

Chapter 3. Assessment of Endpoints: Kinetics and/or Dynamics

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I. INTRODUCTORY REMARKS

A crucial, yet often limiting, factor in the advance of the majority of sciences is the ability to measure and analyze those variables that are truly relevant to a particular field, as for example, in pharmaceutical science, the concentrations in plasma and the effect of a drug. These measurements would be of little use were it not for the development and progress of collateral disciplines such as pharmacokinetics (PK) or pharmacodynamics (PD). The PK examines the relation between the dose administered and the achieved concentration in blood or at the biophase, or “what the body does to the drug”, and the PD deals with the relation between drug amount and effect, or “what the drug does to the body”. In its turn, progress in PK or PD is due mostly to the availability of sensitive and specific analytical methods in order to determine the evolution of the levels of drug and its metabolites in biological fluids and tissues as well as to quantify the drug’s therapeutic effect.

In parallel, the mathematical techniques and methods used in pharmaceutical science to characterize the kinetic processes have also developed considerably, mostly borrowing from other disciplines such as chemistry, physiology or enzymology. The application of these analytical and mathematical techniques to specific studies has enhanced the knowledge of the PK behavior of many drugs, constituting an important advance in adjustment of the dose. But, unpredictability remains a problem since the kinetics usually show large interindividual variation, mainly due to genetic, environmental and pathophysiological factors. For this purpose, state of the art statistical methods generally denominated as “population” PK/PD models have been introduced in the field aiming to evaluate the between (inter) and within (intra) individual differences in the PK/PD in medicated populations of subjects.

The dose is related to the effect through the PK and also through the PD which relates the concentration with the effect. Comprehension of these relations is truly essential for the rational development of therapeutics because the PD determines the target concentration required to produce a specific effect or endpoint, while the PK specifies the appropriate dose regimen to reach that target. This constitutes the basis of what in therapeutics is called “Target Concentration Intervention” (TCI), in contrast to the therapeutic drug monitoring (TDM) methods based solely on a single concentration and Bayes informed PK.

As in the case of PK, methods have also been developed to assess the pharmacological effect *in vivo* and provide biomathematical model descriptions of the PD. It is already well known that the interindividual differences in PD are numerous and are related to factors such as age, race or disease. Therefore, future efforts must lead to the exploration of covariate models for predicting individual PD parameters. This knowledge together with its PK counterpart could constitute the basis for rational individualized therapy. The combination of PK and PD models permits predicting the temporal evolution of the effect at any dose or regimen.

Also, PK/PD analysis of concentration – time – effect data assists in detecting a series of underlying complications. For example, tolerance development, the formation of active metabolites or the desynchronization between the evolution of the drug concentration and that of the effect. The latter can be due either to drug distributing into an effect compartment or because of an indirect effect mechanism. In all these conditions the plasma concentrations could not be used directly as “targets” (TDM) but integrated PK/PD models would permit estimation of the drug concentration at the effect site as well as the relationship between them.

II. OBJECTIVE

The main objective of therapy is to achieve maximum efficacy avoiding the risk of toxicity. This can be achieved via empirical adjustment of the dose, based either on observations of effects (effect – time

evolution) and/or concentrations (concentration – time), or optimally based on knowledge of the pharmacokinetic (PK) and pharmacodynamic (PD) parameters.

II.1. Effect – time evolution

The measurement of the pharmacological effect and the adjustment of the dose as a function of the effect appears, in principle, as the most sensible and intuitive approach. However, there are several problems. The observed pharmacological effect, as well as the time it takes to achieve it and its duration, are measurements that change with the dose and the mode of administration, therefore they are variables. This implies that they cannot be used to make predictions in other situations, different from those of the observations at hand.

Additionally, from the knowledge of the effect *vs* time evolution there can be no direct deduction of parameters, thus having to employ integrated PK/PD analyses as will be discussed below. Parameters are considered those characteristics of the drug that do not change with time, dose or administration route and therefore can be used to adjust or predict adequately any therapeutic regimen. For example, the classical dose - effect relationship provides a useful estimate, that of the dose producing 50% of the maximal effect, ED₅₀. Yet, this is not a true parameter since it depends on the time post-dose when the effect was measured.

Dealing with the pharmacological effect is complicated further because of the difficulty to obtain a precise, objective, and continuous measurement of the effect. The use of biomarkers attempts to relieve this problem although we are still far from having the ideal (and validated) biomarker for the majority of drugs.

The use of biomarkers in drug development and clinical practice was revised in the Ninth European Federation of Pharmaceutical Science Conference on Optimizing Drug Development held in Basel 2001 (1). The terminology has been put up to date and the differences between the terms “end point”, “surrogate endpoint” and “biomarker” clarified. Biomarkers are now defined as “physical signs or laboratory measurements that may be detected in association with a pathologic process and that have putative diagnostic and or prognostic utility”. They are, therefore, factors which can be measured objectively and are evaluated as indicators of biological or pathological processes and/or indicators of the response to a therapeutic intervention. In general, biomarkers have a much wider range than surrogate endpoints. We understand as endpoints those variables which can be used to measure how a patient feels after a specific treatment or how a specific body function evolves, “the clinical impact of therapeutic intervention”. The term surrogate endpoint implies that some variables related to the endpoint or the clinical response have been used as biomarkers but are not the final response of the drug (2,3). For example, blood pressure is a biomarker for prevention of hypertension and, at the same time, a surrogate endpoint for the prevention of myocardial infarction and stroke. The measurement of the degree or percentage of prevention would be the endpoint. This example serves also to demonstrate the difficulty in selecting adequate biomarkers since it is still discussed whether the systolic, diastolic or mean blood pressure is the one of interest. The most widely used biomarkers are plasma concentrations of drugs that are used as guides to dosage in clinical practice (e.g. TDM).

Biochemical and molecular biomarkers, such as leukotrienes, angiotensin I and II, or CD4 cell count are of great utility but are hampered by complexity in their mechanism of action which is widely interconnected to other processes. Consequently, no single such biomarker can predict a significant proportion of the observed clinical endpoint. For example, CD4 cell count explains 30% of the survival to HIV and CD4 count plus viral load explains 70%.

Something similar occurs with the gene biomarker products which are also under rapid development. Genes and their function are identified in the genome. Then the proteome is used to identify proteins from

selected genes. Evaluating how mutations cause disease as a result of protein differences, between healthy and diseased subjects, appears to provide candidate gene biomarkers. However, the complexity of the genome or of the pathway from expression to phenotype to macroscopic reality has deflated initial hope.

Adversities aside, the development of adequate biomarkers for a drug apart from better characterization of its PK/PD for analysis or prediction, now appears crucial for the drug development effort. Valid biomarkers help completing the proof-of-concept in the early phases, facilitating decision on continuation with the new drug. Eventually, biomarkers permit reduction of the number of patients in later phases (II or III) and adjustment of the dose in specific populations or in individuals receiving the drug through different routes or dose regimen. Recall however, that even when the ideal biomarker is known, it is necessary to have the PK/PD parameters permitting to make predictions.

II.2. Concentration – time evolution (PK)

The observations of plasma or blood concentration are also non generalizable in their pure form. The maximum concentration reached after a specific dose or the peak or trough after repeated dosing, are measurements useful exclusively for adjusting the dose in situations reproducing the one where they were obtained (body – drug whole). Nevertheless, the measurement of the concentration evolution with time is advantageous, compared to the evolution of the effect, since from this kinetics the PK parameters can be derived that are of great use in adjustment of the dose.

Pharmacokinetics has advanced considerably with the use of mathematical models and computer packages which aid in the analysis and processing of the information generated in clinical practice. The PK also permits the simulation of conditions affecting a particular patient. These models and accessory packages are *tools* which assist in obtaining parameters but add no scientific surplus value to the information than that input by the experiment, including the user. Modelling will simply reflect the knowledge of the physicochemical characteristics of the drug, the precision of the analytical techniques for drug assays. With more complex models, the qualifications of the user also become more important.

The basic or primary PK parameters are apparent volume of distribution (V) and clearance (CL). V is defined as the relation between dose and initial concentration ($V = \text{Dose} / C_0$). CL is a relation between elimination rate (or distribution rate, for intercompartmental clearances) and concentration in blood or plasma. With passive phenomena (or first order kinetics), which are the most usual in the body, a larger dose implies a larger concentration in blood or plasma and consequently larger elimination rate, thus V and CL are always constant. They are therefore considered parameters and permit prediction of the dose which would be necessary in a patient to produce a specific effective concentration level.

For example, knowing V we could predict the dose needed to reach a specific target (loading dose = target concentration x V). Additionally, CL is a parameter independent of the complexity of the kinetic model (mono, bi or tricompartamental) and is calculated simply as Dose / AUC (assuming complete absorption of the drug). In steady state, after an infusion or multiple dosing, CL is related to the steady state concentration (C_{ss}) or average C_{ss} ($C_{ss} = \text{infusion rate} / \text{CL}$), so this parameter can be used to predict the dose regimen (maintenance dose = target C_{ss} x CL). Both parameters, CL and V, are primary parameters and are directly related to the physiological processes of the organism. They give us an idea of the relative importance of the space where the drug is distributed and of the organs which eliminate it.

Another PK parameter, commonly employed in dose adjustment, is the elimination rate constant (Kel). It is a mixed parameter that depends on CL and V ($K_{el} = \text{CL} / V$) and is often recast in the form of the half-life parameter as $t_{1/2} = \ln(2) / K_{el}$, now with units of time. This parameter gives an idea of when the steady state is reached and can be used to predict when a C_{ss} monitoring sample can be taken.

A facet which should not be neglected when performing a kinetic study or using the concentrations as markers is to know a priori what needs to be finally estimated from the observations. The parameters obtained depend on what has been measured, for example, metabolites, unbound or total drug, enantiomers or racemic mixture.

Another frequently encountered issue is the necessity or not to measure unbound drug (not bound to plasma proteins). It is important to recall that on some occasions the unbound concentration in plasma should be considered since binding in plasma and tissues (the effect site) may not be equal. Nevertheless, if the binding is linear in the range of therapeutic or toxic concentrations, the free and total concentration are simple ratios one of the other and in these cases it is not necessary to measure the free concentration. In contrast, nonlinear plasma protein binding (free drug concentrations increase disproportionately with increasing total drug concentrations) can create havoc in analysis unless free drug concentrations are measured. Perhaps an advantage of parameter estimation as a function of free concentration is that it supplies information about the (intrinsic) behavior of the drug excluding possible differences in the degree of binding. For example, the V of unbound drug, V_u , corrected for the weight, can be extrapolated from animal to man permitting estimation of the first dose in humans. An approximate value of CL for the unbound drug, CL_u , can be obtained from “in vitro” studies with microsomes in the initial stages of development.

In conclusion, V and CL are fundamental parameters for establishing a dosing regimen, but, depending on the physicochemical characteristics of the drug, the models for the distribution can become complicated and the number of parameters may increase, particularly there may now be more than one volume of distribution. In this case, the volume used to adjust the therapeutic dose, should be the one closer to the effect site (and could also be the steady state volume, V_{ss}) (see Propofol example below). It is important to remember that the basic concepts reflected in V and CL are always applicable independently of the complexity of the complete model.

II.3. Programs for data analysis

Depending on the experiment, the intentions may range from obtaining estimates of PK (PD) model parameters in a single subject or the mean for multiple subjects (a population), or up to the rigorous resolution of the inter and intra subject variability in a population as reflected into statistical distributions of the parameters (Bayesian priors). A subsequent, yet very important task, is usually that of relating the parameters, or their inter subject variability with individual specific covariates, so that a priori prediction of the individual PK (PD) characteristics, and hence the dose, can be improved.

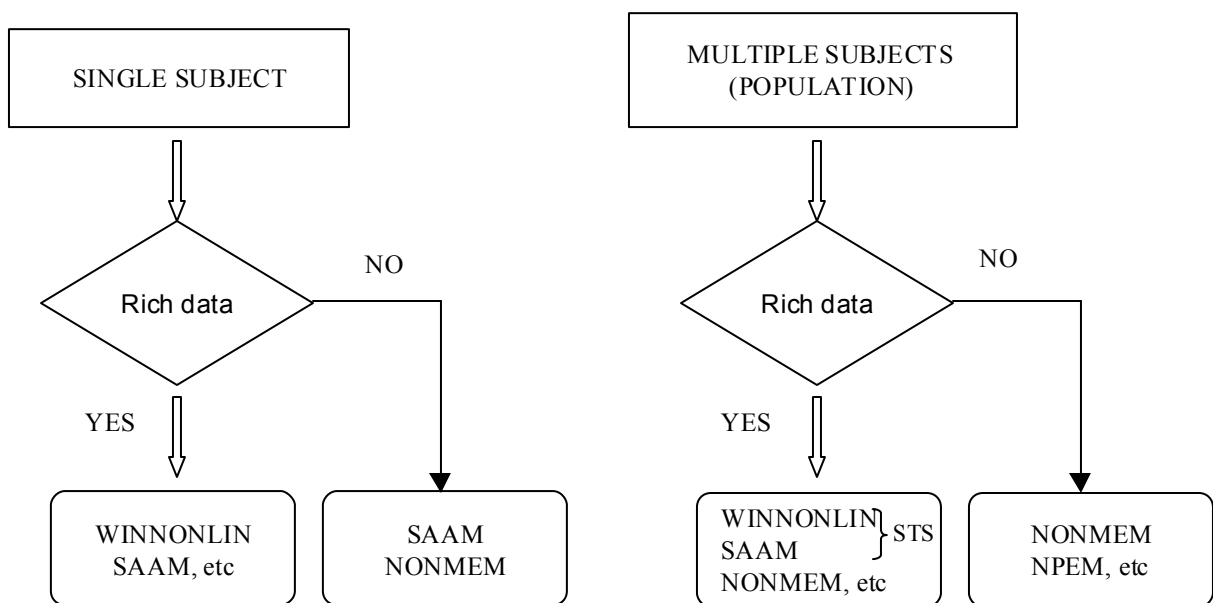
Several software packages have been designed and marketed, mainly in the last 20 years and can be distinguished as single subject analysis programs, or population analysis programs, although both can be used, with the exceptions of some occasions, to perform single or multiple subject analyses. The decision about which approach to use depends, in order of significance, on the density of the observations (*rich* or *sparse* data), the scope of the study (e.g. obtaining estimates for a single subject only, or Bayesian priors for a population), and *ad hoc* criteria regarding modelling.

Rich sampling is when there are several drug concentration observations per individual, necessarily more than or equal to the parameters in the model and well distributed in time (e.g. phase I). Sparse sampling is when there are fewer data points per subject than parameters in the PK model to be estimated. Sparse data occur frequently, e.g. in the clinic, due to monitoring restrictions or in phases II, III or IV, due to logistical or ethical considerations. It is also frequent in dose escalation experiments with drug levels below the quantification limit, particularly in small animals, and also in toxicological single point per animal studies.

With rich sampling, individual fitting programs can always be used in single or multiple subject problems, in addition to population specific packages for the latter case (Fig. 1). In sparsely sampled designs, some knowledge about the population at large is always required for estimation of the individual PK model. In single subject sparse PK model estimates, a Bayesian prior from the population has to be introduced at some point in order to inform or support the algorithm on the data gaps. Single subject PK/PD modelling packages are WINNONLIN (Pharsight Corp., Mountain View, CA) and SAAM (Saam Institute Inc., Seattle, WA), this latter permitting the introduction of Bayesian priors from the literature or earlier studies when the data is sparse (e.g. a monitored concentration for each subject).

When many individuals are treated as one within a single subject fit, the analysis performed is known as *naïve* and produces estimates of the mean parameter without any indication of statistical spread. If that information is desired, with rich data and for more than one individual the single subject model fitting run can be simply repeated or iterated for each case. An improvement is a population – like analysis known as standard two stage (STS), because in the first stage all subject specific PK or PD parameters are obtained and in the latter, their centering and dispersion (mean, standard deviation) are simply calculated. With STS, relationships with covariates (e.g. age, weight, sex, and creatinine) can be assessed with standard commercial statistical analysis packages (SPSS, SAS, S-PLUS etc).

Figure 1. Schematic of decision tree for use of population methods in data analysis.



For sparse data and multiple subject experiments, the use of *population* or *population modelling* approach is most appropriate. Belonging to the well known statistical problem of *mixed effects*, population algorithms use the information from the remaining population to complete the model of each subject in an iterative process of enriching a prior parameter distribution at each step. Population methods eventually produce estimates of the complete distribution for the PK model parameters (population mean, standard deviation), useful as Bayesian priors, as well as the distribution of the residual error: measures of the inter- and intra-individual variability respectively. Population analysis programs are NONMEM (nonlinear mixed effect modelling, NONMEM Project Group, UCSF, CA) (4) or NPEM (non parametric expectation maximization, Laboratory of Applied Pharmacokinetics, USC, CA) (5) and have been validated and compared (6,7). Recently, a package with a more user friendly interface has been introduced (WINNONMIX, Pharsight Corp., Mountain View, CA), and although not widely used so far, it is lately gaining ground (8,9).

Population fits are usually followed by a maximum a posteriori (MAP) Bayesian estimation step, where the PK (PD) model parameters are obtained for each individual based on the just obtained population priors.

Generally, population analyses are far more complex than single subject approaches, in terms of expertise required and man hours and computer time invested, mainly because there is no single pharmacostatistical model solving the parameter estimation problem. Neither is there a single best fit criterion and the solution process includes visual inspection of residuals or evaluation of confidence intervals. Thus the analyst becomes part of a loop in successive model improvement steps. “Turn of the crank” modelling is impossible with population methods. A simple PK model which may take minutes in a computer and a single subject algorithm to solve may require days or weeks for its population counterpart, even excluding computer time delays due to the size of the sample. The time and knowledge invested is largely extended when multiple occasions of the same subjects and covariate models for the PK parameters are created within the package. The complete population analysis, beginning with the design of the samples, collection, analysis, covariate modelling and possibly simulation, is a highly demanding task.

The PK parameters depend on demographic and physiopathological covariates such as renal insufficiency, diabetes, age, sex and weight and this finally affects the dose. The causality or pathways of such variation are usually not well known and thus, unpredictability remains a problem for most drugs. Covariate model development is a very important effort subsequent to any characterization of the individual kinetics in a population. Some population packages allow introduction of a theoretically unlimited number of covariates in the population fit, thus permitting immediate reduction of the inter-individual variability (NONMEM, WINNONMIX); others allow only a limited number of covariates to be introduced in the fit, thus covariate modelling is performed externally.

Even drugs in use for years, like methadone, in clinical practice, show variability in the response (10). In recent experimental work with methadone, it was observed that sex (11), protein binding (12) and P-glycoprotein (13) modify the PK/PD of methadone and it has been suggested that these covariates could be implicated in the variability observed in the clinic. Another example is propofol. Many studies report on the influence of various covariates on the kinetic parameters of propofol, but without a consensus as to the importance of each one (14). Weight, age and formulation have been associated with the variability. Additionally, plasma protein binding, mainly to lipoproteins, is modified in thyroid, diabetic or critical patients, which could finally impinge on the kinetic parameters (15-17). Models have been developed between these variables and the unbound fraction of propofol “in vitro”, which could aid in the inclusion of lipoprotein levels in population PK analyses (17).

Immunosuppressant medication also shows worrisome variability in the kinetics which is further complicated by the standard oral administration. Much of the variability described in the parameters appears to be associated with the bioavailability which may also vary with post transplantation time (18). In this situation, in addition to estimating the mean parameters it is important to quantify the variability, which is important in adjusting the dose and the regimen. A variation in CL, for example, would immediately reflect in a change in the required maintenance dose or the dosification rate. Population covariate modelling often deals with inter occasion variation within the same subject, in addition to the inter intra individual variabilities.

These concepts are important to the Target Concentration Interval (TCI). In NONMEM, for example, the overall variability for each patient can be summarized in three parts, the between subject variability (BSV) and the inter occasion variability (IOV) for each PK parameter and the within subject variability (WSV) for the concentrations. Then covariate models can be developed to reduce the BSV and IOV. If the dose is to be adjusted between different subjects the BSV and IOV must be treated for the parameter of interest (e.g. CL), but if the adjustment is within the same subject the WSV has to be considered.

The population approach has been used for years, and increasingly, in all phases of drug development (19) and in postmarketing studies. Some of the most recent studies with NONMEM, which have implied an advance in the adjustment of the dose in the clinic, are listed in Table 1.

Table 1. Some of the latest studies where population methods are employed for dose adjustment.

Drug	Reference
Efavirenz	Chantal et al. Clin Pharmacol Ther 2003; 73: 20-30
Galantamine	Piotrovsky et al. J Clin Pharmacol 2003; 43: 514-523
Levosimedan	Jonsson et al. Br J Clin Pharmacol 2003; 55: 544-551
Mycophenolic acid	Shum et al. Br J Clin Pharmacol 2003; 56: 188-197
Ciprofloxacin	Payen et al. Antimicrob Agents Chemother 2003; 47:3170-3178
Zidovudine	Capparelli et al. J Clin Pharmacol 2003; 43: 133-140
Enoxaparin	Bruno R et al. Br J Clin Pharmacol 2003; 56: 407-414
Nedaplatin	Ishibashi et al. Br J Clin Pharmacol 2003; 56:205-213

Population methods are intimately tied to simulation, deterministic when random components are absent or stochastic when they are not. The latter is used in drug development for “in silico” dosing regimen and risk assessment, facilitating the reduction of trial patients in the later phases. Most of the above packages can be used to perform simulations in a population setting (20).

The applicability of the models, the presentation of concise final reports, and the relevance of the study are factors which should be considered in order for population analyses to be useful in clinical practice and in learning about the behavior of the drug in its development phases (21).

In conclusion, dose predictions are usually simple applications of the elementary PK principles discussed above and permit estimating a target concentration after administration of a specific dose or a particular regimen. Nevertheless, the target concentration should not be far removed from knowledge of the concentration – effect relationship because, in fact, that is where the target originates. The selection of a target concentration requires exploration of the PD relation not only for the desired effect but the range from undertherapeutic to toxic effects. In clinical practice, there is debate regarding the utility of two approaches: TCI which is based on the above discussed PK/PD based principles and Therapeutic Drug Monitoring (TDM) which is based on adjusting the dose to maintain a range of drug levels, perhaps losing sight the concept of target and its immediate relation to an effect.

II.4. Concentration – effect relationship (PD)

The concentration – response relationships lead to the estimation of PD parameters, useful in drug development as well as in the clinic. The most common PD parameters are the maximum effect that can be reached (E_{max}) and the EC_{50} , or concentration in plasma or blood capable of producing 50% of the maximum effect, known as *potency* in “in vivo” studies. These parameters are independent of the dose or time and therefore allow prediction of the effect at any concentration independently of how it was generated. These parameters, like with the PK, are obtained from models, although these are typically diagnostic at steady state rather than with explicit time dependence. The classical models are the E_{max} or hyperbolic model and the sigmoid model which ensue from the classical drug – receptor relations. The E_{max} model is expressed as,

$$E = E_{max} \frac{C}{EC_{50} + C}$$

In this form it is seen that once the parameters are obtained the effect at any concentration (C) can be estimated. Additionally, the parameter EC_{50} , expressed as free (unbound to protein) concentration of drug, offers valuable information since it usually is of the same order of magnitude as the “in vitro” potency of the drug, IC_{50} , obtained from receptor binding studies in early drug development stages. As such, it can be considered as the target for therapy.

In a recent study with lerisetron, a new $5HT_3$ antagonist in phase III development, the PD was measured using its surrogate effect, the Bezold-Jarish reflex. The observed value for the EC_{50} “in vivo” was in the range of the affinity of lerisetron in binding studies published earlier, which allowed the assumption that the activity of lerisetron was due to the parent product and not to the presence of possibly active metabolites, as had been suggested for other compounds of the same family (22). This approach has also been used for comparing adenosine and other lipophilic derivatives as well as various benzodiazepines (23,24).

In spite of the importance of knowing the concentration - effect relationship, for many drugs there is little documentation regarding their PD parameters, including those which are commonly monitored such as aminoglycosides, cyclosporin, phenytoin and digoxin. An exception is theophylline for which the E_{max} value is known (expiratory flow rate) and as well as the EC_{50} . These parameters have been used successfully to estimate the target concentration in the clinic (25).

There is also ample evidence that interindividual differences in PD are sizeable and are associated with variables such as age, race and pathologies. Therefore in the future, effort should focus on resolving the population PD (e.g. with mixed effects approaches) hoping to eventually employ covariates for predicting individual PD characteristics. These studies must be designed in accordance with basic epidemiological principles, i.e. with populations where all the possible covariables (demographic or pathophysiological) can be completed for all subjects. The results from these PD studies together with their PK counterpart would constitute the basis for adequate use of concentrations and effects as therapeutic targets.

II.5. Complex PK/PD situations and applicability of integrated models

The concentration – effect relations can also be used to detect situations where there is an apparent lack of relation between concentrations and effect, when plasma concentrations can not be used directly as biomarkers. Such conditions exist, for example, when there is temporal disequilibrium between plasma and biophase, tolerance, presence of active metabolites, or enantiomers with distinct pharmacological activity, e.g. tramadol (26,27) and methadone (28). But even in these situations, the use of integrated PK/PD models could permit the prediction of the effect, reached after a certain drug dose or through a specific administration route, since the PD diagnoses the concentration necessary for a specific effect and the PK informs us of the dose corresponding to that concentration.

Since the kinetic and dynamic processes are intimately related to the temporal evolution of the pharmacological effect, combined PK/PD models have been developed in place of characterizing separately the concentration vs time and effect vs time relations.

Of all PK/PD models dealing with complicated conditions, the most developed and amply used is the effect compartment model. It resolves the possible disequilibrium between the central distribution compartment and the effect site via an empirical equilibration rate ke_0 in a “link” model which allows the estimation of the concentrations at the effect site. In clinical practice, it is used for dose adjustment, particularly in anesthesia with propofol and phentanyl, since they are drugs with complex kinetics of multiple compartments posing the problem, discussed earlier, of choice of appropriate parameter for dose adjustment. For example, with propofol the effective concentration range for hypnosis is 2 - 3 mg/L. If, for

estimation of the therapeutic dose, we were to select the central V in a hypothetical patient of 70 kg weight with $V = 10$ L, the dose provided would be 30 mg, completely ineffective. If we were to select V_{ss} for the same purpose ($V_{ss} = 466$ L), the estimate would be 1428 mg, well above the therapeutic dose. The solution lies in performing the same task employing the peak-effect volume ($V_{pe} = 20$ L) estimated via the link model. The correct dose would then be 60 mg. This concept is actually programmed into the target controlled infusion pumps used in the operating room for anesthesia with propofol.

Another practical example of the importance of PK/PD integration is evident in a study where two oral formulations of ibuprofen were compared. The PK had been studied in healthy volunteers (typical study of bioequivalence) and the evolution of the effects (fever) in children with hyperthermia. At first, the kinetic study appeared to indicate that the two formulations were different in absorption as reflected in the time needed to assess C_{max} (T_{max}). Nevertheless, observation of the effect - time evolution in the children did not show any difference. Integrated PK/PD analysis of both populations jointly, with the use of NONMEM, allowed the determination of the causes of this discrepancy. Due to the nature of the indirect response mechanism, via which the fever process proceeds, the differences in the plasma concentration were not reflected in the observed therapeutic response. Eventually, the two formulations were bioequivalent (29).

The integration of PK and PD is key to understanding the use of TCI as an alternative to TDM. This latter approach, widely used at present, often fails precisely because it does not consider the pharmacological effect. The time seems ripe to start paying attention to the concentration effect relation and to think of strategies to individualize the dose with the help of the concentrations but without losing sight the synthesis of the PK/PD concepts.

In conclusion, the appropriate combination of biomarker identification and selection, and bioanalytical methods for development and validation and the use of PK/PD models (including population approaches) for fitting data and predicting future clinical endpoints, can provide powerful insights and efficacious guidance for individual patients.

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