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Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine

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In veterinary drug development procedures, pharmacokinetic (PK) and pharmacodynamic (PD) data have generally been established in separate, parallel studies to assist in the design of dosage schedules for subsequent evaluation in clinical trials. This review introduces the concept of PK/PD modelling, an approach in which PK and PD data are generated in the same study, and used to derive numerical values for PD parameters based on drug plasma concentrations. The PD parameters define the efficacy, potency and slope (sensitivity) of the concentration–effect relationship. It is proposed that the parameters derived from PK/PD modelling may be used as an alternative and preferred approach to dose titration studies for selecting rational dosage regimens (both dose and dosing interval) for further evaluation in clinical trials. In PK/PD modelling, the explicative variable for effect is the plasma concentration profile. The PK/PD approach provides several advantages over dose-titration studies, including determination of a projected dosage regimen by investigation of a single dose, in contrast to dose-ranging studies which by definition require testing of multiple dosage. Implementation of PK/PD modelling in the veterinary drug development process is currently constrained by the limited number of veterinary studies performed to date, and the consequently limited understanding of PK/PD concepts and their absence from regulatory authority guidelines. Nevertheless, PK/PD modelling has major potential for rational dosage regimen determination, as it considers and quantifies the two main sources of interspecies variability (PK and PD). It is therefore applicable to interspecies extrapolation and to multiple species drug development. As well as the currently limited appreciation of PK/PD principles in the veterinary scientific community, a further constraint in implementing PK/PD modelling is the need to validate PK/PD approaches and thereby gain confidence in its value by pharmaceutical companies and regulatory authorities.

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INTRODUCTION

Predictions, from data generated in preclinical studies, of dosage schedules for evaluation in clinical trials, are based on linking, in some manner, pharmacokinetic (PK), pharmacodynamic (PD) and toxicological data. There are three main possibilities, PK/PD integration, dose titration studies and PK/PD modelling. The former has been used for many years and involves, for example for an antimicrobial drug, integrating an *in vitro* PD measurement such as minimum inhibitory concentration (*MIC*) with one or more PK parameters such as C_{max}, *AUC* or $T > MIC$ generated in a separate PK study (*vide infra*). The objective is to administer a drug dose which achieves plasma concentrations which reach or exceed a breakpoint value of one of the ratios C_{max}:*MIC* and *AUC*:*MIC* or the time $T > MIC$. Commonly this evaluation of a dose is obtained from a titration study, in which two or more doses of drug are administered, either to one group of animals using a cross-over design or to separate groups of animals using

a parallel design. The outcome is usually measured by one or more clinical responses, but, as no blood samples are taken, there is no information on plasma concentration–time profile and therefore neither PK parameters nor surrogate PK/PD markers can be derived. All that is known is administered dose and clinical response at each dose level.

The third approach, PK/PD modelling, is a very versatile tool, which can be used as a more effective and less expensive alternative to dose titration studies. The difference between integration and modelling is that the former brings together data from separate (or the same) PK and PD studies, whereas the latter is normally based on *in silico* modelling of PK and PD data derived from the use (usually) of a single dose of a drug in a single investigation. PK/PD modelling addresses the two key questions asked in drug discovery and development programs: (a) has the best compound been selected as a drug candidate, and (b) has not merely an effective dose but the optimal dosage regimen been established?

Drug action depends on the concentration–time profile at the site of action. Generally this will not be identical to the plasma–concentration profile but it bears a proportional relationship to concentration in plasma. This approach therefore provides the basis for improved drug development through the use of PK/PD modelling. The PK and PD information generated in the single dose study is bridged using a link model. Therefore, three models are used, the PK, the PD and the link models.

The PK/PD modelling concept can be applied at all stages of drug discovery and development. For example, in the earliest stages compounds having acceptable *in vitro* potency, but for which there is insufficient *in vivo* exposure, can be rejected. In subsequent studies the main objective of PK/PD analysis is to generate estimates of the three relevant *in vivo* properties of the drug, namely potency, efficacy and selectivity. In addition, PK/PD modelling provides an attractive basis for extrapolating data both between species and from *in vitro* to *in vivo* studies. When applied to clinical trial data, PK/PD modelling also takes account of the two main sources of intra- and inter-animal variation, thus providing the basis for individualizing dosage regimens by using relevant PK and PD co-variables (population PK/PD modelling).

This review outlines the limitations of dose titration studies for dose determination, summarises the scientific principles of PK/PD modelling and discusses the application of the PK/PD approach to studies of mechanism of drug action as well as dosage determination in drug product development programs. This article complements other recent reviews on this subject (Toutain, 2002, 2003; Toutain *et al.*, 2002; Lees *et al.*, 2004a,c).

DOSE TITRATION STUDIES

In both human and veterinary medicine, dose selection historically has generally been based on dose ranging/titration studies, in which the simple parallel design has been used. Animals, either healthy or experimentally infected, are randomly allocated to several dose groups (at least 3 but preferably more) and the responses compared using a standard statistical test of the hypothesis (Fig. 1). The limitations to this design are twofold: it fails to provide data on the shape of the individual dose–response relationship (such information is important for determining drug selectivity) and; the dose selected as the most effective is not necessarily and indeed is unlikely to be the optimal dose (Toutain *et al.*, 2002). The dose selected in a dose titration study depends critically on the power of the design, which is controlled by the number of animals. Trials in which sample size is small commonly lead to selection of high doses. With small numbers it can be difficult to demonstrate dose dependency, creating the false assumption that the response is maximal. Because of low statistical power, to reach a statistically significant effect, doses greater than those required for optimal efficacy are likely to be selected.

The parallel design is necessarily used in studies of anti-parasitic and antimicrobial drugs, as an irreversible drug effect, the eradication of the pathogen, is the pivotal outcome. In contrast, drugs acting on physiological systems almost invariably act reversibly and a cross-over design is then strongly preferred. In cross-over studies each animal receives several doses and this enables generation of individual dose–response curves. For each animal, the dose–effect relationship is expressed by the equation:

$$\text{Effect} = E_0 \pm \frac{E_{\max} \times \text{Dose}^n}{\text{ED}_{50}^n + \text{Dose}^n} \qquad (1)$$

in which Effect, the dependent variable, is the effect predicted for a given dose, which is the independent variable. E_0 is the effect obtained in the absence of drug. For example, this may be a

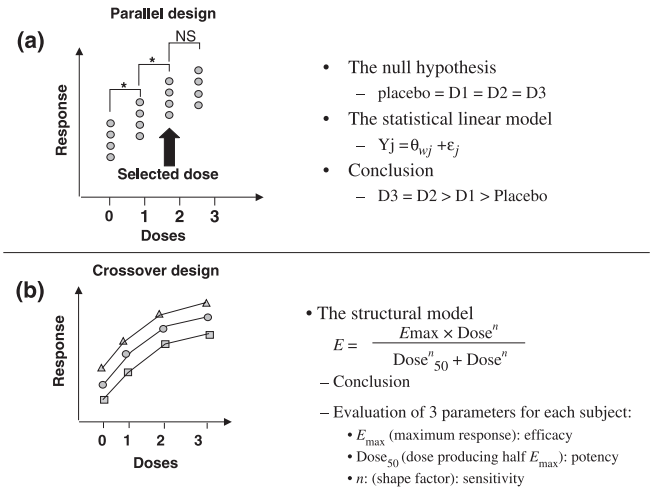

Fig. 1. Comparison of parallel and cross-over designs for dose titration studies. In the parallel design, animals are randomly allocated to one of the selected dose levels (0, 1, 2, 3 where 0 = placebo or no treatment). Data analysis is performed by a test of hypothesis and the selected dose must be one of those tested as there is no possible interpolation. In the cross-over design every subject receives every dose (0, 1, 2, 3) and dose–effect curves are generated for each animal. For each separate curve, PD parameters ($E_{\max}$, ED_{50}) are determined and any dose within the tested range can be selected (not necessarily an actual dose tested).

placebo effect or the basal response of the system. E_{max}, the measure of efficacy, is the maximum possible effect, whilst ED_{50}, a measure of drug potency, is the dose which produces half E_{max}. For drugs with a narrow therapeutic index, E_{max} may not be directly measurable *in vivo*. Generally, an exponent (n) is incorporated in the model and this measures the slope of the dose–effect relationship. It provides information on selectivity of the drug for the effect under study (*vide infra*).

The essential difference between parallel and cross-over design investigations is that, with the latter, any dose within the range of those evaluated can be selected as an optimal dose because interpolation is possible. This is not possible for studies based on parallel designs (Sheiner *et al.*, 1991).

COMPARISON OF DOSE-TITRATION AND PK-PD MODELLING

From Eqn 1 it will be noted that ED_{50} is not a true PD parameter but a hybrid PK/PD variable. In fact, ED_{50} is based on two PK and one PD parameters, as indicated by the equation:

$$ED_{50} = \frac{Cl \times EC_{50}}{F} \quad (2)$$

where Cl is plasma clearance, F is systemic bioavailability for any extravascular route of drug administration and EC_{50} is the steady state plasma concentration providing half of E_{max}. For intravenous dosing $F = 1$ and ED_{50} is then solely dependent on Cl and EC_{50}.

Figure 2 illustrates the essential differences between dose titration and PK/PD modelling studies. The objective of both is to establish the relationship between administered dose and response. However, in the PK/PD approach, dose is replaced by the plasma concentration–time profile and potency is expressed in terms of EC_{50} rather than ED_{50}. EC_{50} is a true PD parameter, in contrast to ED_{50}. For a given endpoint, there is only one steady-state EC_{50} and this, unlike ED_{50}, is independent of PK parameters, formulation and administration route. Thus, EC_{50} is a drug-dependent PD parameter, whereas ED_{50}, is a formulation-dependent variable. Therefore, if a new formulation of the drug is to be developed, there is no requirement to undertake a new PK/PD trial. All that is required is a PK study to establish the influence of a new bioavailability on effect. Hence, EC_{50} has much wider application than ED_{50}, and determination of EC_{50} is a primary objective of PK/PD studies.

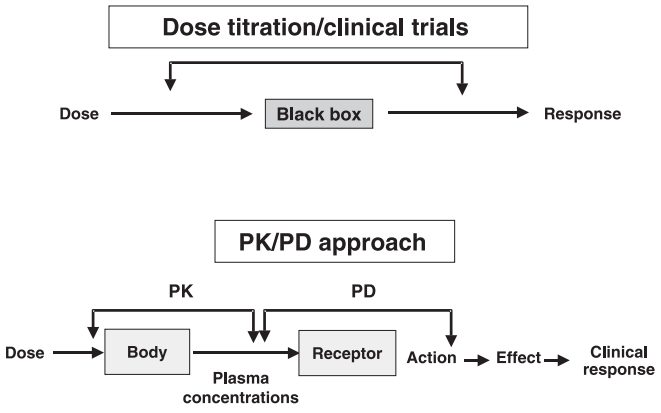

Fig. 2. Diagrammatic comparison of dose–effect relationship and pharmacokinetic (PK)/pharmacodynamic (PD) modelling. The objective of both is to define the relationship between dose and drug response. The dose–effect relationship may be regarded as a black box approach for which dose is the explicative variable of drug response. PK/PD modelling opens the black box to define the two primary processes interposed between dose and response. As a first step dose is transformed into a plasma concentration–time profile (PK model). As a second step plasma concentration–time profile replaces dose as the variable which accounts for the drug response.

PLASMA CONCENTRATION–EFFECT AND DOSE–EFFECT RELATIONSHIPS

Plasma concentration–time profiles provide more information than administered dose. Dose may be described as a mass (e.g. mg/kg) selected by the clinician. It provides no intrinsic pharmacological information. Concentration–time profiles, on the contrary, are dependent on both dose (clinician determined) and the animal receiving the dose (clearance, distribution…). Furthermore, plasma concentration–time data incorporate a temporal element, so that PK/PD modelling is appropriate to establish not only a dose amount but also a dosage interval.

A major advantage of PK/PD modelling to establish dosage regimens is that both efficacy and potency, E_{max} and EC_{50}, can be estimated after administration of a single dose, because establishing the entire plasma concentration–time profile provides single-sweep coverage of the whole concentration–effect relationship. The PK/PD modelling approach is dependent on the temporal relationship between plasma concentration and the measure of drug effect (*vide infra*). This is exemplified by the study of Toutain *et al.* (2001), in which comparison between dose-ranging and PK/PD modelling of data for the nonsteroidal anti-inflammatory drug (NSAID) nimesulide in the dog indicated that PK-PD modelling effectively determined both dose and dosage interval after a single oral dose administration. The PK/PD predictions were consistent with those provided by a separate dose titration study.

ESTABLISHING PK/PD MODELS

Generally PK/PD modelling requires consideration of three separate models (Fig. 3). First, a conventional PK model is used to transform administered dose into a concentration–time profile. Secondly, a link model is applied to describe the passage of drug from plasma to the biophase. Thirdly, a PD model then relates biophase concentration to the effect (Holford & Sheiner, 1981).

The PK model is generally the traditional compartmental model and PK parameters are estimated in the conventional way. The PK model is used to provide concentration data for the

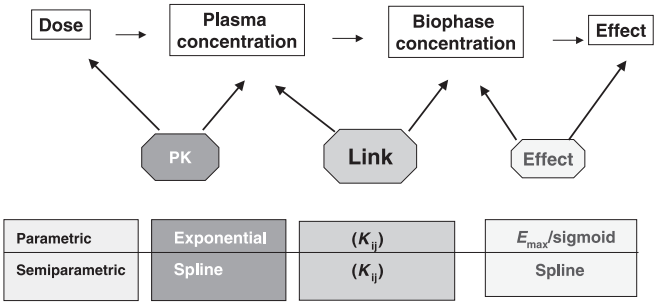

Fig. 3. The three models for a pharmacokinetic (PK)/pharmacodynamic (PD) effect compartment model. Effect compartment modelling involves three models: a PK, a link and a PD model. Modelling is said to be parametric when the three models can be described by a set of parameters. PK model is usually a classic compartmental model described by a poly-exponential equation. The link model is generally described by a first order rate constant and the PD model can be any of the classical models (E_{max}, Hill…). The models are said to be semi-parametric when the PD or both the PK and PD models are not fully parameterized. For example, the PK model can be replaced by the smoothing functions (e.g. cubic spline). This avoids specifying a PK model and can be useful when the plasma concentration driving effect cannot be described by a conventional PK model (e.g. for an endogenous substance with episodic release). Similarly, the first order rate constant (link model, parametric) can be directly estimated without the need to specify a specific PD (parametric) model by only searching the best value collapsing the hysteresis curve.

PD model. If the plasma concentration–time relationship cannot be described by a conventional PK model, it can be replaced by a smoothing procedure (e.g. cubic spline).

For most drug responses the plasma concentration–time profile and the effect–time relationship are not in phase. In general, the effect lags some time behind plasma concentration. Hence, concentration cannot be incorporated directly into a PD model. This is illustrated by plotting effect (*Y*-axis) against plasma concentration (*X*-axis) (Fig. 4). Following the data points (1–6) in chronological order reveals a hysteresis loop. The term derives from a Greek word translated as 'coming late'. Less commonly the effect may be smaller at a later time point for a given plasma concentration. This is termed proteresis and means 'coming early'. It is described by a proteresis clockwise loop (Fig. 5).

In a hysteresis loop, the mechanistic basis for the delay should be identified as this dictates the appropriate type of link model to use. The delay may be of PK origin, for example involving slow distribution to the biophase or metabolism of a pro-drug to an active metabolite. If drug response is related directly to concentration of drug in the biophase, an effect compartment link model is selected. This is interposed between the PK and PD models. Normally, the link model describes drug transfer from plasma to the biophase by a first order rate constant (K_{eo}). K_{eo} and $t_{1/2keo}$ are the parameters of the link model. They are estimated from the time course of drug effect.

For most drugs, the combination of drug with its receptor is followed by a cascade of time-dependent biochemical reactions (Lees *et al.*, 2004a). Thus, the measured response does not result instantaneously from binding of the drug to the receptor. The delay in response may be very short (msec) when gated ion channels are opened, short (sec) when secondary chemical messengers follow drug–receptor interaction but much longer (possibly hours) when transcription processes are involved. Under the latter circumstance, the delay between plasma concentration and the response is because of the intrinsic temporal responsiveness of the system. For this type of response, indirect response models are used.

Dayneka *et al.* (1993) have proposed four basic indirect response models based on the following equation, which describes the rate of change of response with time when no drug is present:

$$\frac{dR}{dt} = K_{in} - K_{out}R \qquad (3)$$

where dR/dt is the rate of change in the response variable (R). It is assumed that the response is formed at a constant rate (K_{in}) and that it disappears in a first-order manner (K_{out}). It is further assumed that the indirect action of the drug involves inhibition or stimulation of the physiological pathways that regulate production or dissipation of the monitored effect as described in the Equation:

$$\frac{dR}{dt} = K_{in} \times \{\text{stimulation or inhibition function}\} - K_{out} \times \{\text{stimulation or inhibition function}\} \times R \qquad (4)$$

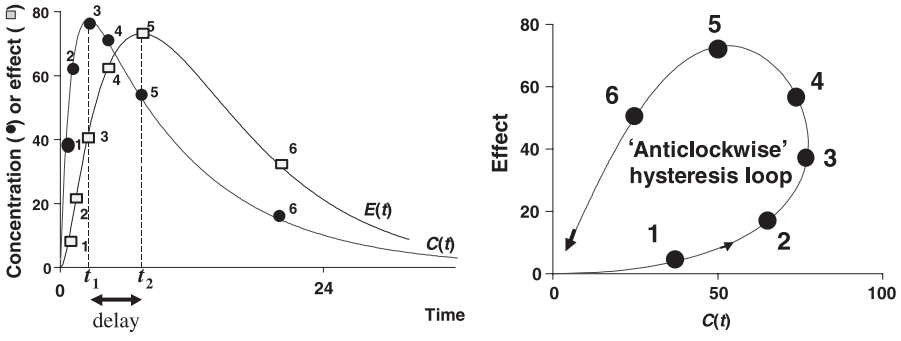

Fig. 4. In general, plasma concentration–time and effect–time relationships are not in phase. The peak concentration occurs at time t_1 and peak effect occurs at a later time t_2. An arithmetic plot of effect vs. time reveals a hysteresis loop.

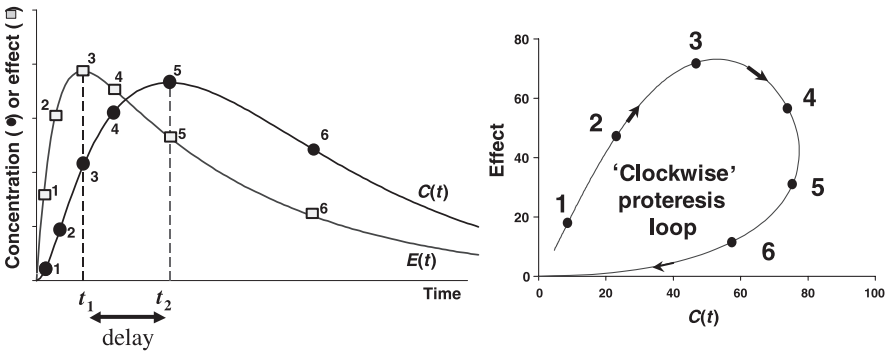

Fig. 5. Occasionally peak drug effect, occurring at time t_1, may precede the achievement of peak plasma concentration at time t_2. An arithmetic plot of effect vs. time reveals a proteresis loop.

Concentration lags behind effect (infrequent)

- e.g. tolerance phenomenon /accumulation of a metabolite having antagonist properties
- e.g. effect driven by arterial concentration but delay to equilibrium in venous blood

Stimulation or inhibition function can be the classical E_{max} model, so that

$$\frac{dR}{dt} = K_{in}\left(1 + \frac{E_{max} \times C}{EC_{50} + C}\right) - K_{out}R \quad (5)$$

or for an inhibitory effect:

$$\frac{dR}{dt} = K_{in}\left(1 - \frac{I_{max} \times C}{IC_{50} + C}\right) - K_{out}R \quad (6)$$

In Eqn 5 E_{max} (a number) is the maximum effect attributed to drug. In Eqn 6 I_{max} is often fixed to 1 (fractional I_{max} model).

The third and final model in PK/PD modelling is the PD model, of which there are two principal types. The first describes a concentration–effect relationship which is graded, whilst the second, quantal concentration–response relationship, involves an all-or-none type response. The graded model is applicable when the response to changing drug concentrations can be determined and quantified on a scale (e.g. heart rate, blood pressure, body temperature, survival time, PGE2 concentration). In the quantal or fixed-effect model the measured effects are categorical (e.g. alive or dead, bacteriological cure or not, presence or absence of side-effects, etc.). For quantal concentration or dose–response and exposure–response relationships, there is no correlation between concentration (dose) or exposure and magnitude of the effect but rather to frequency of the effect, which is all-or-none. Quantal responses are commonly clinical end-points, whilst graded responses are usually surrogates.

The most useful and widely applied model for graded effect relationships is the Hill (sigmoidal E_{max}) model described by the equation:

$$E(t) = E_0 + \frac{E_{max} \times C^n(t)}{EC_{50}^n + C^n(t)} \quad (7)$$

where $E(t)$ is the effect obtained for the concentration $C(t)$ at time t, E_{max} is the maximal effect obtainable, EC_{50} is the plasma concentration producing 50% of E_{max} and n is the Hill coefficient, which represents the slope of the concentration–effect relationship. When $n = 1$, the model corresponds to a hyperbolic function and reduces to the E_{max} model (Fig. 6). The E_{max} model is derived from classical drug-receptor theory but, when used in PK/PD modelling, its primary function is as an empirical model.

Most drug-induced effects involve modulation of some physiological variable (e.g. heart or respiratory rate, rectal temperature etc.). Incorporation of the term E_0 in Eqn 7 then denotes the baseline effect. However, E_0 can also represent a placebo effect.

The drug effect may be due to inhibition rather than stimulation of a physiological or biochemical process. The drug effect is then subtracted from the baseline (E_0) and the corresponding equation is:

$$E(t) = E_0 - \frac{I_{max} \times C^n(t)}{IC_{50}^n + C^n(t)} \quad (8)$$

where IC_{50} is the concentration which produces 50% of the maximum inhibition effect (E_{max}). Equations 7 and 8 contain several parameters (E_0, E_{max}, EC_{50} or IC_{50}, and n) and the final objectives of PK/PD modelling are to determine the means and variances of each parameter from the values of $E(t)$ derived over a range of $C(t)$ values (Toutain, 2002, 2003).

PARAMETERS AND DEPENDENT VARIABLES OF THE HILL PD MODEL

Dependent variables (action, effect, response and surrogates)

The dependent variable E refers to the generic term 'effect'. It is, however, more useful to refer to three terms, drug action, drug effect and drug response (Holford, 1992). As an example, the 'action' of an antimicrobial drug comprises, say, inhibition of bacterial protein synthesis, the 'effect' of this is to lyse and thereby kill bacteria, whilst the clinical 'response' may comprise reduced mammary gland swelling, suppression of fever or abolition of dyspnoea. If elucidation of mode of action is the objective of a PK/PD study, the drug action at a major

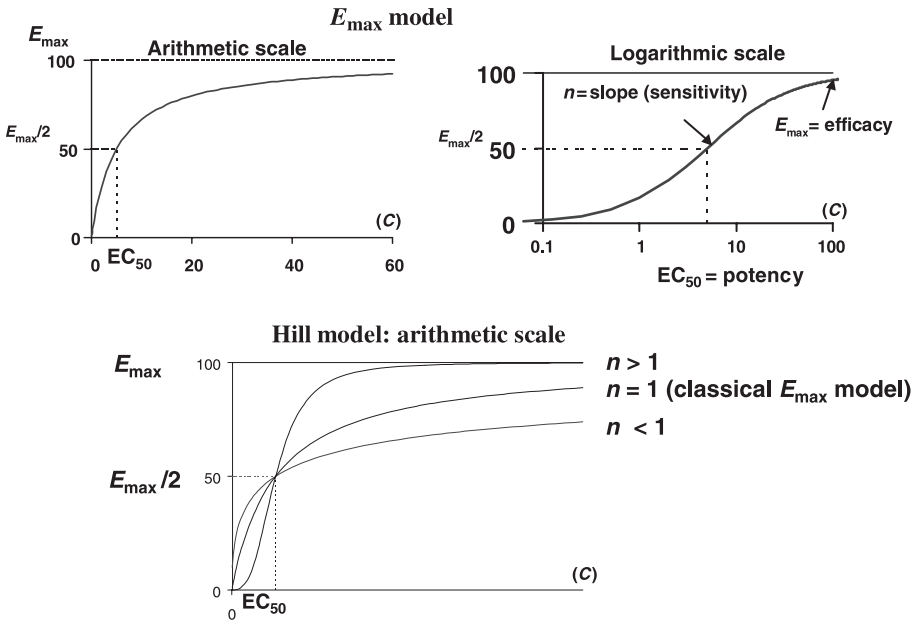

Fig. 6. In the E_{max} and Hill models the concentration–response curves are hyperbolic when plotted on an arithmetic scale and sigmoidal when plotted on a logarithmic scale. The relationships are described by Eqn 7 (see text) in which $n = 1$ in the E_{max} model and n can have any value greater or less than 1 in the Hill model. In the Hill model when $n > 5$ the relationship is described as quantal or all or none. The maximal possible effect (*Y*-axis) is E_{max} and this parameter describes efficacy. The concentration corresponding to $E_{max}/2$ (*X*-axis) is EC_{50} and this parameter describes potency. The parameter describing the sensitivity of the concentration–effect relationship is the slope (n) and this parameter influences drug selectivity.

site in or on bacteria, for example on an enzyme, will be determined. On the contrary, if the purpose of the study is determination of a dosage regimen, a clinically relevant response will be measured.

In practice, the clinically relevant drug response may be difficult or even impossible to measure. For example, for an antimicrobial drug, measurement of bacteriological cure may not be possible, because the site of infection is not accessible in the living animal. In other cases there may be difficulty in taking accurately and objectively a quantitative measure (e.g. pain in an animal which has received an analgesic). In yet other cases there may be a significant time delay in response (e.g. survival time for cancer chemotherapy). In these circumstances, it may be necessary, in place of the effect of ultimate interest, to record a surrogate end-point. This may be defined as a biomarker that can be objectively measured and validated and which serves as an indicator of a pathological or normal process (Colburn, 2000; Anonymous, 2001).

Substitution of the clinical end-point by the surrogate end-point should be validated. In veterinary medicine several PK/PD indices for antimicrobial drugs (e.g. C_{max}:*MIC* ratio, *AUC*:*MIC* ratio, *AUIC* and $T > MIC$) have been proposed as predictors of bacteriological cure and clinical outcome (McKellar *et al.*, 2004). Inhibition of angiotensin converting enzyme (ACE) has been used as a surrogate in studies of ACE inhibitors (Toutain *et al.*, 2000; Toutain & Lefebvre, 2004); the clinical requirement of ACE inhibitor therapy being increased survival time of animals with disease (e.g. congestive heart failure) and improvement in the quality of life.

Parameters of the Hill equation

Three PD parameters estimated from the Hill equation are drug potency, efficacy and sensitivity (Fig. 7).

Efficacy (E_{max}) is defined as the maximum effect that can be produced by or in that system (e.g. maximal possible heart rate). Clinically this is a crucial parameter. For example, the analgesic efficacy of morphine, an agonist for opioid receptors, will be greater than that of butorphanol, a partial agonist for opioid receptors. In an equine study the efficacy of flunixin, on stride length, a surrogate marker of pain in a model of inflammatory arthritis, was greater than that of phenylbutazone (Toutain *et al.*, 1994). There is no necessary correlation between efficacy and potency, so that a drug of lower potency can produce a greater E_{max} than a drug which is more potent.

Potency (EC_{50}) is defined as the intensity or magnitude of drug activity relative to its concentration. Estimates of EC_{50} (for stimulation) and IC_{50} (for inhibition) are obtained by application of the Hill equation. There is an inverse relationship between potency and the concentration needed to produce the effect (Fig. 7). Potency *per se* is of limited importance to the clinician. Low potency of a drug becomes disadvantageous in therapy only if the size of the effective dose is so large that it is difficult or very inconvenient to administer.

Sensitivity is defined by the shape coefficient (n), that is the slope, of the concentration–effect relationship. The term n has a precise meaning in terms of drug binding to target sites in drug receptor theory, but *in vivo* the experimentally determined value of n should not be interpreted in mechanistic terms. However, *in vivo* the numerical value of n has clinical significance in relation to drug selectivity and sensitivity. It can provide essential data on the range of useful concentrations or doses required to elicit the required effect whilst avoiding unwanted toxic or side-effects (Fig. 8).

In vivo, when n has a low value (<1), the PD profile is relatively flat and relatively small changes in effect are obtained over a wide concentration range. This shallow concentration–effect relationship explains the very persistent action of some

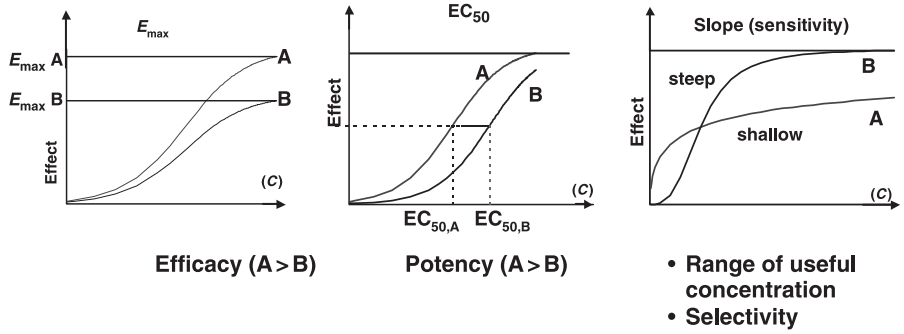

Fig. 7. The structural parameters of the dose–effect relationship. *Left*: Efficacy (*Y*-axis) describes the drug response of the system. This is the parameter of interest for the clinician. Drug A is more efficacious than drug B because $E_{max}A > E_{max}B$. *Middle*: Potency (*X*-axis) expresses the intensity of the drug activity in terms of concentration (EC_{50}) or dose (ED_{50}). Drug A is more potent than Drug B because EC_{50}, $A < EC_{50}$,B. *Right*: Sensitivity (slope): the slope of the concentration–effect curve can be more or less shallow (or steep) and the slope (*n* of Hill with a E_{max} model when $n = 1$) describes the sensitivity of the concentration–effect relationship. The *n* of Hill is the midpoint slope of the concentration effect relationship. The sensitivity is of relevance when considering the range of useful concentrations, a shallow curve (drug A) being characterized by a larger range of efficacious concentrations than a steep curve (drug B). The sensitivity also influences the selectivity i.e. the possibility of achieving a desired effect without unwanted side effects (see Fig. 8).

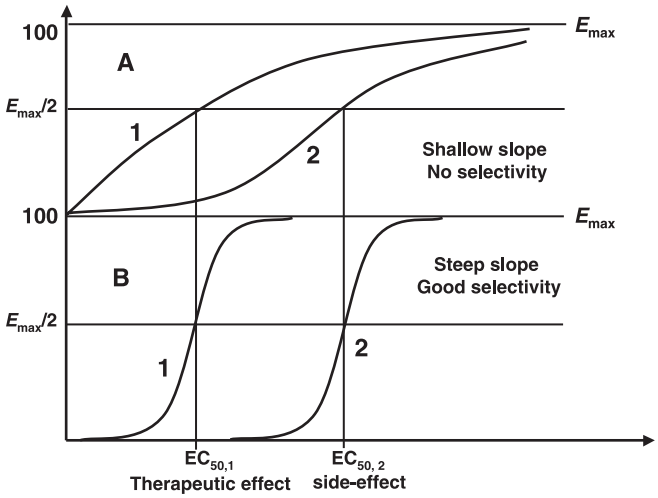

Fig. 8. The selectivity of a drug is defined by the slope of the concentration–effect relationship. In this example drug A (top) and drug B (bottom) have the same potency (EC_{50}) and the same efficacy (E_{max}) for the therapeutic effect (curve 1). A and B also have the same efficacy and potency for the side effect (curve 2). For the two drugs selectivity differs. Full efficacy is obtained with drug B (but not drug A) without significant side-effects.

drugs. For such drugs, the terminal half-life is generally a good predictor for the duration of action. On the contrary, when the slope is steep relatively small concentration changes on either side of EC_{50} produce effects ranging from no effect to almost maximum effect. Such a steep relationship has been demonstrated for phenylbutazone and flunixin for lameness inhibition in horses (Toutain *et al.*, 1994). When *n* has a high value but the therapeutic index is low, careful drug monitoring is required to ensure efficacy without toxicity.

As *n* increases to attain high values (>5), the concentration range for a given effect tends towards a simple threshold for a critical concentration just above EC_{50} and the graded PD model converts to a quantal model when $n \gg 1$.

For further discussion and a glossary of terms used in pharmacodynamics see Neubig *et al.* (2003) and Lees *et al.* (2004a).

THE QUANTAL CONCENTRATION–EFFECT RELATIONSHIP (FIXED-EFFECT MODEL)

For some drugs the effect obtained may be all-or-none as a consequence of the mechanism of action. For example, for an anti-epileptic drug, seizures may be controlled or not controlled in some subjects (although in others there may be reduction in frequency of seizures). For other drugs the effect may be monitored to a selected end-point (animal recumbent or standing, cured or not cured). For such effects the concentration–response relationship indicates frequency of the occurrence which a given concentration of a drug produces. The EC_{50} (or ED_{50}) is then the median effective concentration or dose for which 50% of animals exceed the threshold, and the slope of the relationship represents the dispersion or variance of the threshold in the population.

The ratios of two median effective concentrations, each for two separate end-points (required effect and undesirable effect) provides essential data on drug selectivity. However, as well as median ratios, slope must also be considered, because wide inter-animal dispersion (as indicated by a shallow slope) results in likely overlap of clinically desired and unwanted effects in the population.

APPLICATIONS OF PK/PD MODELLING IN VETERINARY DRUG DISCOVERY

The PK/PD modelling may be applied at all stages of drug discovery and development in veterinary medicine. In the early stages of discovery, initial estimates of drug potency

(EC_{50}) may be obtained using *in vitro* test systems for a group of new agents. It is then necessary to establish if the candidate drug is likely to be usable at a dose which is acceptable and inexpensive. An estimate of dose can be obtained rapidly if drug clearance is known and *AUC* has been determined, using Eqn 2. Either the cassette dosing (or n-in-one dosing) study or the cocktail approaches can be used. Cassette analysis (Hop *et al.*, 1998) involves pooling of several plasma samples obtained at different sampling time points to provide a single sample whose concentration is directly proportional to *AUC*. In this method it is possible to use an abbreviated calibration curve. In the cocktail approach several compounds are administered simultaneously to one animal (Allen *et al.*, 1998). *AUC* estimates indicating clearance are placed in rank order and this enables compounds to be prioritized on the basis of potencies.

Plasma clearance data should always be obtained at an early stage in veterinary drug development; it gives an approximate estimate of the final dosage regimen and therefore permits drugs with clearance values too high or too low for the intended use to be eliminated. As an example, low clearance will normally be required for an antimicrobial drug with time-dependent activity, whilst rapid clearance will be a requirement for an injectable anaesthetic, where the requirement is for short duration of action.

A defined plasma clearance value expressed in mL/kg/min might be considered high in a large animal species but low in a small species (Toutain, 2002; for further details on the interpretation of a plasma clearance see Toutain & Bousquet-Melou, 2004).

EXTRAPOLATION BETWEEN *IN VITRO* AND *IN VIVO* STUDIES AND INTERSPECIES EXTRAPOLATION

Pharmacokinetics is the main source of between species variability. In those circumstances and for those species for which PD variability is small or absent, the PK/PD approach offers the prospect of inter-species extrapolation. This is possible when drug potency is independent of species and when the same overall exposure as indicated by *AUC* produces the same effect in two or more species. As the only factor determining *AUC* for intravascular administration is plasma clearance, dose estimation for a second species 2 is derived from the effective dose in species 1, by application of the equation:

$$\text{Dose}_{\text{species2}} = \frac{\text{Dose}_{\text{species1}} \times Cl_{\text{species2}}}{Cl_{\text{species1}}} \qquad (9)$$

where Cl_{species1} and Cl_{species2} are plasma clearances for species 1 and 2, respectively.

When required, Eqn 9 can be modified (a) by introduction of a bioavailability factor, *F*, for nonvascular administration and (b) if drug binding to plasma protein differs between the species, the ratio of free fraction, fu, for the 2 species should be introduced, as only free concentration produces pharmacological effects. Therefore, the modified equation is:

$$\text{Dose}_{\text{species2}} = \frac{\text{Dose}_{\text{species1}} \times fu_1 \times Cl_{\text{species2}}}{fu_2 \times Cl_{\text{species1}}} \qquad (10)$$

where fu_1 and fu_2 are, respectively, free fractions for species 1 and 2. Similarly, extrapolation from *in vitro* or *ex vivo* data to *in vivo* conditions can be made when a validated effective concentration is derived from *in vitro* or *ex vivo* assays. The EC_{50} or IC_{50} data are incorporated directly into eqn 2. However, *in vitro* studies generally use a protein free matrix and therefore study only free drug concentration, so that a correction for drug binding to plasma protein will be required to estimate the appropriate *in vivo* plasma EC or IC.

DETERMINATION OF EFFECTIVE AND SAFE DOSES OF NSAIDS

In veterinary medicine PK/PD data have been generated for drugs of the NSAID class. The action of several licensed NSAIDs (including flunixin, ketoprofen, vedaprofen and tolfenamic acid) in a range of farm animal species (including sheep, calf, horse, goat and pig) as inhibitors of the cyclo-oxygenase (COX) isoforms, COX-1 and COX-2, has been investigated in an experimental model of acute inflammation (Lees *et al.*, 1994; Landoni *et al.*, 1995; Landoni & Lees, 1995; Landoni *et al.*, 1996). The PD parameters EC_{50}, E_{max} and the Hill coefficient and the link model parameter $t_{1/2keo}$ were computed using an effect compartment model. Two effects of the drugs were monitored: (1) *ex vivo* inhibition of synthesis of serum thromboxane (TxB_2) indicative of COX-1 activity and (2) *in vivo* inhibition of inflammatory exudate PGE_2, tentatively indicative solely of COX-2 activity. The selected endpoints represent drug actions at the molecular (mediator synthesis) level. They are surrogates and do not indicate outcomes of *direct* clinical relevance. However, the magnitude of COX-2 inhibition is relevant to clinical outcome and can be used to compute a tentative clinical dose. Warner *et al.* (1999) have suggested at least 80% COX-2 inhibition is required for clinical efficacy, whilst Landoni *et al.* (1995, 1996) have proposed inhibition of at least 90–95% COX-2 may be needed to produce a clinical response. For instance, in calves tolfenamic acid was shown to have an IC_{50} of 0.077 μg/mL for PGE_2 inhibition (Landoni & Lees, 1995); the slope exponent (*n*) was 2.38. Using these data, 95% PGE_2 inhibition corresponds to a plasma concentration of 0.245 μg/mL. If this EC_{95} is incorporated in Eqn 2 and as plasma clearance of tolfenamic acid was found experimentally in the same study to be 0.30 L/kg/h a dose of 1.76 mg/kg/24 h is obtained (Lees, 2003). This is approximately equal to the manufacturer's recommended daily dose of 2 mg/kg.

Generally, the principal objective of a PK/PD study is to project a dosage regimen and the best approach is to relate NSAID concentration in plasma to clinically relevant indices, for example, for fever – body temperature, for locomotor inflammation – lameness, for hypertension – reduction in arterial blood pressure. In the horse, stride length in a Freund's adjuvant-induced carpitis has been used to establish both potency and efficacy of the phenylbutazone (Toutain *et al.*, 1994). Thus,

plasma clearance of phenylbutazone was 41.3 mL/kg/h, EC_{50} was 3.6 μg/mL and the slope factor (*n*) was steep (almost quantal). These data indicate that the dose required to suppress lameness by 50%, obtained from Eqn 2, is 3.6 mg/kg/day. This is slightly below the manufacturer's recommended daily dosage of 4.4 mg/kg. Because of the very steep slope, the EC_{50} for phenylbutazone should be regarded as a threshold value, with almost maximal effects occurring at higher concentrations.

The IC_{50} values for nimesulide for lameness and antipyresis in a Freund's adjuvant model in the dog were 6.26 and 2.72 μg/mL, respectively (Toutain *et al.*, 2001). As plasma clearance of nimesulide was 15.3 mL/kg/h and oral bioavailability was 47%, the calculated ED_{50} for lameness treatment was 4.9 mg/kg/day. This is virtually identical to the manufacturer's recommended dose of 5 mg/kg.

For multiple dosing regimens, the time interval between doses can be determined by PK/PD modelling. It is possible to simulate a large number of dose rates and dosage interval possibilities to identify regimens optimizing efficacy and safety without the need for additional studies. For example, modelling predicted greater antipyretic efficacy for nimesulide in the dog with a dosage of 2.5 mg/kg twice daily compared with 5 mg/kg once daily, although both dosage schedules were equivalent in their ability to suppress lameness (Toutain *et al.*, 2001). For further discussion of PK/PD modelling of NSAIDs see the article by Lees *et al.* (2004b) in this issue.

DETERMINATION OF OPTIMAL DOSAGE SCHEDULES OF ANTIMICROBIAL DRUGS

Ideally, antimicrobial therapy should not only provide clinical cure but also eradicate pathogenic organisms to achieve total bacteriological cure. If this is not achieved, the sub-population of organisms that are less susceptible may divide during or at the end of therapy to establish a population of organisms of increased *MIC* value and therefore potentially resistant, even to high drug concentrations. Hence, it is now recognized that a major additional goal of therapy (in addition to optimizing efficacy) is to minimize opportunities for the selection and spread of resistant organisms (Lees & Shojaee Aliabadi, 2002; Toutain *et al.*, 2002; McKellar *et al.*, 2004).

Published data indicate that indices of clinical outcome are insufficiently sensitive for determining the optimal dosage regimen for bacteriological cure. This criterion is fundamental to strategies designed to minimize the selection of resistant organisms. The use of PK/PD approaches and surrogate indices in healthy animals has provided a new means of addressing this problem. Murine lung and thigh infection models have generated empirical PK/PD indices, which have been used to predict the effectiveness of therapy (Leggett *et al.*, 1991; Craig, 1998). Three indices have been used, *AUC*/*MIC* ratio for quinolones, C_{max}/*MIC* ratio for aminoglycosides, and *T* > *MIC*, the time for which plasma concentration exceeds *MIC*, for betalactams. These have been proposed as PK/PD indices of efficacy, since they comprise a PK parameter (*AUC*, *T* > *MIC*, C_{max}) together with a PD parameter (*MIC*). They therefore encompass dual dosage individualization, since they incorporate both microbiological susceptibility and drug disposition pharmacokinetics (Schentag *et al.*, 1985; Hyatt *et al.*, 1995).

Each of the three PK/PD predictive indices of *in vivo* efficacy is based on unbound plasma drug concentrations and not on total plasma or total tissue concentration. Because pathogens of clinical relevance are usually located extracellularly, the biophase for antimicrobial drugs is most commonly extracellular fluid (Schentag, 1989). However, for intracellular pathogens and when there is a barrier to drug diffusion (for example into the central nervous system, prostate, eye, abscesses, etc.), plasma concentration may be less useful in predicting infection site drug concentration. Each of the three PK/PD indices is a surrogate marker of what is required clinically, namely clinical recovery and bacterial eradication. The clinical validity of the surrogates is currently based on evidence from two sources, data from human prospective or retrospective clinical trials and the mechanistic links between the surrogates and bacterial eradication, which is the final objective of antibiotic therapy. When validity of the surrogate indices has been confirmed in veterinary medicine, their breakpoint or critical values must be established. PK/PD breakpoints have not yet been clearly validated in veterinary medicine. However, as the indices reflect differences in host species pharmacokinetics and bacterial species *MIC* values, it is likely that the breakpoints normally will not differ significantly between animal species (Craig, 1998). Therefore, data derived from clinical human trials or animal infection models should provide a sound basis for the design of dosage regimens for new antibacterial drugs and new species. On the contrary, the animal's immune status, including both specific and innate immunity, will influence the ability to eradicate an infection. Continued bacterial challenge from the environment will also have an impact.

For betalactams acting by time dependent killing mechanisms, it is recommended in human medicine that *T* > *MIC* should be at least 50% and possibly ≥80% of the dosage interval to ensure an optimal bactericidal effect. For drugs highly bound to plasma proteins, this criterion applies to the free concentration.

In several infection models with a range of gram-positive and gram-negative bacteria, for fluoroquinolones *AUC*/*MIC* ratios of <30 h were associated with more than 50% mortality, whereas with *AUC*/*MIC* ratios of ≥100 h there was almost no mortality (Craig, 1998).

The optimal C_{max}/*MIC* ratio of aminoglycosides for efficacy should be in the range 8–10 (Moore *et al.*, 1984). This can be achieved with a single high daily dose, and this also minimizes renotoxicity.

An important current and future role for veterinary pharmacology is to establish breakpoint values for the three PK/PD indices discussed above for individual animal species and pathogens causing disease. One approach has been to assess antibacterial activity of antimicrobial drugs *ex vivo* in serum and in inflamed and noninflamed tissue cage fluids (Aliabadi & Lees,

2002; Aliabadi *et al.*, 2003). The response to the drug, measured as reduction of initial bacterial count is regressed against the surrogate marker $AUC_{24\ h}/MIC$, using the inhibitory form of the classical inhibitory Hill equation:

$$\text{Ex vivo antibacterial response} = \frac{[\text{maximal possible drug effect}] \times (\text{surrogate})^n}{(\text{surrogate})^n_{50} + (\text{surrogate})^n} \qquad (11)$$

where antibacterial response is measured as the decrease in bacterial count from the initial count. The independent variable is the surrogate (in this case $AUC_{24\ h}/MIC$) for which a breakpoint is to be determined. Three clinically useful PD parameters can be derived from Eqn 11. These are maximal effect which is bacterial eradication, the $(\text{surrogate})_{50}$, the value of $AUC_{24\ h}/MIC$ producing 50% of the maximal effect, which is a measure of antimicrobial drug potency, and n, the Hill coefficient, which describes the slope of the concentration–effect relationship. The model can be applied to determine any level of antibacterial response ranging from bacteriostasis through bactericidal effect to eradication and corresponding breakpoint values for the surrogate can be calculated. For example, in a study in camels, the breakpoint value for $AUC_{24\ h}/MIC$ for danofloxacin for bactericidal effect was 21.1 h and the corresponding value for eradication of bacteria was 68.7 h. This is less than the breakpoint generally recommended for fluoroquinolones in human medicine of 125 h (Aliabadi *et al.*, 2003).

DETERMINATION OF IRRELEVANT PLASMA AND URINE DRUG CONCENTRATIONS FOR DOPING CONTROL

The high level of sensitivity, precision and accuracy of analytical techniques used in relation to drug control in horses has led to the concern that trace concentrations of drugs used in legitimate therapy of racehorses may be detected on the day of racing. To distinguish between relevant and irrelevant plasma or urine concentrations, a PK-PD approach based on Eqn 2 has been proposed. Plasma clearance is used to convert an effective dose (as recommended in the manufacturer's literature) into an effective concentration. From this effective concentration an irrelevant plasma concentration is calculated by application of a safety factor (Toutain & Lassourd, 2002a,b). The irrelevant plasma concentration can be reviewed by racing authorities in relation to current analytical thresholds for each drug.

POPULATION PK/PD STUDIES

A detailed description of the principles and applications of population PK-PD modelling is outside the scope of this review, but the essential features are as follows. For a given species the sources of PK variability include breed, sex, age, diet, renal and hepatic function, physiological/pathological states and route of drug administration. These have been extensively studied, whereas sources of PD variability have been much less investigated. Nevertheless, available data indicates that PD variability may for some drug classes be greater than PK variability. For example, for antimicrobial drugs the clinical response depends not only on penetration of the drug to the site of infection but also on PD variability, which includes both host response to the invading pathogen and susceptibility of the bacterial species to the administered drug.

A major benefit of PK/PD modelling is that it has enabled separate evaluation of the two principal sources of variability (PK and PD) through the application of population PK/PD. Through population analyses it is possible to evaluate variations between both animals and groups of animals in terms of drug exposure and also on the basis of drug response. For example, population PK/PD may replace the dubious body surface area rule as a means of optimizing drug efficacy and safety in cancer chemotherapy (Toutain, 2002).

Antimicrobial drug dosage regimens for use in veterinary medicine should be built on population PK/PD approaches. These allow for PK variability of exposure in the animal population including animals with disease with knowledge of population distribution of *MIC* values for the target pathogen rather than a single *MIC* value as at present. By this approach the percentage of animals for which a given breakpoint can be achieved for a given dosage regimen and *MIC* value can be established.

In summary, PK/PD modelling has major potential for rational dosage regimen determination as it separates the two main sources of interspecies variability (PK and PD). It is therefore applicable to inter-species extrapolation and to multiple species drug development. The principal constraints in implementing PK/PD modelling are (1) limited understanding of PK/PD principles within the veterinary scientific community and (2) the need to validate PK/PD approaches, particularly with respect to relevant surrogate end points and the models relating drug concentration to effect. When these constraints have been overcome we anticipate that PK/PD modelling will gain acceptance by pharmaceutical companies and regulatory authorities.

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