REVIEWS

Drug Concentration, Binding, and Effect In Vivo

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Abstract: It is an axiom of pharmacodynamics that drug effects are determined by drug concentration at the site of action. The link between concentration and effect can often be described by empirical models, but the ability to measure binding to the target tissue permits a more detailed description. The action and interaction of drugs at identifiable receptor sites can then be predicted from a knowledge of their binding properties and the law of mass action. The time course of drug effect is determined not only by drug disposition reflected in the blood circulation but also by the equilibration rate between blood and the effect site, and the steps linking the direct actions of the drug to their expression as an observable drug effect. Models encompassing these phenomena have been developed and have been applied in many situations to describe the kinetics of pharmacological response.

Introduction

Early attempts to quantitate drug action (1) were based upon *in vitro* experiments and were formulated in terms of the theory of adsorption isotherms. Subsequently the development of the science of pharmacokinetics has allowed drug concentrations to be predicted *in vivo*. Measurement of drug effects *in vivo* can now be correlated to drug concentration using concepts similar to those used to interpret *in vitro* experiments. The time varying nature of concentrations *in vivo* has, however, required the development of special methods to describe the time course of the *in vivo* response.

The concept of a receptor was developed early in the history of modern pharmacology to explain the specificity of drug action. The adsorption isotherm theory developed by Langmuir, based upon the law of mass action, provided an obvious link between concentration and the extent of drug binding. The theory proved to be extremely successful in quantitatively predicting the interaction between drugs competing for the same binding site (2). While experiments were being done relating concentration to effect, others were using physicochemical methods to measure drug binding. The ability to quantitate the binding site concentration and the affinity of drugs for these sites was an important step in exploring further the relationship between concentration, binding and effect. The link between these events clearly involves a receptor, but it must be borne in mind that receptors are defined by their *functional* properties and must be distinguished from binding sites which are defined by their *physico-chemical* properties.

Binding Models

The simplest model of binding assumes that one molecule of drug combines with each binding site. A binding site may be formed from one or more molecules or there may be more than one binding site per molecule of binding substance.

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The Dissociation Constant

The binding of a drug (D) to a binding site (S) may be derived by proposing a simple bi-molecular reaction forming the drug-binding site complex (DS):

$$D + S \rightleftharpoons DS \qquad (1)$$

The law of mass action predicts the rates of formation and breakdown of DS from the concentrations of the 3 species. At equilibrium these rates are equal:

$$DS \times k_1, = D \times S \times k_2 \qquad (2)$$

where k_1 and k_2 are rate constants. If the ratio of k_1 to k_2 is defined to be $KD_{D,S}$ (the equilibrium dissociation constant), then it may be defined by rearranging equation *(2)* as follows:

$$KD_{D,S} = \frac{S \times D}{DS} \qquad (3)$$

It should be noted that the dissociation constant is a property of the interaction between a particular binding site and a particular drug. The notation used here is to use a subscript for the drug followed by a subscript for the site. In the simple case of only one site and one drug this can be simplified to KD. The affinity of a drug for its binding site is the reciprocal of the dissociation constant.

The Concentration of Binding Sites

Specific binding sites are recognized by drug binding approaching a maximum value as drug concentrations are increased. This asymptotic concentration of bound drug is usually called Bmax and is directly proportional to the concentration of binding sites. Bmax is a property of the binding site and is independent of the drug. For completeness it may be subscripted to denote the particular binding site e. g. $Bmax_S$, the concentration of binding site S.

If the molar concentration of the binding site substance is known by independent means, e. g. binding to a protein of known molecular weight is studied, then the ratio of Bmax to this concentration is the number of binding sites per molecule of the binding substance.

The Equilibrium Binding Model

Because of conservation of mass the sum of unbound sites and bound sites must equal the total concentration of sites, Bmax. The concentration of unbound sites (S) in the definition of KD (equation *3)* can therefore be replaced by Bmax – DS. The concentration of bound sites, DS, is then defined by:

$$DS = \frac{Bmax \times D}{D + KD_{D,S}} \qquad (4)$$

The Binding-Effect Model

What is the expected relationship between the extent of drug binding and the degree of drug effect? The simplest answer is to assume that the effect (E) is directly proportional to binding, i. e. the direct binding-effect model:

$$E = i_{D,S} \times DS \qquad (5)$$

where $i_{D,S}$ is a constant reflecting the efficacy of the drug-binding site complex in producing an effect. This model implies that the maximum effect (Emax) will be achieved when DS is equal to Bmax, i. e. all binding sites are occupied. It further predicts that half-maximal binding should produce half-maximal effect.This direct link between binding and effect indicates that the receptor is a transducer *directly* converting the drug-binding site complex into effect.

Many drug effects are known to require at least one additional step between formation of the drug-binding site complex and the expression of effect, e. g. the generation of the messenger substance cyclic AMP. If we assume that there is a direct link between drug binding and messenger concentration we must then consider how the messenger is related to the extent of effect. If we imagine that the messenger binds to a transducer (T) which directly converts *bound* messenger into effect, then the effect becomes a function of messenger concentration (M), the efficacy of the messenger bound to the transducer ($i_{M,T}$), and the dissociation constant describing messenger binding to the transducer:

$$E = i_{M,T} \times \text{bound M}$$

$$= i_{M,T} \times \frac{M}{M + KD_{M,T}}$$

From equation *(5)* for a direct link between bound D and M:

$$M = i_{D,S} \times DS$$

Therefore

$$E = i_{M,T} \times \frac{i_{D,S} \times DS}{i_{D,S} \times DS + KD_{M,T}} \qquad (6)$$

The effect is now an indirect function of the drug-binding site complex even though we have assumed messenger concentration is directly related to the concentration of this complex.

Pharmacodynamic Models

Using the expressions defining binding as a function of drug concentration (equation *4*) and effect as a function of binding (equation *5* or *6*), we can now define corresponding pharmacodynamic models, i. e. the relationship between drug concentration and effect:

Direct Binding-Effect Model:

$$E = \frac{i_{D,S} \times Bmax \times D}{D + KD_{D,S}} \qquad (7)$$

Indirect Binding-Effect Model:

$$E = \frac{i_{D,S} \times Bmax \times \frac{i_{M,T}}{i_{D,S} \times Bmax + KD_{M,T}} \times D}{D + KD_{D,S} \times \frac{KD_{M,T}}{i_{D,S} \times Bmax + KD_{M,T}}} \qquad (8)$$

Both of these expressions can be simplified to the form of the Emax model *(3)*:

$$E = \frac{Emax \times D}{D + EC50} \qquad (9)$$

where Emax is the maximum response produced by the drug, and EC50 is the concentration producing 50 % of Emax. In the case of the direct binding effect model:

$$Emax = i_{D,S} \times Bmax$$

and

$$EC50 = KD_{D,S}$$

or

$$\frac{EC50}{KD} = 1 \qquad (10)$$

If effect is an indirect consequence of drug binding then:

$$Emax = i_{D,S} \times Bmax \times \frac{i_{M,T}}{i_{D,S} \times Bmax + KD_{M,T}}$$

and

$$EC50 = KD_{D,S} \times \frac{KD_{M,T}}{i_{D,S} \times Bmax + KD_{M,T}}$$

or

$$\frac{EC50}{KD} = \frac{KD_{M,T}}{i_{D,S} \times Bmax + KD_{M,T}} \qquad (11)$$

The Identification of Binding Sites and Receptors

The relationship between EC50 and KD is the most revealing in identifying a receptor with its binding site. The EC50 can be estimated from measurements of drug effect and KD estimated from measurements of drug binding. If there is a direct binding-effect link, then the EC50 of the receptor producing the drug effect will be identical to the dissociation constant of the binding site. If there is an indirect link, then the EC50 must always be less than the KD (equation *11*). The observation of an EC50 less than the KD is commonly explained (4) by the "spare receptor" hypothesis but can be equally well understood as due to a multi-step binding-response relationship.

Comparison of the EC50 and KD for a single drug and binding site will only allow the site to be identified with the receptor if EC50 and KD are equal. If the EC50 is less than the KD, no assertions can be made, because it is possible that the KD reflects binding to a site unrelated to the receptor binding site. However, the use of two drugs producing similar effects may provide stronger support. Whether the link between binding and effect is direct or indirect the ratio of EC50 to KD should be the same for different drugs which bind at the same site and have the same efficacy (equations *10* and *11*). This ratio is 1 for drugs with a direct link and is always less than 1 for drugs acting indirectly. It is determined by parameters independent of the drug, except for $i_{D,S}$ which will be the same for drugs with equal efficacy.

This prediction has been used by Rosenbaum et al. on the basis of *in vivo* binding studies (5) to identify which of two opiate binding sites in rat brain was associated with the receptor mediating analgesia. Sufentanil and etorphine are

opiate analgesics with similar efficacy (Emax), but they differ in potency (EC50) by 2.5 fold when used to produce analgesia. The ratio of EC50 to KD at the first site (μ site) for sufentanil was 0.022 and for etorphine was 0.021, yet this ratio differed by a factor of 50 at the other site.

In contrast the ratio of EC50 to KD at the first site for buprenorphine was 0.85 (6). Could this 40 fold difference from the ratio seen with sufentanil and etorphine be explained by a lower efficacy of buprenorphine? By rearranging equation *11* the value of $i_{D,S}$ is given by:

$$i_{D,S} = \frac{KD_{M,T} \times (KD_{D,S}/EC50 - 1)}{Bmax}$$

The ratio of $i_{S,1}$ (sufentanil binding to site 1) to $i_{B,1}$ (buprenorphine binding to site 1) is then:

$$\frac{i_{S,1}}{i_{B,1}} = \frac{(KD_{S,1}/EC50_S - 1)}{(KD_{B,1}/EC50_B - 1)}$$

Substituting the known values for the ratios of EC50/KD for sufentanil and buprenorphine predicts that the ratio of the intrinsic activity of sufentanil to buprenorphine must be 252. However, measurements of the maximal analgesic effect of buprenorphine compared with sufentanil suggest the intrinsic activity of buprenorphine is at least 50 % of that of sufentanil (7). The observed ratio of EC50/KD for buprenorphine of 0.85 must, therefore, be explained by another mechanism than differences in intrinsic activity. The same group has already suggested that buprenorphine analgesia may reflect the combination of effects arising from 2 sites with one site promoting analgesia and the other inhibiting it (7).

The Kinetics of Pharmacological Effect

The preceding section has described aspects of concentration-effect relationships which are independent of time. The ability to predict drug action *in vivo* requires additional considerations of the time course of pharmacological effect. The next section introduces two simple concepts which can be used to link both time and concentration to drug effects. In particular, the kinetics of direct effects of a drug will be distinguished from the kinetics of indirect effects. Notice that this use of direct and indirect is different from the earlier use in connection with direct and indirect binding-effect links.

The Kinetics of Direct Drug Effects

The time course of drug effect can be predicted by combining a pharmacokinetic model with a pharmacodynamic model (3). For example, if we assume a simple pharmacokinetic model involving a single compartment and a bolus dose input, then plasma concentration as a function of time (C(t)) is given by:

$$C(t) = \frac{Dose}{V} \times e^{-CL/V \times t} \qquad (12)$$

where CL is drug clearance and V is the volume of distribution. The effect of the drug as a function of time (E(t)) can now be predicted by a pharmacodynamic model e. g. the Emax model (equation *9*), by substiting D with C (t):

$$E(t) = \frac{Emax \times C(t)}{C(t) + EC50} \qquad (13)$$

The Effect Compartment Equilibration Model

The simplest way of linking changes in drug levels to effect is to assume concentrations at the receptor are the same as those in plasma; however, when effects are measured frequently the time course of drug response lags behind plasma concentrations. This suggests a delay in equilibration between plasma and the receptor site determining the effect.

The rate of equilibration of plasma concentration with that at the receptor can be described by an extension to conventional compartmental pharmacokinetic models which proposes the existence of an *effect compartment*. Input to this compartment is from plasma and both input and loss are first order processes. Using the one compartment bolus input model for plasma concentration (equation *12)*, the concentration of drug in the effect compartment, as a function of time (Ce(t)), can be shown to be:

$$Ce(t) = \frac{Dose \times Keq}{V} \times \left(\frac{e^{-CL/V \times t}}{(Keq - CL/V)} + \frac{e^{-Keq \times t}}{(CL/V - Keq)} \right) \qquad (14)$$

where Keq is the rate constant describing loss of drug from the effect site (3). The time course of drug effect is now defined by:

$$E(t) = \frac{Emax \times Ce(t)}{Ce(t) + EC50} \qquad (15)$$

The equilibration half-time is simply determined from Keq, and its value has been estimated to be only a few minutes for drugs with rapid actions, such as quinidine (8), to several hours for drugs with a slower onset, such as digoxin (9). The rate of equilibration is determined in physiological terms by the drug removal rate constant which is defined by organ blood flow and the volume of distribution as a function of the physical size of the organ and the tissue/blood partition coefficient. For drugs such as quinidine and digoxin which both have effects on the heart but are delivered at the same rate, the differences in blood/effect site equilibration rates are explained by differences in affinity for heart tissues.

A similar argument can be made to explain differences in the duration of action of opiate analgesics which have similar plasma pharmacokinetics. Delivery to and clearance from the brain will be determined by cerebral blood flow, but the duration of action will also be proportional to the brain/blood partition coefficient. Thus more lipophilic drugs will be active for longer times, even though they are delivered and removed from the brain at the same rate.

The Kinetics of Indirect Effects

In some cases the drug effect that is most readily observable is only indirectly related to the drug's action. For example, the anti-coagulant effect of warfarin is usually measured by changes in the prothrombin time, which in turn reflects the prothrombin activity in the blood. Warfarin reduces prothrombin activity by inhibiting the synthesis of prothrombin precursors, and it is this action which is the *direct* effect. At steady state the prothrombin activity will be proportional to the synthesis rate of the precursors, but changes in synthesis will not be reflected immediately in prothrombin activity, because the elimination half-lives of the precursors are several hours.

The time course of prothrombin activity can be predicted from the time course of warfarin concentrations by the use of a model incorporating not only the effect of warfarin on prothrombin synthesis but also the elimination kinetics of the already synthesized precursors (10). Application of this model to the observations of O'Reilly et al. (11) predicts that the

EC50 for warfarin is 1.5 mg/L, and the apparent elimination half-life of prothrombin activity is 14 hours.

This approach can be applied quite generally to drug responses which are influenced by the time course of an intermediate substance whose kinetics are altered by a direct action of the drug.

Conclusion

The ability to predict drug effects *in vivo* has as its cornerstone the role of concentration linking drug to its receptor binding site. The consequence of binding may be expressed directly or indirectly in order to produce a drug response. The time course of drug concentrations *in vivo* is determined by the kinetics of drug distribution to the effect site and the kinetics of intermediates which express the final effect. Appreciation of the quantitative relations between time and concentration, and concentration and effect, can extend understanding of drug actions in man.

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Nephelometric Immunoassay for Therapeutic Drug Level Monitoring

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Abstract: Nephelometric immunoassay for the determination of drug levels in blood is based on the inhibition of immunoprecipitation by a hapten (drug). It represents a homogeneous method that does not require any separation steps nor radioisotopes. Precipitation in an aqueous solution can be quantitated by nephelometry (scattered light measurement) or turbidmetry (traversed light measurement). Advantages over other drug assay methods include its simplicity, speed and low cost. Only two reagents are added, and the subsequent reaction can be monitored optically with the potential for full automation. The reaction is usually completed in less than 15 minutes. The two reagents, anti-drug antibody and polyhaptenic antigen, can be easily prepared and are highly stable. Therefore, precipitation inhibition immunoassays and in particular nephelometric immunoassays are being commercially developed for routine therapeutic monitoring of drugs such as anticonvulsant drugs, aminoglycoside antibiotics and theophylline. The specificity is high, though depending on the cross-reactivity of the anti-drug antibody as is the case with other immunoassays. The sensitivity depends on a variety of factors such as antibody-hapten affinity, detection mode of the precipitation, and intrinsic turbidity of the test sample. But the sensitivity is sufficiently high for serum drug concentration greater than 1 µg/ml when less than 10 µl of serum are used. Variations of this assay technique include rate analysis for precipitate formation instead of endpoint analysis. Agglutination-, or particle aggregation-inhibition immunoassay is also a useful and more sensitive method. Finally, use of monoclonal antibodies can serve to enhance the specificity of nephelometric immunoassay of drugs.

Introduction

Therapeutic drug level monitoring (1) is widely performed in clinical laboratories for anticonvulsant drugs, cardiac glycosides, antiarrhythmics, aminoglycoside antibiotics, anticancer drugs, theophylline and lithium. Serum drug levels can serve as a guide to optimize dosage regimens for individual patients. A critical requirement for successful drug level monitoring is a reliable and accurate analytical method. For clinical use, the method should be simple, rapid, inexpensive, and require a small volume of blood. Therefore the clinical need for drug level data has stimulated development of novel analytical techniques.

Immunoassays and high performance liquid chromatography are now considered to be most suitable for routine therapeutic drug level monitoring. Each of these two methods has its own advantages and disadvantages. In general, immunoassay is more suitable for the analysis of a large number of samples than high performance liquid chromatography. Therefore various immunoassays have been applied to routine drug level monitoring such as radioimmunoassay, enzyme-labeled immunoassay and fluorescence-labeled immunoassay including fluorescence polarization immunoassay and fluorogenic substrate-labeled immunoassay. In this review, the nephelometric immunoassay for therapeutic drug level monitoring is described and discussed.

Assay Principle

Nephelometric immunoassay is based on the inhibition of precipitation by a hapten (Fig. 1). As Landsteiner described in his classical and pioneering studies (2), a hapten may be defined as a low molecular weight substance, too small to be immunogenic, but which can react with an antibody of appropriate specificity. When a small molecule is covalently conjugated with a large immunogenic protein and the conjugate is

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