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Research Paper

Mechanism-Based Pharmacokinetic–Pharmacodynamic Modeling—A New Classification of Biomarkers*

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Abstract. In recent years, pharmacokinetic/pharmacodynamic (PK/PD) modeling has developed from an empirical descriptive discipline into a mechanistic science that can be applied at all stages of drug development. Mechanism-based PK/PD models differ from empirical descriptive models in that they contain specific expressions to characterize processes on the causal path between drug administration and effect. Mechanism-based PK/PD models have much improved properties for extrapolation and prediction. As such, they constitute a scientific basis for rational drug discovery and development. In this report, a novel classification of biomarkers is proposed. Within the context of mechanism-based PK/PD modeling, a biomarker is defined as a measure that characterizes, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect. The new classification system distinguishes seven types of biomarkers: type 0, genotype/phenotype determining drug response; type 1, concentration of drug or drug metabolite; type 2, molecular target occupancy; type 3, molecular target activation; type 4, physiological measures; type 5, pathophysiological measures; and type 6, clinical ratings. In this paper, the use of the new biomarker classification is discussed in the context of the application of mechanism-based PK/PD analysis in drug discovery and development.

KEY WORDS: genotype; molecular target activation; molecular target occupancy; pathophysiological measures; physiological measures.

INTRODUCTION

The application of pharmacokinetic/pharmacodynamic (PK/PD) modeling in drug development is well established. The primary objective of PK/PD modeling is prediction of the time course of the drug effect *in vivo* in health and disease. As such, PK/PD modeling constitutes the scientific basis for optimization of the dosing and the delivery profile of new and existing drugs in phase 2 clinical trials. Furthermore, PK/PD modeling is also widely applied as the basis for the design and the evaluation of phase 3 clinical trials (1).

PK/PD modeling is increasingly applied in drug discovery and preclinical development. Specific applications include a) the selection of drug candidates with the most favorable pharmacokinetic and pharmacodynamic properties and b) the prediction of exposure response in humans with the aim to optimize the design of (early) clinical trials. The use of PK/PD modeling in this context relies on prediction of the time course of drug effect in man, using information from nonclinical investigations. Not surprisingly, there is a clear trend toward the development of mechanism-based PK/PD models, which have much improved properties for extrapolation and prediction. This concerns the extrapolation and prediction 1) from *in vitro* test systems to the *in vivo* situation, 2) from *in vivo* animal studies to humans, and 3) from healthy volunteers to patients, and 4) the prediction of intra- and inter-individual variability in drug effects.

Mechanism-based PK/PD models differ from empirical descriptive models in that they contain specific expressions to characterize, in a strictly quantitative manner, processes on the causal path between drug administration and effect. This

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includes 1) the target site distribution, 2) the target interaction and activation, 3) the transduction, and, finally, 4) the influence of *in vivo* homeostatic feedback mechanisms. Ultimately, mechanism-based PK/PD models will also consider the effects on 5) disease processes.

In the following paragraphs, the principle of mechanism-based PK/PD modeling is reviewed and discussed. It is shown that not only mechanism-based PK/PD modeling depends on the use of biomarkers, but that at the same time mechanism-based PK/PD modeling also constitutes a scientific basis for the selection and evaluation of novel biomarkers in drug discovery and (early) drug development.

MECHANISM-BASED PK/PD MODELING

Target Site Equilibration

An important factor in mechanism-based PK/PD modeling is the distribution between blood plasma and the target site in peripheral tissues. In PK/PD modeling, biophase equilibration is usually characterized on the basis of an effect compartment model, on the assumption that in steady state, the drug concentration in the biophase is identical to the free plasma concentration (2). This assumption, however, is not always valid. In theory, the target site equilibration of drugs depends on 1) the target site delivery (blood flow, plasma protein binding) and 2) the target site distribution (filtration, diffusion, functionality of transporters). Particularly for relatively large hydrophilic molecules and for compounds that are substrates for specific transporters, target site distribution is restricted. This is particularly important for drugs with an intracellular target (i.e., cytostatic drugs) or drugs that act in tissues that are protected by specific barriers (i.e., the central nervous system). Recently, several specific transporters have been discovered that may restrict the access of a drug to the site of action (3). Furthermore, increased expression of such transporters may explain loss of efficacy, as exemplified by drug resistance in cancer chemotherapy (4). This underscores the need for measures of target site exposure (drug and metabolite concentration) as biomarkers in mechanism-based PK/PD modeling. A novel technique to obtain information on the target site exposure in the central nervous system is intracerebral microdialysis. An example of the application of intracerebral microdialysis is in the investigations on the PK/PD correlation of morphine, where P-glycoprotein and possibly other transporters restrict the distribution into the central nervous system (5).

Target Interaction and Activation

Descriptive PK/PD models mostly use empirical models such as the Hill equation to describe drug concentration–effect relationships *in vivo*. A limitation of the application of the Hill equation is that it provides only limited insight into the underlying factors that determine the shape and the location of the concentration–effect curve. Specifically, the potency (i.e., the EC_{50}) and the intrinsic activity (i.e., maximal effect, E_{max}) of a drug are functions of compound-specific (i.e., receptor affinity and intrinsic efficacy) and system-specific properties (i.e., the receptor density and the function relating receptor occupancy to pharmacological effect). Classical receptor theory explicitly separates drug-specific properties and system-specific properties as determinants of the drug concentration–effect relationship (6) and therefore constitutes a theoretical basis for the prediction of this relationship.

Not surprisingly, receptor theory is increasingly applied in mechanism-based PK/PD modeling to explain and predict (variability in) *in vivo* drug concentration–effect relationships (7). In the meantime, receptor theory has successfully been incorporated in mechanism-based PK/PD models of A_1 adenosine receptor agonists (8), OP_3 opioid receptor agonists (9), 5-HT_{1A} serotonin receptor agonists (10) and $GABA_A$ receptor agonists (11). The studies on the hemodynamic and the antilipolytic effects of A_1 adenosine receptor agonists in rats demonstrate the concept of partial receptor activation as a mechanism to improve the selectivity of action *in vivo* (12). The utility of mechanism-based PK/PD modeling in interspecies extrapolation of drug effects is illustrated in the prediction of the pharmacodynamics of the anesthetic opioid remifentanil and its active metabolite GI 90291 in humans based on information from rats (13). Moreover, in a recent PK/PD study, the influence of receptor knock down on the *in vivo* concentration–effect relationship of alfentanil was analyzed, illustrating the importance of receptor expression as a determinant of intra- and interindividual variability in *in vivo* concentration–effect relationships (14).

Transduction

Transduction refers to the processes of the translation of the receptor activation into the ultimate pharmacological response. Specifically, the binding of a drug to a biological target initiates a cascade of biochemical and/or electrophysiological events resulting in the observable biological response. For most receptors (i.e., G-protein-coupled receptors), phospholipases (i.e., 1,4,5-inositol triphosphate, diacylglycerol) and nucleotide cyclases (i.e., cAMP) serve as second messengers. For other receptors (i.e., glucocorticoid receptors), transduction is mediated through an interaction with DNA, thus regulating the expression of second messengers, proteins, or enzymes. For some drugs (i.e., antidepressants), the molecular mechanisms underlying transduction are complex and still poorly understood.

There can be wide differences in the rates at which the various transduction processes occur *in vivo*. In many instances, transduction is fast (i.e., operating with rate constants in the range of milliseconds to seconds), relative to the rate constants governing the disposition processes (typically minutes to hours). In that situation, the transduction process determines the shape and the location of the *in vivo* concentration–effect relationship (6,7), but it does not influence the time course of the drug effect relative to the plasma concentration. In contrast, transduction *in vivo* can also be slow, operating with rate constants in the order of hours to days, in which case transduction becomes an important determinant of the time course of drug action.

As an approach to account for a delay of the drug effect relative to the concentration, a family of four indirect-response models has been proposed (15). In these models, the drug effect is described as stimulation on or inhibition of the factors controlling either the input or the dissipation of drug

response in a direct concentration-dependent manner. In these models, the rate constants for the input and the dissipation of the drug response are the important, system-specific parameters governing the time course of the drug response. In the meantime, numerous useful applications of various forms of the indirect response model have been reported (16).

As a next step in the modeling of complex transduction mechanisms, models have been proposed in which transduction is modeled mechanistically on the basis of intermediary processes between pharmacokinetics and response. In terms of mathematical modeling, the so-called transit compartment model has been proposed. This model relies on a series of differential equations to describe the cascade of events between receptor activation and final response (17). Well-known examples of applications of this type of modeling are the modeling of the genomic effects of corticosteroids (18) and the modeling of hematologic toxicity in cancer (19). The transit compartment model is attractive because of its flexibility, but for it to become fully mechanistic, pertinent information on the processes on the causal path is required. This underscores the need for biomarkers to characterize transduction mechanisms.

Homeostatic Feedback

Apart from being able to describe a delay in the pharmacological response relative to the drug concentration in plasma, there is a growing need for methods to describe and predict complex pharmacological effect *vs.* time profiles. Such complex profiles may be observed when drug exposure leads to tolerance/sensitization or when homeostatic feedback mechanisms are operative. An example of a model to describe complex effect *vs.* time profiles is the so-called "push-and-pull" model. Because of its plasticity, the push-and-pull model could be successfully applied to describe tolerance to the diuretic response on repeated administration of furosemide (20).

Another example of a PK/PD model describing tolerance is the "precursor pool" model. The precursor pool model can conceptually be considered a description of a tachyphylactic system and has been successfully applied to describe the effects of neuroleptic drugs on the prolactin balance (21).

Attempts to model physiological counterregulatory mechanisms have resulted in a series of advanced models describing complex behavior. These models are in part based on the work by Ekblad and Licko (22). An example is the model proposed by Bauer *et al.* to characterize tolerance to the hemodynamic effects of nitroglycerin in experimental heart failure (23). In the meantime, this type of physiological counterregulatory effect model has been successfully applied to describe tolerance and rebound to the effects of drugs such as alfentanil and omeprazole (24–26). Moreover, a dynamical systems model has been proposed, which can account for the complex hemodynamic effects of arterial vasodilators (i.e., nifedipine) for which rate of administration is a major determinant of the effects (27,28).

The most recent development in the incorporation of dynamical systems analysis in PK/PD modeling was the conceptualization of the so-called set point model (29). This model was designed to describe complex effect *vs.* time profiles of the hypothermic response after the administration of 5-HT_{1A} receptor agonists to rats. In this model, the indirect physiological response model is combined with a thermostat-like regulation of body temperature. Specifically in the model, body temperature and set point temperature are interdependent through a feedback loop. A unique feature of this feedback loop is that it can give rise to oscillatory behavior, allowing characterization of the complex hypothermic response *vs.* time profiles that are often observed on administration of 5-HT_{1A} agonists.

Disease Processes

At present there is a growing interest in the development of disease progression models, which are particularly important for drugs that interact in a highly specific manner with the disease process and that may have no direct observable effects in healthy subjects. Furthermore, application of disease progression analysis is imperative when drug treatment is specifically intended to modify disease progression. Chan and Holford (30) and Holford and Peace (31) were among the first to propose disease-progression models for clinical rating scales. In these models, the signs and/or symptoms of disease and their response to treatment are modeled directly, without consideration of the underlying biological system. Such a descriptive approach is applicable when only information on clinical symptoms or outcome is available. However, if relevant biomarkers have been identified, a more detailed and mechanistic description of disease progression can be obtained. Recently, a theoretical framework for mechanism-based disease progression models has been proposed (32). In the meantime, steps have been taken toward the application of such mechanistic models for the effects of thiazolidinedione insulin-sensitizing agents (i.e., pioglitazone) in type 2 diabetes mellitus, using biochemical indices such as fasting plasma glucose concentration, plasma insulin and split proinsulin concentrations, and percent glycosylated hemoglobin (HbA_{1C}) as biomarkers (de Winter *et al., unpublished observations).*

BIOMARKERS AND PK/PD MODELING

There is an increasing interest in the use of biomarkers in drug development as reflected in the various review papers and commentaries that have recently appeared on this subject (33–37). Much of the discussion on biomarkers focuses on their validity and validation as surrogate endpoints for the clinical effects of a drug, particularly within the context of regulatory decision making (35). It should be realized, however, that the use of biomarker information for regulatory decision making is restricted to drugs with a specific mechanism of action and a well-established clinical effect where relatively rich information is available on the relationship between the biomarker responses *vs.* the clinical response. For most drugs, such information is lacking. Furthermore, for innovative products, the pertinent information becomes available at the earliest at the completion of phase 3 clinical trials. As a result, the use of information on biomarkers for regulatory decision making is generally of limited value, particularly for innovative products with novel mechanisms of action.

Recently, in publications by Peck and Wechsler (38) and Peck *et al.* (39), the concept of "causal-chain biomarkers" has been proposed within the context of use for confirmatory evidence. Here the underlying concept is again the extrapolation of drug effects from a biomarker to clinical effectiveness. Key factors in this respect are the strength of clinical data in studies of other drugs in a class or related diseases that share a similar action or disease mechanism (36).

In this contribution, we take the use of biomarkers forward toward research aiming at understanding and mathematical modeling of the functioning of the integral biological system *in vivo*. Thus, here we discuss the use of biomarkers in the context of biological systems analysis aiming at the prediction of drug effects in man. This is important in drug discovery and development, particularly in relation to extrapolation and prediction within the trajectory from target identification (*in vitro* and in *in vivo* animal studies) to proof of concept (in phase 2 clinical trials). Specifically, it is proposed that within this paradigm, biomarkers can also be of value in the development of drugs acting at novel targets, where the link with clinical effectiveness has not yet been demonstrated.

As was outlined above, the use of PK/PD modeling has in recent years progressed from empirical to mechanism-based models. Mechanism-based PK/PD models contain specific expressions for processes on the causal path between drug administration and response and have much improved properties for extrapolation and prediction. In a strict sense, mechanism-based PK/PD modeling is identical to biological systems analysis.

The concept of mechanism-based PK/PD modeling adds a novel dimension to the selection, evaluation, and validation of biomarkers, with a strong emphasis on "construct validity" as defined by Rolan (40). Within the context of mechanism-based PK/PD modeling, we define a biomarker as a measure that characterizes, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect. In this respect, a 7-point mechanistic classification scheme is proposed based on the location of the biomarker in the chain of events from underlying subject genotype or phenotype through to clinical scales (Table I). The proposed division into seven levels is logical in mechanistic terms, in the sense that it reflects the major intermediate steps in pharmacodynamics. An important point is that it is not always necessary to obtain information on each of the intermediate steps. The principle of parsimony can be (and in fact often is) applied in mechanism-based PK/PD modeling. For different drugs and/or modes of administration, different types of biomarkers may be more or less readily available (39).

Within the proposed classification, a type 0 biomarker refers to genotype and/or phenotype as a determinant of the drug response. This may be related to either a factor in the disposition of the drug that determines the target exposure (i.e., the expression of a specific enzyme or transporter) or a factor determining the response directly (i.e., the expression of a specific receptor). In a strict sense, a type 0 biomarker is traditionally considered a covariate rather than a biomarker.

Table I. Mechanistic Classification of Biomarkers

Type 0: Genotype or phenotype
Type 1: Concentration of drug and/or metabolite
Type 2: Target occupancy
Type 3: Target activation
Type 4: Physiological measures or laboratory tests
Type 5: Disease processes
Type 6: Clinical scales

A type 1 biomarker refers to the concentration of the drug and/or a drug metabolite. As such, drug concentrations in blood or blood plasma serve as useful and probably the most widely used biomarkers in drug development. Ultimately, however, one is specifically interested in the (free) target site concentration. Because for most drugs the target is located in peripheral tissue, free target site concentrations may not be readily accessible. Novel technologies (including microdialysis) may offer new opportunities for quantification of drug concentrations at the target site (5).

A type 2 biomarker refers to the target occupancy. In theory, drug effects may occur at different degrees of target occupancy. Information on the relationship between target occupancy and response is therefore important for the prediction of *in vivo* concentration–effect relationships (6,7) and for the understanding of intra- and interindividual variability in drug response (14). Neuroleptic drugs are an example where the relationship between receptor occupancy and therapeutic response is particularly well established (41). For certain drugs, information on the degree of target occupancy can be obtained on the basis of *ex vivo* bioassays (42). Alternatively, novel imaging techniques (i.e., positron emission tomography scanning) open new avenues for the assessment of *in vivo* target occupancy, provided that a suitable and specific ligand is available (41).

A type 3 biomarker refers to quantification of the target site activation. According to receptor theory, this target site activation is determined by the intrinsic efficacy of the drug in combination with the level of receptor expression in the target tissue (6,7). It has been demonstrated that differences in target site activation can be an important determinant of tissue selectivity of drug action (12). An example of type 3 biomarkers is quantitative electroencephalogram (EEG) parameters, which have been validated as biomarkers characterizing $GABA_A$ receptor activation *in vivo* (11). Likewise, for synthetic opioids, quantitative EEG parameters have been proposed as biomarkers reflecting OP_3 opioid receptor activation (8). Interestingly, in a series of investigations in healthy volunteers, evidence for the validity of quantitative EEG parameters as a surrogate for depth of anesthesia has been obtained (43–45).

A type 4 biomarker refers to physiological measures in the integral biological system. Hemodynamic variables characterizing the *in vivo* effects of drugs with an action on the cardiovascular system are probably among the most well known examples of type 4 biomarkers. An important feature of type 4 biomarkers is that the biomarker response is often influenced by *in vivo* homeostatic control mechanisms. The utility of the application type 4 biomarkers in phase 1/2 clinical investigations is amply illustrated in the studies on nifedipine, where rate of administration was found to be an important determinant of the cardiovascular effect (27). This provided a scientific basis for the design of novel sustained-release preparations with not only a prolonged duration of action but, more importantly, also a much improved selectivity of action.

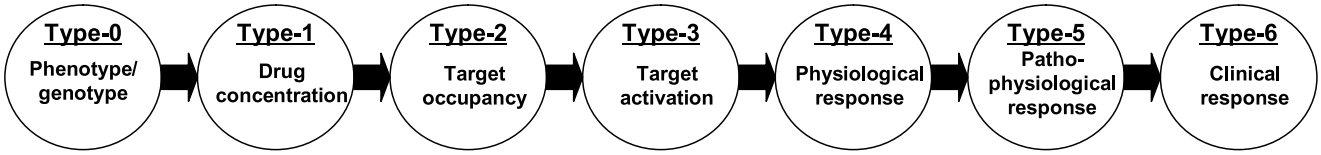

Fig. 1. Schematic representation of the concept of a "cascading" PK/PD model for prediction of *in vivo* drug effects on the basis of intermediary biomarker responses.

Type 5 biomarkers are parameters that characterize in quantitative manner disease processes. Examples are biomarkers characterizing inflammatory processes or type 2 diabetes mellitus. Recently, *ex vivo* PGE_2 and thromboxane B_2 inhibition have been proposed as biomarkers in the development of COX inhibitors (46). The utility of disease biomarkers in drug development is illustrated in the recent investigations on mechanism-based modeling of type 2 diabetes mellitus showing the beneficial effects on disease progression of metformin and thiazoledinedione insulin-sensitizing agents (de Winter *et al.* unpublished observations).

Finally, type 6 biomarkers are clinical scales. In a strict sense, these biomarkers can be regarded as clinical endpoints rather than biomarkers *per se*. The utility of clinical scales as pharmacodynamic endpoints in PK/PD modeling is illustrated in the modeling of disease progression of neurodegenerative diseases (i.e., Parkinson's disease, Alzheimer's disease) (30,31).

CONCLUSION

A new classification of biomarkers is proposed that is based on the mechanism of drug action and, ultimately, in the ideal situation, the interactions with the disease process. This classification is useful in reducing disagreement on the potential impact or role of a biomarker in drug discovery and development (33). In this respect, it is important that biomarkers in many instances do not reliably predict clinical response and may therefore not qualify as a surrogate marker (31). The concept of mechanism-based PK/PD modeling, however, brings an entirely new dimension to the prediction of drug response because it constitutes a basis for better understanding of the biological system of interest providing a basis for extrapolation and prediction.

The proposed classification of biomarkers into seven levels is particularly useful in conjunction with the concept of mechanism-based PK/PD modeling. As was outlined above, mechanism-based PK/PD models contain specific expressions for processes on the causal path between drug administration and response and typically have much improved properties for extrapolation and prediction. Biomarkers constitute a basis for the characterization, in a strictly quantitative manner, of processes on the causal path between drug administration and response. This allows in principle the development of cascading models in which the effect of drugs from one process in the chain of events to the next can be described (Fig. 1). This is important because the relationships between the various processes depend solely on the functioning of the biological system and are therefore independent of the drug. Mechanism-based PK/PD modeling constitutes, therefore, the scientific basis for prediction of the ultimate clinical effects of novel drugs based on a biomarker response. Furthermore, studies on biomarker responses can provide the basis for the understanding, in mechanistic terms, and the prediction of *variability* in drug response. Ultimately, after proper validation, such biomarkers might be used in clinical practice in the individualization of drug treatment.

Thus, biomarkers are invaluable for the development and application of mechanism-based PK/PD modeling in drug development. At the same time, however, mechanism-based PK/PD modeling constitutes also a scientific basis for the selection, evaluation, and validation of biomarkers, particularly in nonclinical investigations. This is illustrated in investigations on the effects of $GABA_A$ receptor agonists in rats. On the basis of the observed *in vitro*/*in vivo* correlation, amplitude in the 11.5- to 30-Hz frequency band of the EEG was validated as a biomarker reflecting in a direct quantitative manner modulation of GABA-ergic inhibition *in vivo* (11).

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