k-1} t}}{\int_0^t e^{\alpha_1 t} * \sum_{k=1}^{m} \alpha_{2k} e^{\alpha_{2k-1} t} dt}, \quad \sum_{k=1}^{m} \alpha_{2k} = 1 \tag{64}$$

and

$$E(t) = S(C_e(t), \beta) \tag{65}$$

where S indicates a natural cubic spline (this has second derivatives at the first and last breakpoint set to zero). From a historical perspective

[6] As a personal recollection: "the joke" between Lew (Sheiner) and Davide (Verotta) "was that after PD without effect compartment concentrations, and now PD without PK, in the next publication we were going to have PD without the drugs … and just look at the data. We never got around doing it".

reference (73) is somewhat significant because it breaks away from the rather obsolete "collapse the loop" algorithms: the values of the vectors of parameters α, β are simply chosen to minimize the sum of squared residuals between predicted and observed E, an obvious solution which allows a semi-parametric approach (the relationship E vs. C_e is nonparametric) to be embedded in any statistically sound model.

Finally, we mention that disentangling the estimation from "collapsing the loop" allowed the proposal (see (74)) of a whole class of semi-parametric models describing drugs interactions in the form of additivity, synergism or antagonism (2). The models were called semi-parametric because they used nonparametric functions (splines), which were forced to obey certain functional forms and constraints corresponding to reasonable assumptions about drug interactions, and were embedded in otherwise parametric (mechanistic) structures.

For example, a simple semi-parametric model for uncompetitive interaction between two drugs (C_1, C_2) takes the form:

$$E = E_0 + E_{\max} \frac{C_1}{C_1 + \mathrm{EC}_{1,50}} (1 = -S(C_2)) \tag{66}$$

where $0 \leq S(C_2) \leq 1$, but otherwise of arbitrary shape.

Population PK/PD Modelling

An important problem in drug therapy is the variability of response among individuals in a population, which can manifest itself both in the pharmacokinetics and the pharmacodynamics (75). Numerous studies have demonstrated a contribution of kinetic and dynamic variabilities to the overall variability in clinical response (76). A general approach to using patient data was put forth by Sheiner *et al.* as early as 1972 (77). They suggested the use of non-linear mixed effects regression models to analyse the data pooled over all sampled individuals to allow, in particular, the quantification of inter-individual and intra-individual variability. The concept developed into a computer program, NONMEM, first released by Stuart Beal and Lewis Sheiner in 1980 (for IBM mainframe), and in 1984 exportable for all computers (78).

Since 1972, there has been great deal of work in population PK/PD using nonlinear mixed effects models, both methodologically and in applications (see (79) for a bibliography review). The usefulness of population PK/PD modeling has been shown, in particular in clinical settings for Bayesian dosage individualization (80, 81) as well as in drug development (75, 82). The population approach has increased during the last

15 years with the availability of several "pharmacostatistical" programs (NONMEM V (83), WinNonmix® (84), ADAPT (85), PopKinetics (86) and PKBugs (87)).

COMPLEX MODELS

The major pharmacodynamic models have been presented in terms of basic theory, operable equations and essential model features. More sophisticated pharmacodynamic models based on the basic concepts outlined above have been described during the last ten years. The complexity of many these models precludes their full description in this review. The transduction model reported above is one example of a complex model; we give further examples: a feedback model (88), briefly describe models for HIV-1 therapy, and a recent model describing the time course of 8-OH-DPAT-induced hypothermia (89).

An Example of Feedback Modelling

Fattinger *et al.* (88) shows a sophisticated PK/PD model to characterize the input–response relationship between gonadotropin-releasing hormone antagonist (antide) and luteinizing hormone (LH) and testosterone concentrations (90).

A nonlinear compartmental model including feedback was developed using PK and PD data from experiments in which different short intravenous antide infusions were given. Because of the control interdependence between serum LH and testosterone, a separation principle was used: testosterone and LH were first modeled separately, conditioning on the other observed response. This revealed that that LH effect on testosterone depended on previous LH exposure and that LH depended on previous testosterone exposure. Both submodels were combined into one global model, which in addition included a model for testosterone circadian variation. The final model was as follows:

$$\frac{dA_3}{dt} = -k_{30}A_3 + A_6\left(1 - \frac{p_{31}C_1^{p_{32}}}{p_{33}^{p_{32}} + C_1^{p_{32}}}\right) \tag{67a}$$

$$\frac{dA_4}{dt} = -k_{40}A_4 + A_5(1 + p_{41}(\sin(2\pi t/24 + p_{45}))\left(\frac{A_3^{p_{43}}}{p_{42}^{p_{43}} + A_3^{p_{43}}}\right) \tag{67b}$$

$$\frac{dA_5}{dt} = p_{51} - p_{52}A_5\left(1 - p_{53}\frac{A_3^{p_{54}}}{p_{55}^{p_{54}} + A_3^{p_{54}}}\right) \tag{67c}$$

$$\frac{dA_6}{dt} = -k_{60}A_6 + p_{61}\left(1 - p_{62}\frac{A_4^{p_{63}}}{p_{64}^{p_{63}} + A_4^{p_{63}}}\right) \tag{67d}$$

where the p_{ij} and k_{ij} are parameters (to be estimated). The first two compartments (not shown) describe antide PK, compartment 3 represents LH concentration, compartment 4 testosterone concentration, compartment 5 represents the production rate of testosterone and compartment 6 the LH production rate.

LH depends on the production rate expressed by A_6, times the part in brackets on the right side of Eq. (67a), which in turn is a function of which is described by a two-compartments model: the concentration of antide saturably decreases LH production. The effect of testosterone on LH production is modeled by the effect of testosterone on its production rate A_6 as described by Eq. (67d): testosterone saturably decreases A_6 "production". The effect of LH on testosterone production depends on the current LH concentration (through the second brackets on the right side of Eq. (67b), but also on former LH concentrations. This second influence is modeled by a saturable decreasing effect of LH on (Eq. (67c), the current production rate of testosterone). Diurnal variation in observed testosterone concentration is modeled by the first part in brackets on the right side of Eq. (67b). Figure 15 shows a schematic of the pharmacokinetic/dynamic model, and Fig. 16 the fit of the model to the LH data.

HIV-1 Modelling

The modeling of HIV-dynamics has also raised a great interest in the recent years. The advent of highly active antiretroviral therapy has provided a wealth of information on the interaction between HIV and the human immune system and is continuing to stimulate the debate on the basic mechanisms of viral pathogenesis. However, most of the models developed by biomathematicians and biologists are too complicated and contain too many unknown parameters to be used to analyse real clinical data (91–97). Recently, several simplified models have been proposed and applied to real virological data from clinical trials (96, 98–101). Those models are generally used to explain the decay of the virus following antiviral treatment and do not describe the resurgence of the virus. Recently (102) proposed an identifiable model that can characterize not only the decay of the virus but also its resurgence. In respect to

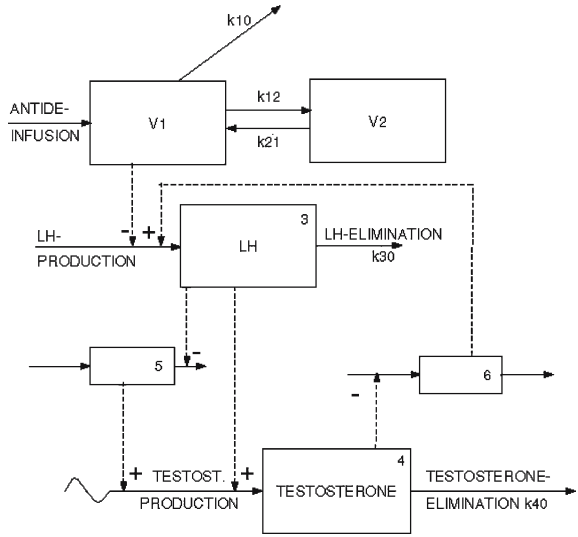


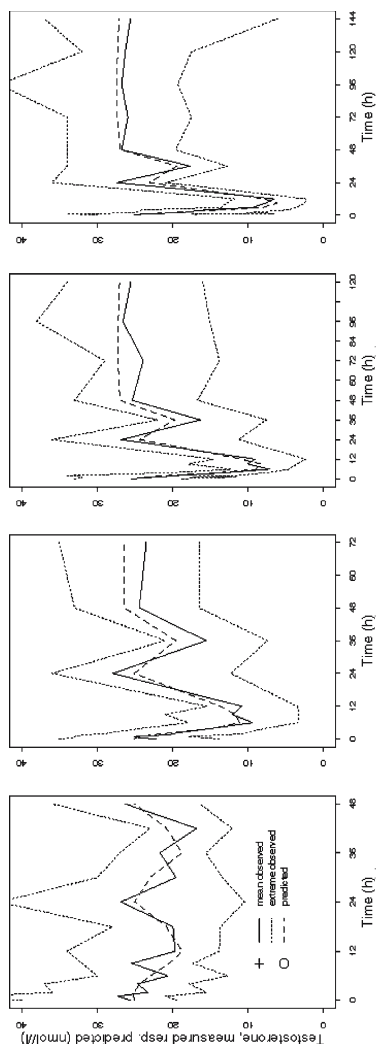
Fig. 15. Schematic of the dynamic control model for testosterone/LH. Antide disposition is described by a two-compartment model, compartment 3 represents LH concentration, compartment 4 testosterone concentration, compartment 5 represents the production rate of testosterone and compartment 6 the LH production rate. (see text).

physiologically based models (which are generally used in simulation studies), the models are simplified to yield estimated "macro-parameters": combinations of underlying non-identifiable micro-parameters characterizing the physiology of HIV-1 dynamics.

The models can incorporate, drug concentration, compliance and viral mutation and are based on classic predator–prey model (103) as described in (92,97). The dynamics include the interaction of non-infected T-cells, infected T-cells, infective virus, and cytotoxic-T-lymphocytes. An infection of target T-cells by the virus results in infected, virus producing cells. Infected T-cells stimulate the immune response by inducing the production of cytotoxic-T-lymphocytes. Cytotoxic-T-lymphocytes kill infected T-cells and consequently reduce the amount of virus produced. A possible HIV-1 dynamics model (see also (104)) is as follows:

$$\frac{dT}{dt} = \delta_1(1 - VT - T) \tag{68a}$$

$$\frac{dT^*}{dt} = \delta_2(VT - T^*) - \delta_8 T^* + \delta_9 T_1 \tag{68b}$$



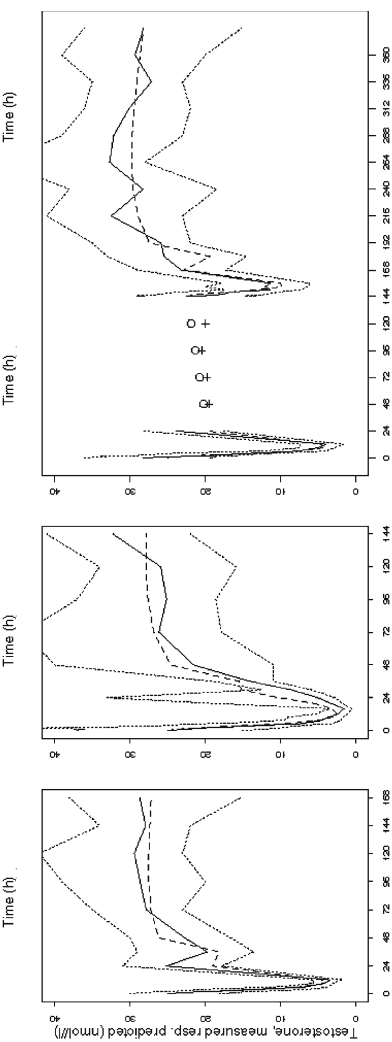


Fig. 16. LH response predicted by the model reported in the text (dashed line and circles), average observed response (solid line and +) and observed extremes (dotted line). The experiments are from left to right: upper row: placebo, 1, 2 and 4 mg antide, lower row: 8 mg, 20 mg and repeated dose occasion.

$$\frac{dT}{dt} = \delta_3 \left(\delta_4 \left(1 - \frac{\lambda_1 M_j(t)}{\lambda_2 + M_j(t)} \right) T^* - V \right) \tag{68c}$$

$$\frac{dCTL}{dt} = \delta_8 T^* - \delta_9 T_1 \tag{68d}$$

where T indicates non-infected T-cells, T^* infected T-cells, V free virus, and CTL-cytotoxic-T-lymphocytes. The unknown initial conditions at time $t = 0$ (beginning of treatment) are $T(0) = T_0$, $T^*(0) = T_0^*$, $V(0) = V_0$ and $T_l(0) = T_l(0)$; δ_1 is the production rate of infectable T-cells, δ_2 represents the average number of new infected cells resulting from a single infected cell before any depletion of target cells, for the other parameters see (104).

The part in brackets of Eq. (68c) describe the effect of compliance to treatment (generally available in the form of questionnaire, or pill count, or electronic monitoring see (105,106) for in depth analysis of the issues related to compliance in clinical trials). For each individual compliance to treatment can be summarized by means of a function of time, $M_j(t)$, which satisfies $0 \leq M_j(t) \leq 1$, where 0 corresponds to no compliance, and 1 to perfect compliance to therapy, λ_1 is the (fractional) decrease in reproductive ratio induced by the drug, and λ_2 is the level of compliance yielding 50% of λ_1: this part of the model clearly resembles an indirect action model. The model just described can of course be directly used to incorporate pharmacokinetics, when these are measured; in this case one can simply use predicted drug concentration ($C_j(t)$) instead of $M_j(t)$, so that now (68c) is as follows:

$$\frac{dV}{dt} = \delta_3 \left(\delta_4 \left(1 - \frac{\lambda_1 C_j(t)}{\lambda_2 + C_j(t)} \right) T^* - V \right) \tag{68c*}$$

the parameter λ_2 can in both cases incorporate some form prior knowledge in the form of, e.g., 50% inhibition of viral production *in vitro*. Figure 17 shows a graphical depiction of a predator–prey dynamics model of HIV-1 infection.

Set-point Model for Oscillatory Behavior

The model reported in (89) provides another example of complex nonlinear relationships which results in oscillatory behavior. Similarly to the dumped oscillations observed in HIV-1 response profiles approaching secondary (not below quantification limit) steady-states, after administration of potent multi-drugs "cocktails", the time course of 8-OH-DPAT induced hypothermia shows dumped oscillations when temperature returns toward pre-administration steady state.

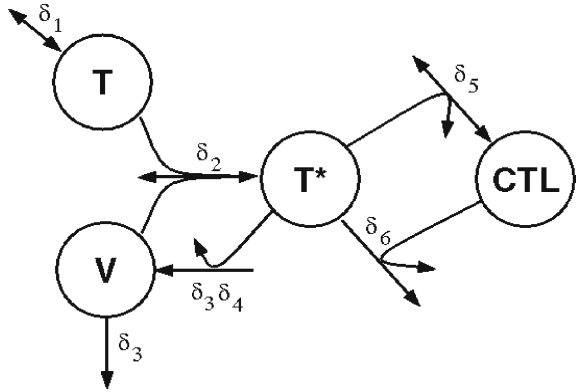


Fig. 17. Graphical depiction of a model of predator/prey dynamics of HIV-1 infection. Non-infected T-cells (T) are produced by the body at a constant rate and have a natural death rate. When the HIV-1 virus (V) infects T the product is an infected, virus-producing cell T^*.T^* produce a certain (generally large) amount of virus during their lifetime. T^* can also stimulate the production of cytotoxic T-lymphocytes (CTL), which in turn enhance the elimination of T^* by CTL interaction (killing). Circles indicate compartments; straight arrows indicate fluxes from/to compartments; curved arrows indicate control actions on the fluxes; the merging curved arrows indicate transformation of two precursors to a product.

The model chosen in (89) is based on a thermostat-like regulation of body temperature, in which body temperature is compared with a reference (set-point) temperature to retain steady-state; the set point temperature is decreased by drug action. Changing notation, and slightly adapting the model to suit the feedback modeling section below, the model is as follows:

$$\frac{dE}{dt} = k_{\text{in}} - k_{\text{out}} E h(X) \tag{69a}$$

$$\frac{dX}{dt} = k_x \left[E_{\text{set-point}} (1 - g(C)) - E \right] \tag{69b}$$

where E indicates the temperature, X is the thermostat signal, $h(X)$ is, monotonic, function describing the association of E with X Ch(x) = x (is the simplest solution, (89) uses $h(X) = X^{\gamma}$), k_x is the rate of change of the thermostat signal, and $E_{\text{set-point}}$ is the reference set-point for the regulator.

Note that the function should have the property $h(X(0)) = 1$, so that $E(0) = E_0 = k_{\text{in}}/k_{\text{out}}$, and this implies that initial conditions for X should be 1 for the case $h(X) = X$, and for general $h(X)$ as well; at time zero steady state, before administration of drug, Eq. (69b) also implies $E_{\text{set-point}} = E_0$, so that $E_{\text{set-point}}$ is not an extra parameter in the model. Thus the set-point model equations (69a)–(69b) is an indirect action model

in which drugs alters the thermostat regulator signal by decreasing the reference point from E_0 to $E_0(1 - g(C))$.

We refer to (89) for a discussion of problems associated with the fit of their model to their data set, and in particular to the model simplifications that were adapted in the process.

DISCUSSION

In the past 40 years the field of PK/PD modeling has evolved from the simple use of empirical functions, strictly associated with linear regression, to the employment of a diverse array of complex nonlinear models which reflect the essential underlying rules of pharmacology and physiology, models which are in turn embedded in a complex statistical frameworks. We will not play Cassandra (107) in the following, but we will simply try to discuss some possible future directions specifically from a modeling and statistical viewpoint. Others, more closely involved with actual PK/PD studies, are much more qualified to forecast the scientific directions of the field.

Computing

From a historical perspective it is quite striking to notice to what extent the development of the personal computer, together with software combining differential equation solvers with non-linear minimization routines accelerated the PK/PD modeling progress.

Up at least to the mid seventies there was just one sophisticated data modeling and analysis tool available: the NONLIN program by Carl Metzler that was the most popular one in pharmacokinetics (108) and the SAAM program developed by Mones Berman at the National Institutes of Health (20). Without computers modeling was mostly an exercise in simplification to coerce complex processes into linear relationships of sorts, so that the estimation of useful parameters could be achieved, literally, using pencil and paper. Many of the limitations of the early PK/PD models are therefore of purely technological nature, related to lack of tools, rather than lack of insight on possible mechanisms of action.

The explosion of activity in the last quarter of a century begs the question: are the current computers and software limiting the development of the field? One cannot fail to notice that all computer programs available today share, to different degrees, the same limitations: (i) user unfriendliness, (ii) severe computational limitations, (iii) single task orientation. User unfriendliness is not only limited to the interface, that is data entry and

model specification (which is often obscured under massive help manuals), but extends to the need to specify initial estimates for the parameters, the generally very poor quality of diagnostics available to the user to evaluate the results both in terms of convergence (a frequently overlooked issue which has dire consequences in terms of reported results) and in terms of goodness of fit of the model.

Computational limitations are severe: a simple PK/PD population model combined with a modestly sized data set can easily generate computation times of the order of days or weeks, using any of the fastest desktop computer CPU available. Similarly, relatively complex models cannot be developed without investing exorbitant amount of times. For example, for a complex HIV-1 model, keeping track of different viral sub-populations, the number of differential equations quickly becomes intractable for all practical purposes.

Finally, all the computer programs are devised to fit a single model at a time: this completely contradicts the reality of modeling, which requires fitting many (often hundreds for a population analysis) models to the same data set. The result, in our experience, is that a complete analysis of a data set, especially when using a mixed effect model, requires weeks if not months of time in the hands of very experienced data modelers.

It is easy to forecast that any improvement in (i)–(iii) will generate more complex and at the same time more reliable models in the future. The ideal is one of a completely automatic procedure, which fits a large class of models to (multiple) data sets and provides foolproof minimizations, diagnostics, measures of goodness of fits and posterior probabilities of the different models, and can update results, in a Bayesian context (109), when new data become available.

Semi-parametric Modelling

Pharmacodynamics are often much more complex than pharmacokinetics, and in general require much more varied models: there is little equivalent to the ubiquitous use of the one-, two- or three-compartmental models in PK. Linear, or non-linear clearance, time-invariant compartmental models describe a large number of drugs disposition, and often absorption. In PD one has a minimum of five, conceptually very distinct, basic models (direct action, and the four types of indirect action models) just to begin with. The complexity and large number of different pharmacodynamic responses, compared to absorption and disposition, explains the variety of the models required in PD.

This situation, basically rooted in physiology, helps explain why there is little equivalent in PK/PD to "non-compartmental" analysis (110),

and why there has been, perhaps fortunately, comparatively little interest in "sparse sampling" designs in PK/PD population modeling, since the uncertainty about the PD model precludes the use of sub-optimally informative experimental designs.

For this reasons most of the non-parametric attempts described in parametric and sem: parametric paragraph might end up having only historical interest: a non-parametric procedure must be model-independent to be useful, and all the approaches described in (27,64–68,70), besides their statistical limitations, are not so, in the sense that they only apply to direct action models.

A truly non-parametric approach has been described in (111), where Volterra series (112) are used to relate Doses or PK to an arbitrary PD response, without any prior knowledge on the structure, direct or indirect or otherwise, of the system. Although the methodology is very sophisticated, and it can be used within a maximum-likelihood (including mixed effects), or Bayesian, statistical framework, it is rather doubtful that the it will be used in a lot of applications, mostly because it requires large volumes of data to be applied successfully, and because equivalent approaches, in different fields (113), have not been able to overcome the justifiable scientists' resistance to methods which explain little of the structure of systems underlying data.

Semi-parametric models, might find a better reception, because they maintain a high degree of physiological interpretability and allow easier data exploration as well. Since one of us (Verotta) spent a good deal of his post-doctoral training with his dear mentor Lewis developing non- and semi-parametric models, we now spell out a few possibilities which can be developed by somebody willing to put his/her time and creativity in such an enterprise.

First, and rather obviously, indirect action models can easily be recast into a semi-parametric approach (a direct action model is a special case of the semi-parametric models found in (74) by simply using a spline in place of a parametric sub-model. This is achieved by changing Eqs. (33) as follows:

$$\begin{aligned} \frac{dE}{dt} &= k_{\text{in}}[S_1(C)] - k_{\text{out}}E \\ \frac{dE}{dt} &= k_{\text{in}} - k_{\text{out}}[S_1(C)]E \end{aligned} \tag{70}$$

where $S_1(\cdot)$ is a spline, $S_1(0) = 1$, but is otherwise unconstrained. More interesting is to incorporate a convolution operator, as in

$$\frac{dE}{dt} = k_{in}\left[S_1\left(C(t) * \alpha_1 e^{-\alpha_1 t}\right)\right] - k_{out}E \tag{71a}$$

$$\frac{dE}{dt} = k_{in} - k_{out}\left[S_1\left(C(t) * \alpha_1 e^{-\alpha_1 t}\right)\right]E \tag{71b}$$

so that the semi-parametric indirect action model can be driven by "effect compartment" concentrations. From Eq. (71) it is just a simple step to modify the PD model "without PK" reported in (73), see Eqs. (64) and (65), to work with an underlying indirect action model of operation:

$$\frac{dE}{dt} = k_{in}\left[S_1\left(L(t) * I(t)\right)\right] - k_{out}E \tag{72}$$

Returning to Eq. (70), extensions of the model to a non-linear E "elimination", is easily achieved using

$$\frac{dE}{dt} = k_{in}\left[S_1\left(C\right)\right] - k_{out}S_2(E) \tag{73}$$

where $S_2(\cdot)$ is a spline, $S_2(0) = 1$, $S_2(E) \geq 0$.

A hinged bivariate spline (114), indicated with abuse of notation $S_2(\cdot)$, could be used to further generalize the indirect action models (71a)–(71b). Now we have just one indirect action model:

$$\frac{dE}{dt} = k_{in}\left[S(C, E)\right] \tag{74}$$

where

$$S(0, E) = 1 - k_{out}/k_{in}E \tag{75}$$

which implies $S(0, 0) = 1$, is the hinge of the spline. A bi-dimensional plot of the estimated bivariate spline will provide insight in the mode of action of drugs. Extension of (74) as done in Eqs. (72) and (73) for (71) is of course possible but we, mercifully, omit details.

Regulatory Feedback Modelling

Pharmacodynamic responses are almost invariably subject to some sort of physiological homeostatic control. The only reason why feedback is not more often observed is probably due to the fact that the PD system is not pushed far enough from equilibrium by drug administration. In the same spirit of the previous paragraph, which is to provide some conceptual inspiration for future research in the area, we provide (somewhat

novel) general equations for mechanistic and semi-mechanistic regulatory feedback models for direct and indirect action models.

A general equation for regulatory feedback direct action models is very similar to the formulation found (56), it is follows:

$$E = E_0 + g(Ce) + \alpha X \tag{76}$$
$$\frac{dX}{dt} = k_f(E - X)$$

where when $\alpha \leq 0$ we have a negative feedback system, and when $0 \leq \alpha \leq 1$ we have positive feedback. At steady state we obtain

$$E^{ss} = \frac{E_0 + g(Ce^{ss})}{1 - \alpha} \tag{77}$$

For indirect action models we need to rewrite Eqs. (33) to re-introduce the distinction between "endogenous-substance" (which we now indicate E_e) and observed pharmacological effect (which retains the symbol E), the feedback model is as follows:

$$E = E_e + \alpha X \tag{78a}$$

with either

$$\frac{dE_e}{dt} = k_{in}[1 \pm g(C)] - k_{out}E_e \tag{78b}$$

or

$$\frac{dE_e}{dt} = k_{in} - k_{out}[1 \pm g(C)]E_e \tag{78c}$$
$$\frac{dX}{dt} = k_f(E - X) \tag{78d}$$

At steady state the model predicts

$$E^{ss} = \frac{E_e^{ss}}{1 - \alpha} \tag{79}$$

Note how both Eqs. (78a) and (79a) use a linear additive relationship between effect of drug ($g(C_e)$ and E_e, respectively) and regulatory feedback.

For direct action models a non linear feedback can be introduced by using instead of Eq. (78a) the following:

$$E = (E_0 + g(C_e))\,h\,(X) \tag{78a*}$$

where $h(X)$ a monotonic function in X.

More interestingly, nonlinear feedback models for indirect action models which take inspiration from the set-point model described above take the form:

$$\frac{dE}{dt} = k_{\text{in}} - k_{\text{out}} E \; X \tag{80a}$$

$$\frac{dX}{dt} = k_x \left[E_0 \left(1 \pm g(C)\right) - E \right] \tag{80b}$$

which describes a negative or positive effect (depending on $(1 \pm g(C))$) of drug on the regulator by decreasing or increasing the reference set-point. Taking inspiration from indirect-action model where drug acts on k_{out} the model can be modified to

$$\frac{dX}{dt} = k_x \left[E_0 - E \left(1 \pm g(C)\right) \right] \tag{80c}$$

where drug now acts not by changing the set-point, which might be physiologically unlikely, but by modifying the response of the regulatory subsystem to the size of departure from the set-point (thus reducing or increasing the capacity of reacting to a change).

Final Conclusions

The PK/PD field is clearly moving toward mechanistic modelling, since this allows a deeper understanding of the action of drug and, importantly, opens the possibility for a variety of future applications in which *in-silico, in-vitro*, animal, and pharmacogenomics information can be incorporated as prior knowledge in a predictive model of PD response (we note that semi-parametric models allow the incorporation of prior knowledge almost as well as mechanistic models). The forecast is that complex, mixed-effects, mechanistic PD models combined with Bayesian estimation and incorporating different sources of prior knowledge will provide a paradigm for future model development.

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