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REVIEW

An overview of pharmacodynamic modelling, ligand-binding approach and its application in clinical practice

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Abstract   The study of the magnitude and variation of drug response is defined as pharmacodynamics (PDs). PD models examine plasma concentration and effect relationship. It can predict the archetypal effect (E) of a drug as a function of the drug concentration (C) and estimate an unknown PD parameter (θ_{pd}). The PD models have been described as fixed, linear, log-linear, E_{max}, sigmoid E_{max}, and indirect PD response. Ligand binding model is an example of a PD model that works on the underpinning PD principle of a drug, eliciting its pharmacological effect at the receptor site. The pharmacological effect is produced by the drug binding to the receptor to either activate or antagonise the receptor. Ligand binding models describe a system of interacting components, i.e. the interaction of one or more ligands with one or more binding sites. The E_{max} model is the central method that provides an empirical justification for the concentration/dose-effect relationship. However, for ligand binding models justification is provided by theory of receptor occupancy. In essence, for ligand binding models, the term *fractional occupancy* is best used to describe the fraction of receptors occupied at a particular ligand concentration. It is stated that the *fractional occupancy = occupied binding sites/total binding sites*, which means the effect of a drug should depend on the fraction of receptors that are occupied. In the future, network-based systems pharmacology models using ligand binding principles could be an effective way of understanding drug-related adverse effects. This will facilitate and strengthen the development of rational drug therapy in clinical practice.
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Contents

1. Introduction to pharmacodynamic modelling aspects

1.1. Pharmacodynamics

The study of the magnitude and variation of drug response is defined as pharmacodynamics (PDs). To be explicit, it is the study of the drug which reaches the systemic circulation after the onset of administration, its intensity, and duration of action or response or effect, which are related to the drug concentration at its receptor site of action (Rosenbaum, 2011). The relationship between concentration and effect is usually non-linear, i.e. double the concentration does not result in a twofold increase in effect but, it will increase the duration of effect by one half-life. PD encompasses use of quantitative tools to measure affinity and efficacy of drugs. Clinical pharmacology can be divided into pharmacokinetics (PK), PDs and its integration as pharmacokinetic-pharmacodynamic (PKPD) models combine the time course of drug concentration with binding of drug to the target site(s) and subsequent drug effects. The PKPD relationship is presented in Fig. 1. PKPD models are therefore useful to describe, understand, and predict the extent and time course of drug effects. It follows that the greater the endeavour to incorporate mechanistic behaviour into the PKPD model the greater its ability to predict new situations.

1.2. Receptors, ligands, binding

Receptors can be defined simply as specific macromolecules at which a ligand binds and alters the biochemical activity (note that a ligand may do this by inhibiting the effects of endogenous substances from stimulating the receptor). Any macromolecular tissue site where a drug may bind can be considered a *binding site* and if this site has some functional activity then it is a *receptor*. Drug response is a result of chemical interactions between a drug and a binding site. The classification of drug receptors is based on tissue location, specificity of the drug, and the primary amino acid sequence. Majority (95%) of the total receptors are protein in nature. Not all proteins in the plasma membrane are receptors, some serves as transporters, enzymes or ion channels.

A drug can interact with four principle protein targets such as ion channels (nimodipine and voltage-gated Ca^{2+} ion channels), enzymes (neostigmine and acetylcholinesterase), membrane carriers (tricyclic antidepressants and catecholamine uptake-1) and receptors (Lambert, 2004). A drug binds and activates a receptor causing an alteration to a number of intracellular messengers/proteins (effectors). Generally, drugs are considered to bind to receptors and any chemicals that bind to receptors are usually termed *ligands* (e.g. drugs). A ligand is usually considered to be smaller in size than the receptor;
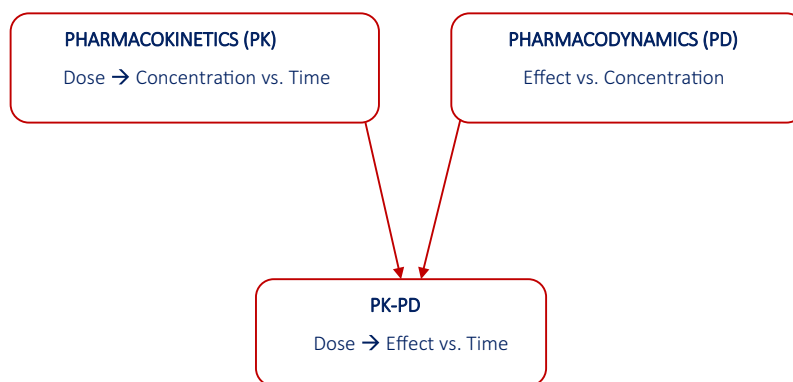
Figure 1   The pharmacokinetic and pharmacodynamic (PKPD) relationship.

however, anything that binds with specificity can be considered a ligand. The chemical structure/moiety of both drugs and receptors are quite important, even though a small change can result in diminished or null response. A ligand can bind either reversibly or irreversibly to a receptor. The action is produced by interacting the drug binding to the receptor to either activate or antagonise the receptor. A drug-receptor interaction can open or close an ion channel across the cell membrane.

The drug concentration at the site of the receptor determines the intensity of a drug's effect; however, the drug response could be influenced by receptor density on the cell surface, signal transmission mechanism into the cell by second messengers (substances within the cell), or regulatory factors that control gene translation and protein production. High concentrations of ligand added to a binding system produce binding, particularly non-specific binding. When a ligand binds to a receptor of non-interest is termed as non-specific binding. For example, a muscarinic receptor antagonist drug can even bind to multiple type receptors such as histamine receptors. Non-specific binding is linearly proportional to unbound ligand concentration and many biological tissues have both saturable and non-saturable components. Further details pertaining to the non-specific binding are outlined in Section 3.3. Binding does not always produce an effect; however, receptors are saturable binding sites that express an effect.

Receptors are usually activated by dimerization. Ligand binding to receptor monomers causes them to dimerize by interactions between the extracellular domains. Dimerization is likely made by the capacity of membrane proteins to move laterally within the membrane bilayer (Heldin, 1995). Dimerization triggers the cytoplasmic domains (signalling pathway) by an autophosphorylation (ability of a species of kinase to phosphorylate itself) in which the kinase activity of each monomer phosphorylates the other monomer (Cunningham et al., 1991).

1.3. Agonist, partial agonist, inverse agonist, biased agonist and antagonist

Agonist: A drug that mimics the endogenous receptor ligand to activate the receptor to produce a biological response is called as an agonist. Several agonists are able to produce the target maximum response without completely occupying all the receptors.

Partial agonist: A drug that binds and activates a receptor but does not elicit a full response is known as a partial agonist.

A partial agonist can block the effect of a full agonist. In the presence of high concentrations of a partial agonist, the action of a full agonist can be reduced to the maximum response elicited by the partial agonist. However, the intrinsic activity would be greater than zero but less than 1 that of a full agonist.

Inverse agonist: An inverse agonist is a molecule or agent that binds to the same receptor site as an agonist and is considered to be a full agonist. However, it exerts the opposite pharmacological response to that of a normal agonist, i.e. demonstrates negative efficacy. Constitutive activity refers to the ability of a receptor in producing its biological response in the absence of a bound ligand (Milligan, 2003). The constitutive activity of a receptor may be blocked by an inverse agonist.

Biased agonist: G protein-coupled receptors (GPCRs) are capable of signalling with different efficacies to their multiple downstream pathways, a phenomenon referred to as biased agonism. Biased agonism is one of the fastest growing areas in GPCR pharmacology. Biased agonism has been primarily reported as a phenomenon of synthetic ligands and the biological importance of such signalling is unclear (Rajagopal et al., 2013).

Antagonist: A drug that binds to a receptor but does not elicit a response is referred to as an antagonist. Importantly, the antagonist must block the action of the agonist at the receptor site. Antagonist can shift the concentration–response curve of an agonist to the right by reducing its fractional occupancy. High concentrations of the antagonist may block the actions of the agonist completely. However, antagonists have no intrinsic activity and therefore they do not produce any effects. There are two main types of antagonists. Competitive antagonists compete with the agonist for same receptor binding site, but the binding is reversible. It shifts the concentration-response curve of the agonist to the right without any reduction in maximal response. Non-competitive antagonists bind irreversibly to a receptor site and thereby reduce the ability of an agonist to bind and produce a response. The non-competitive antagonism is a slow process which resulting in a prolonged antagonistic effect.

1.4. Affinity, potency and efficacy

Affinity can be defined as the extent or fraction to which a drug binds to receptors at any given drug concentration or the firmness with which the drug binds to the receptor. The

mathematical model of affinity of a drug for the receptor was first described by Irving Langmuir Kenakin (2004). Affinity is one of the factors that determine potency. Affinity is inversely proportional to the potency of a drug $\left(\frac{1}{Kd}\right)$, where Kd is the dissociation constant. The strength of the binding (interaction) of a ligand and its receptor can be described by affinity. The higher the Kd value, the weaker the binding and the lower the affinity. The opposite occurs when a drug has a low Kd.

Potency is a measure of necessary amount of the drug to produce an effect of a given magnitude. In general, potency is denoted as the median effective concentration/dose as $EC_{50}/ED_{50}/Kd$.

Efficacy (intrinsic activity) is the ability of a drug to illicit a pharmacological response (physiological) when interaction occurs with a receptor (relationship between response and occupancy of receptor). Efficacy depends on the efficiency of the receptor activation to cellular responses and the formation of number of drug-receptor complexes.

- Full agonists: efficacy = 1.
- Partial agonists: efficacy > 0 and < 1.
- Competitive antagonists: efficacy = 0.

2. Properties of receptors

The two major properties of receptors are binding and signal transduction. Binding observes laws of thermodynamics and is typically stereo-selective, saturable, and reversible in nature. Signal transduction is the second property of a receptor that the binding of an agonist must be transduced into some kind of functional response (biological or physiological). This indicates that two domains exist on a receptor which are 'a ligand-binding domain' and 'an effector domain'.

2.1. Major receptors families

A receptor is any biological macromolecule to which a drug binds and produces a measurable response. Thus, enzymes and structural proteins can be considered to be pharmacologic receptors. However, all plasma proteins are simply binding sites having no function but responsible for transducing extracellular signals into intracellular responses. Receptors are mainly divided into four families:

1. Ligand-gated ion channels and voltage-gated sodium channel.
2. G protein-coupled receptors (GPCRs).
3. Enzyme-linked receptors.
4. Intracellular receptors.

2.1.1. Ligand-gated ion channels (ionophores) and voltage-gated sodium channel

Ligand-gated ion channels are responsible for regulation of the flow of ions across cell membranes. The activity of these channels is regulated by the binding of a ligand to the channel. The nicotinic acetylcholine receptor (for all anaesthetics) and the γ-aminobutyric acid (GABA) receptor are good examples of ligand-gated receptors, whose activation permits Cl^- influx

to produce membrane hyperpolarisation and reduced central transmission.

Voltage-gated sodium channel, is an another important drug receptor other than ligand-gated ion channels for several drug classes (e.g. local anaesthetics). The receptor is made of multiple subunits club together to form an aqueous pore through which (not only) Na+ ions flow. Binding of acetylcholine opens the pore allowing Na+ influx to produce a depolarisation i.e. acetylcholine stimulates the nicotinic receptor which results in sodium influx, generation of an action potential, and activation of contraction of skeletal muscle.

2.1.2. G protein-coupled receptors

The GPCRs, also called "7 Transmembrane (7 TM)" receptors, are integral membrane protein monomers. These receptors comprise a large protein family of transmembrane receptors. Mainly, three protein families which facilitate the function of the receptors: the G protein-coupled receptor kinases (GRKs), the heterotrimeric G proteins, and the β-arrestins. The primary function is to transduce extracellular stimuli into intracellular signals. Activation of a GPCR allows interaction with a G protein, which is composed of α (binds guanosine triphosphate (GTP)), β and γ subunits. These comprise muscarinic, adrenergic, and opioid receptors. Activated Gα subunits then interact with an effector molecule to produce a second messenger, which then brings about a cellular response and also interact with ion channels to modulate ion conductance.

β-arrestins are versatile adapter proteins that form complexes with most of the GPCRs following agonist binding and phosphorylation of receptors by GRKs (Luttrell and Lefkowitz, 2002). They act as vital role in the interrelated processes of homologous desensitisation and GPCR sequestration, which lead to the termination of G protein activation. β-arrestin binding to GPCRs both uncouples receptors from heterotrimeric G proteins and targets them to clathrin-coated pits for endocytosis (Luttrell and Lefkowitz, 2002). Originally discovered GRKs and β-arrestins as molecules desensitise G protein-mediated signalling. However, the previous research on the formulation of GRKs and β-arrestins as simply a desensitisation system has proven inadequate to explain many cellular phenomena. In fact, β-arrestins have emerged as remarkably versatile adaptor molecules that regulate receptor endocytosis and also serve as signal transducers in their own right (DeWire et al., 2007).

2.1.3. Enzyme-linked receptors

These receptors consist of cytosolic enzyme activity as an integral component of their structure or function. Binding of a ligand to an extracellular domain activates or inhibits this cytosolic enzyme activity.

2.1.4. Intracellular receptors

These receptors are entirely intracellular and, therefore, the ligand must diffuse into the cell to interact with the receptor.

3. Ligand binding models

Ligand binding model works on the underpinning PD principle of a drug eliciting its pharmacological effect at the receptor

site (Bardal et al., 2010). The pharmacological effect is produced by the drug binding to the receptor to either mimic or antagonise the receptor. Largely, the drug concentration at the site of the receptor determines the intensity of a drug's pharmacological effect; however, the drug response could be influenced by receptor density on the cell surface, signal transmission mechanism into the cell by second messengers, or regulatory factors that control gene translation and protein production (Spruill and Wade, 2010).

Ligand binding models describe a system of interacting components, i.e. the interaction of one or more ligands with one or more binding sites. The E_{max} model is the central method to describe the concentration/dose-effect relationship. According to the law of mass action, it has robust theoretical support from the physicochemical principles governing binding of drug to a receptor. It predicts a hyperbolic relationship between the drug concentration and response which is linear. When dose increases, at certain point the response becomes saturated and reaches a plateau. All biological responses must reach a maximum and this is a significant prediction of the E_{max} model.

3.1. Theory of receptor binding

In 1926, the drug-receptor binding concept was first introduced by Clark (1926). The occupancy theory of Clark's was elaborated by Ariens and De Groot (1954) and Stephenson (1956) and became a foundation for the field of PDs.

The models used to describe the PD relationship is based on the receptor binding theory. The assumption made in classical receptor theory is that a drug binds to a receptor reversibly, which then provokes a series of biochemical and physiological changes to produce the observed drug effect. The maximum drug effect is achieved once all the receptors are occupied. Binding of drug to receptor is principally the same as drug to enzyme as defined by the Michaelis–Menten equation (Berg et al., 2002).

Michaelis–Menten equation:

$$Bound = \frac{B_{max} \times [L]}{[L] + K_d}$$
$$V_0 = \frac{V_{max} \times [S]}{[S] + K_m} \tag{1}$$

where B_{max} is the maximum binding capacity, $[L]$ is the ligand concentration, Kd is the dissociation constant, V_0 is the initial velocity of the reaction, V_{max} the maximal rate, $[S]$ is the substrate concentration and K_m is Michaelis–Menten constant.

Ligand – receptor binding experiments are usually analysed according to the simple model of law of mass action.

3.2. Law of mass action

The interaction of drug–receptor complex encompasses chemical bonding, which is normally reversible in nature and can be expressed using the law of mass action.

In accordance with the law of mass action, a drug (termed as ligand if it has affinity for a receptor) – receptor interaction is based on the random coupling of ligand-receptor. The basic concept is illustrated by the 'lock and key' model. The effect that may be stimulated by this will be proportional to both the amount of ligand and the amount of receptor. Since, there

may be a finite amount of receptors and concentrations of ligand may be higher than the affinity constant then the concentration-response will behave according to a process of diminishing returns, in the sense that higher concentrations produce less additional response than would be expected if the relationship is linear. From the law of mass action, the number of receptors site $[R]$ occupied by a drug depends on the plasma drug concentration $[L]$ and the association and dissociation rate constants (k_{on} and k_{off}) of drug receptor complex.

Ligand – receptor binding experiments are usually analysed according to the simple model of law of mass action:

Simple binding reaction (reversible):

$$[L] + [R] \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} [LR] \tag{2}$$

where $[R]$, $[L]$, and $[RL]$ represent the free concentration of receptor, ligand, and receptor-ligand complex, respectively, and where it is assumed that the reaction components can freely diffuse within the medium. k_{on} and k_{off} are the rate constants for association and dissociation of the ligand-receptor complex. It is assumed that after dissociation, receptor and ligand are not altered.

Usually, the following assumptions are made for this equation:

- The interaction is reversible.
- The receptor, ligand, and ligand-receptor complex are in equilibrium.
- The receptor contains one binding site for the ligand.
- The ligand and receptor interact rapidly to form the ligand-receptor complex.

Association and dissociation rates are temperature dependent. The reaction is driven by the concentration of the reacting agents. Equilibrium is reached when the rate at which ligand-receptor complexes are formed and dissociate are equal. At equilibrium, the following applies:

$$[L][R]k_{on} = [LR]k_{off}$$

3.3. Fractional occupancy

Pharmacologists often use the term *fractional occupancy* to describe the fraction of receptors occupied at a particular ligand concentration Hucho (1993); Ross (1996). The fractional occupancy can be described in another way as shown in Eq. (3).

Illustration of fractional occupancy:

$$f_{occupancy} = \frac{occupied\ binding\ sites}{total\ binding\ sites}$$
$$f_{occupancy} = \frac{[LR]}{[total\ receptors]}$$
$$f_{occupancy} = \frac{[LR]}{[R] + [LR]} \tag{3}$$

where $[R]$ is the concentration of free receptors and $[LR]$ is the ligand-receptor complex concentration. In accordance with the law of mass action a drug (termed as ligand if it has affinity for a receptor) – receptor interaction is based on the random coupling of ligand-receptor. The equilibrium dissociation constant

(Kd) represents the inverse of the affinity of the drug for the receptor and can be defined as;

Equilibrium dissociation constant:

$$Kd = \frac{K_{off}}{K_{on}} = \frac{[L][R]}{[LR]} \qquad (4)$$

The dissociation constant Kd represents the inverse of the affinity of the receptor for the ligand when at equilibrium and if Kd is large then the receptor ligand does not bind readily but if Kd is small then the receptor binds readily to the ligand. We know from its definition that Kd is given in Eq. (4).

So if we rearrange to get an equation describing $[LR]$ and then substitute this in Eq. (3), we get the following:

Fractional occupancy:

$$f_{occupancy} = \frac{[L]}{[L] + Kd} \qquad (5)$$

Pharmacologists are often concerned about the fractional occupancy because, the effect of a drug should depend on the fraction of receptors that are actually occupied. Therefore, the measured effect should depend on the fractional occupancy, which in turn depends on $\frac{[L]}{([L]+Kd)}$. Similarly, if we have measured the maximum effect (where almost 100% of the receptors are occupied) with known fractional occupancy, then it is easy to determine the effect for any given concentration of a ligand. This effect will be given by the following equation:

Derivation of fractional occupancy from E_{max} model is as follows:

$$Effect = E_{max} \times \frac{[L]}{[L] + Kd}$$

$$From \quad f_{occupancy} = \frac{[LR]}{[R] + [LR]}$$

Multiply by $[L]$,

$$f_{occupancy} = \frac{[L] \times [LR]}{[L] \times [R] + [L] \times [LR]}$$

Divide by $[LR]$ $\qquad (6)$

$$f_{occupancy} = \frac{[L] \times \frac{[LR]}{[LR]}}{[L] \times \frac{[R]}{[LR]} + [L] \times \frac{[LR]}{[LR]}} = \frac{[L]}{[L] \times \frac{[R]}{[LR]} + [L]}$$

Substitute in Kd

$$since, \quad Kd = [L] \times \frac{[R]}{[LR]}, \quad then$$

$$f_{occupancy} = \frac{[L]}{[L] + Kd}$$

Usually, binding of a ligand to receptors provides a fractional occupancy measure (f occupancy) based on two types of binding, (1) specific binding (saturable) and (2) non-specific binding (non-saturable).

Specific binding relates to receptor occupancy as follows:

Specific binding:

$$specific\ binding = f_{occupancy} \times B_{max} = \frac{B_{max} \times [L]}{[L] + Kd} \qquad (7)$$

$$specific\ binding = total\ binding - nonspecific\ binding$$

where B_{max} is the binding maximum which is equivalent to the maximum number of receptors. Specific binding refers to the binding of the ligand to the receptor which follows the law of mass action (mentioned above). The binding curve is iden-

tical to the fractional occupancy curve, except it is scaled by the number of receptors (B_{max}). Since there are a finite number of receptors then the curve reaches an asymptote at the limit of $[L]$. It is important to note that there may be more than one population of receptors (more than one B_{max}) with more than one affinity for the drug (recall Kd is the inverse of affinity).

Non-specific binding refers to the binding of a ligand to components of the experimental matrix other than the receptor. From a pure pharmacological perspective non-specific binding is of no importance but from an experimental pharmacological perspective non-specific binding cannot be avoided. In contrast to specific binding, non-specific binding is considered to consist of a virtually unquenchable supply of binding sites – the relationship is therefore linear. Any experiment that assesses ligand binding will therefore have to contend with non-specific binding. It is therefore necessary to be able to assay total binding of the ligand and non-specific binding, then specific binding (what we are interested in) is calculated as the difference.

Interaction of a drug with a receptor can be described by two parameters. Affinity describes strength of drug binding with receptor ("fit the lock"). Efficacy describes ability of drug-bound receptor to produce a response ("turn the key"). Agonists have both affinities for the receptor as well as efficacy but antagonists have only affinity for the receptors and no (zero) efficacy.

3.4. Applications of ligand binding model (Pollard, 2010)

Ligand binding models have broad applications in clinical pharmacology;

- Ligand binding models can explain a system of interacting elements of multiple ligands with multiple binding receptor sites.
- The time course of binding (association and dissociation) can be described using ligand binding models however, it is more common to describe binding at the equilibrium.
- Receptor binding properties for disease states can be assessed using the experimental setup with neurotransmitters (e.g. acetylcholine), and radioimmunoassay's.
- Ligand binding models are useful in the drug development process to identify the binding sites of receptors.
- Ligand binding models are useful to demonstrate multiple binding sites and receptors simultaneously.
- Ligand binding models are also very useful to compare the affinities of different ligands for a same receptor. The Cheng and Prusoff equation (Cheng and Prusoff, 1973) allows to calculate the K_i (inhibition constant) from the inhibition concentration (IC_{50}).

4. Pharmacodynamic (dose-response) models

PD models generally examine the plasma concentration and effect relationship. It can predict the archetypal effect (E) of a drug as a function of the drug concentration (C) and an unknown PD parameter (θ_{pd}) can be estimated. The PD models have been described as fixed, linear, log-linear, E_{max}, sigmoid E_{max}, and indirect PD response.

The mathematical form of a PD model:

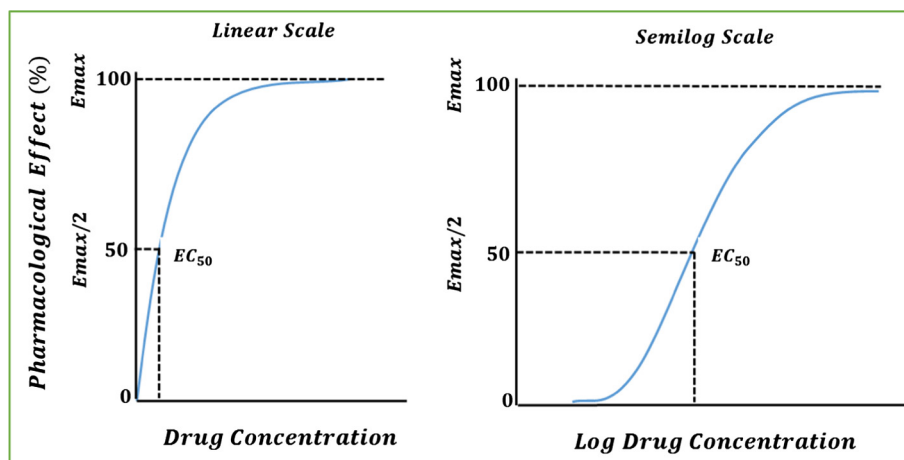$$E(C) = f(C, \theta_{pd}) \qquad (8)$$

Figure 2 The drug concentration-response (pharmacodynamic) relationship.

4.1. Dose-response relationship

It is defined as the relationship between an endogenous and exogenous ligand that binds to a receptor to produce a desired pharmacological effect. This effect can be a maximum (E_{max}) in which further increase in the concentration does not result in higher response called saturation point.

Fig. 2 describes the linear and semi-log approaches to concentration-response relationship. When plotted on a linear scale (left panel), a concentration-response relationship is hyperbolic, and can typically be well described by a Langmuir binding isotherm. The response reaches maximum at high concentrations due to saturation of available receptors by drug. When plotted on a semi-log scale (logarithm of drug concentration vs. effect), the relationship becomes sigmoidal (S-shaped). The semi-log plot is the preferred method for plotting concentration-response relationships because it becomes easier to accurately determine the EC_{50} value (the concentration which produces 50% of the maximum response) by placing it on a linear portion of the curve (semi-log plot). Higher the EC_{50}, lower the potency.

4.2. E_{max} model

This is a nonlinear mathematical model that is derived from the classic drug receptor theory. The E_{max} model is the central method to describe the concentration-effect relationship. According to the law of mass action, it has strong theoretical support from the physicochemical principles governing binding of drug to a receptor. It predicts a hyperbolic relationship between the drug concentration and response which is linear. When dose increases, at certain point the response become saturated and reaches a plateau. All biological responses must reach a maximum and this is a significant prediction of the E_{max} model. A recent study has shown the application of using similar expanded model to examine patient characteristics that predict adverse anticholinergic-type events in older people (Salahudeen et al., 2015).

The E_{max} model:

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

or

$$E = \frac{E_{max} \times D}{ED_{50} + D} \tag{9}$$

where E_{max} is the maximal effect of a drug, C is the drug concentration or D is the drug dose, and EC_{50} is the drug concentration resulting in half maximal effect or ED_{50} is the dose that produce 50% of the maximal effect.

It can be seen from the above equation that EC_{50}/ED_{50} can be directly linked to the kinetics of the drug with the receptor Kd. E_{max} model can be further improved by incorporating E_0 (baseline estimate in the absence of a drug) which account for the baseline physiological conditions such as blood pressure baseline.

The E_{max} model with the baseline of the system E_0:

$$E = E_0 + \frac{E_{max} \times C}{EC_{50} + C}$$

or

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D} \tag{10}$$

4.3. Sigmoid E_{max} model

In addition, many drugs seem to have a steeper relationship of concentration and effect so that a smaller change is required. These steeper relationships can be described by the sigmoidal E_{max} model (Eq. (11)) (Goutelle et al., 2008). This model is an extension of E_{max} model that takes into account for multiple drug binding sites at the same receptor which introduces the Hill coefficient γ to define s-shaped asymptotic behaviour in the model. In fact, 'Hill coefficient' should be referred to as γ if it is an integer that is mechanistically derived based on the theory of allosteric binding of multiple ligands to the same receptor site (Hill, 1910). But, in a modelling perspective it can be estimated as an empirical exponent that is allowed to take any positive value.

The sigmoidal E_{max} model is as follows:

$$E = E_0 + \frac{E_{max} \times C^\gamma}{EC_{50}^\gamma + C^\gamma} \tag{11}$$

The sigmoid E_{max} model was first introduced in 1910 by a physiologist named Hill to explain the association of oxygen with haemoglobin (Hill, 1910). He noted that it was steeper than the simple binding predictions of the E_{max} model when he adds an exponential parameter to the effective concentration, EC_{50}. This equation has been further applied to PDs by Wagner (1968). Mathematically, this equation is an extension of the E_{max} model by adding the Hill coefficient (γ). Hill coefficient determines 'steepness' of the effect versus concentration curve to be steeper (if values of γ is greater than one) or shallower (if values of γ is lower than one). Fig. 3 shows the sigmoid E_{max} model with different values for the Hill coefficient and the consequent effect on the shape of the fractional effect vs. concentration curve.

4.4. Competitive binding model

The interaction between two and three ligands (drugs) at the same receptor binding site can usually be described by a competitive ligand binding model. The term 'competition' in this scenario usually denotes the antagonism in which two ligands are proficient of binding to the same receptor site. The competitive binding models use a labelled concentration of ligand in the presence of various unlabelled ligand concentrations (known as the competitor or inhibitor) and measure binding at equilibrium. The concentration of displacing ligand increase is equivalent to change in the apparent value of the dissociation constant (Kd). Competition binding models are useful for determining whether the unlabelled ligand has affected the ligand's affinity for the receptor and compare the affinities of several ligands for the same receptor to find total versus free concentration or dose for the labelled ligand and unlabelled competitor or inhibitor.

Competitive binding model for two-ligand and one binding site is as follows:

$$E = E_{max} \times \left(\frac{[LR]}{[R]_{total}} \right) = E_{max} \times \left(\frac{[L]_1[R]}{[R] + [L]_1[R] + [L]_2[R]} \right)$$

$$E = E_{max} \times \left(\frac{\frac{[L]_1[R]}{Kd_1}}{[R] + \frac{[L]_1[R]}{Kd_1} + \frac{[L]_2[R]}{Kd_2}} \right)$$
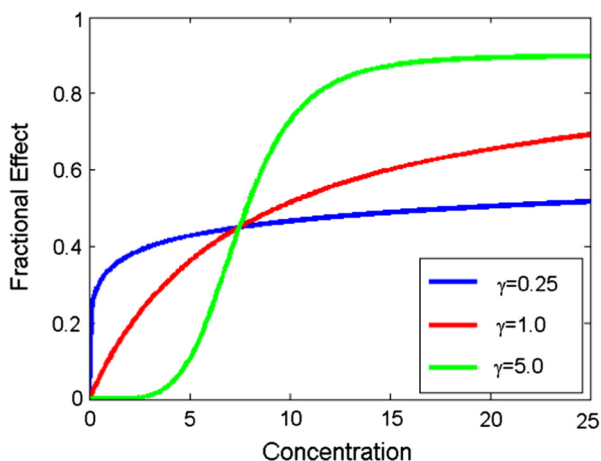

Figure 3 The sigmoid E_{max} model with different values for the hill coefficient.

$$E = E_{max} \times \left(\frac{\frac{[L]_1}{Kd_1}}{1 + \frac{[L]_1}{Kd_1} + \frac{[L]_2}{Kd_2}} \right)$$

$$E = E_{max} \times \left(\frac{[L]_1}{[L]_1 + Kd_1 \left(1 + \frac{[L]_2}{Kd_2} \right)} \right) \quad (12)$$

where the dissociation constants Kd_1 and Kd_2 represent the first and second ligands $[L]_1$ and $[L]_2$.

Competitive binding model for three-ligand and one binding site is as follows:

$$E = E_{max} \times \left(\frac{[L]_1}{[L]_1 + Kd_1 \left[1 + \frac{[L]_2}{Kd_2} + \frac{[L]_3}{Kd_3} \right]} \right) \quad (13)$$

whereas the dissociation constants Kd_1, Kd_2 and Kd_3 represent the first, second and third ligands.

The aforementioned Eq. (12) will change eventually with the following conditions in terms of fractional occupancy:

If the same drug has given twice:
Same drug given twice:

$$f_{occupancy} = \frac{(L_1 + L_2)}{Kd + (L_1 + L_2)} \quad (14)$$

Similar potency of drugs occurs when, $ED_{50(1)} = ED_{50(2)}$
Drugs with same potency:

$$f_{occupancy} = \frac{(L_1 + L_2)}{Kd + (L_1 + L_2)} \quad (15)$$

Different potency occurs when, $ED_{50(1)} \neq ED_{50(2)}$
In this model, the equation is rewritten as;
Drugs with different potency:

$$f_{occupancy} = \frac{\frac{L_1}{Kd_1} + \frac{L_2}{Kd_2}}{\frac{L_1}{Kd_1} + \left(1 + \frac{L_2}{Kd_2} \right)} \quad (16)$$

The above equation is normalised for L_1 and L_2 and works even in the absence of any one drug. If we consider, $\frac{L_1}{Kd_1}$ as 'α' and $\frac{L_2}{Kd_2}$ as 'β', the above equation would change to:
Simplified version of Eq. (16):

$$f_{occupancy} = \frac{\alpha + \beta}{(1 + \beta) + \alpha} \quad (17)$$

Competitive binding model for more than one ligands and two binding sites is depicted below;
Competitive binding model for two-ligand and two binding sites:

$$E = \left(\frac{E_{max(1)} \times [L]_1}{[L]_1 + Kd_{1(1)} \left(1 + \frac{[L]_2}{Kd_{2(1)}} \right)} + \frac{E_{max(2)} \times [L]_1}{[L]_1 + Kd_{1(2)} \left(1 + \frac{[L]_2}{Kd_{2(2)}} \right)} \right) \quad (18)$$

where the dissociation constants $Kd_{1(1)}$, $Kd_{1(2)}$ and $Kd_{2(1)}$, $Kd_{2(2)}$ for the first and second ligands $[L]_1$ and $[L]_2$ with respect to the two receptor binding sites having $E_{max(1)}$ and $E_{max(2)}$.

Competitive binding models become more complex when the number of receptor binding sites and number of ligands increases.

4.5. Applications of E_{max} model

- E_{max} model possess a widespread application in the field of functional receptor pharmacology.
- Re-arrangement of E_{max} equation can lead to a prediction of target concentration or dose to reach the target effect, $C = \frac{EC_{50} \times E}{E_{max} - E}$.
- A useful model and a common descriptor for characterising dose-response relationship.
- Dose-response of drug is monotonic and can be modelled as continuous and also can account for a range of different dose levels.
- E_{max} model can be a useful tool in determining the 'optimal' dose and the 'minimal effective dose'.
- E_{max} model-based drug development and therapeutics is promising and can explain the mechanism-based PD modelling.
- E_{max} model can predict a zero-dose baseline effect (E_0) in the absence of no drug and also follows the 'law of diminishing returns' at higher doses.
- It is straight-forward to implement in any modelling software (for example; NONMEM, S-plus, SAS Proc NLIN).

A recent study employed an E_{max} model to examine patient characteristics that predict adverse anticholinergic-type events in older people (Salahudeen et al., 2015). The authors explored the influence of patient characteristics using a nonlinear model (E_{max}), to test whether they (1) increased the risk of adverse events independent of anticholinergic burden (i.e. they pose a risk even in the absence of anticholinergic burden) by adding patient characteristics to the baseline (β_0), (2) increased the risk of adverse effects in the presence of anticholinergic burden (i.e. an overall greater effect is seen with anticholinergic burden) by including interaction of patient characteristics with E_{max} or (3) increased the apparent potency of the anticholinergic burden (i.e. greater effects were seen for a given anticholinergic burden value) by adding a patient characteristics to ED_{50} that provides 50% of the maximal effect.

5. Population analysis

For predicting a drug response in a given population, a model must be designed that it best describes the relationship between dose, time and effect and also accounts for the variability (e.g. inter/intra-individual) of dose-response among all individuals in the population. However, it is known that data collected from the clinical studies may have huge variability and uncertainty. There are three main population analysis approaches: the two stage approach, Naïve pooled data approach, and a full population based approach.

The two-stage approach is relatively a simple method to estimate the between subject variability (BSV) in addition to the population mean parameters and the data for each individual are analysed separately. In this approach, it requires rich data for each individual and the obtained BSV tends to be inflated compared to the true variability. Naïve pooled approach analyses data as if it has arisen from just a single individual and the whole dataset is pooled together to estimate just one set of parameter values for the model. With regard to computational effort, this method is the least complex approach and does not

account for the BSV. A full population based approach delivers an accurate and precise way of quantification of the population mean parameter estimates including residual unexplained variance (RUV) and BSV for a given dataset. Besides, this approach is able to handle sparse data. The dose-response relationship can be best described using a population model (full population approach) that correlates each individual in that given population. The mean model parameters (RUV and BSV) are also estimated from the population analysis.

5.1. Nonlinear mixed effects modelling

Nonlinear Mixed Effects Modelling (NLME) also called hierarchical nonlinear models is useful for estimating population parameters and variance, accounting for both random and fixed effects Davidian and Giltinan (1995). NLME modelling approach is used routinely to model sparse data particularly relevant to pre-clinical or clinical trials where complete patient data may not be available. The NLME models for fixed effects, where variables in the sample (or population) do not change over time, and for random effects where variables are time-dependent. A full population based approach is desired for precise quantification of the population mean parameter estimates including RUV and BSV. However, the NLME is a reasonable approach for parameter estimation with sparse data and data on some variables are incomplete. NMLE can analyse and quantify data pertaining to a series of individuals with differences in drug response, examining multiple covariates (such as age, sex, ethnicity, comorbidity index, weight, and organ function) that explain the variability between individuals to some extent and also help in dose-individualisation.

Several software programs are available for nonlinear mixed effects modelling, but among these the widely used one is NONMEM (Nonlinear Mixed Effects Modelling) (ICON Development Solutions, Ellicott City, MD), which is a computer program, written using Abbreviated Fortran, designed to fit general statistical (nonlinear) regression models to data for the analysis of population pharmacokinetic and PD information (Beal et al., 2009). NONMEM uses maximum likelihood approach to estimate the population parameters such as mean, RUV, and BSV.

Maximum likelihood estimation (MLE) approach considers that the parameters in the model are fixed but with unknown quantities (values) by maximising the probability of obtaining the observed data. The MLE is an indispensable approach in non-linear modelling assuming the distribution of data is not normal. MLE is employed routinely in inferential statistics, and in well-known model selection criteria such as Akaike information criteria (AIC) and Bayesian information criteria (BIC) use MLE. NONMEM allows for 'mixed effects modelling' by accounting for both unexplainable inter-individual and intra-individual effects (random effects), as well as measured concomitant effects (fixed effects) for proper modelling of such data.

6. Concluding remarks and future direction

Receptor binding has revolutionised the field of drug discovery (Leysen et al., 2010). Drug-receptor binding profiles of certain central nervous system (CNS) drugs such as antipsychotics and antidepressants have revealed that they bind to multiple

receptors within the normal dose range (e.g., atypical antipsychotic clozapine binds to more than twenty receptors and all of them could result in therapeutic response) (Leysen, 2002, 2004). Application of the binding model may help understand relative effect of these responses. Current research on central nervous system focuses on *ex vivo* autoradiography using measured fractional receptor occupancy in the brain by the drugs that are administered to animals. It allows to identify the dose-range of a drug at which the central receptors are occupied and are beneficial to determine the delivery of drugs into the CNS (Leysen et al., 2010).

Ligand binding model can incorporate multiple drugs affinity to the receptor till the receptor occupancy is saturated and exhibits maximum threshold effect. Once after saturation of these receptors, there will be no further pharmacological response. Also, the model would be helpful in predicting the relative effect of single or multiple medicines.

For ligand binding models, the term *fractional occupancy* used to best describe the fraction of receptors occupied at a particular ligand concentration. It is stated that the *fractional occupancy = occupied binding sites/total binding sites*, which means that the effect of a drug should depends on the fraction of receptors that are actually occupied. So, the measured effect would depend on the fractional occupancy. If the measured effect is maximum (where almost 100% of the receptors are occupied) and a known fractional occupancy, the effect for any given concentration of a ligand could be determined.

In the future, network-based systems pharmacology models could be an effective way of understanding drug-related adverse effects (Berger and Iyengar, 2011). The advantages of network-based systems pharmacology models comprise the following: increase in drug efficacy, regulation of the signalling pathway with multiple channels, increase in drug efficacy, increase in the success rate of clinical trials, and decrease in the costs of drug discovery (Wu et al., 2013). Network pharmacology models make two main approaches in the drug development process. One is to establish a pragmatic network model and predict the drug target based on public databases or available data of earlier researches. The second approach is to reconstruct a ''drug target disease'' network prediction model using the high-throughput screen technology and bioinformatic methods. In this approach, the mechanism of drugs in the biological network could be analysed by comparing the interaction between the drug and the model. The emphasis on mechanistic models may open new opportunities for clinical researchers to rationalise drug therapy in clinical practice.

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