Drug Metabolism and Pharmacokinetics in Drug Discovery: A Primer For Bioanalytical Chemists, Part II

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The rapidly evolving drug discovery process requires the analytical chemist to design new analytical procedures that maximize the efficiency of lead compound selection. Pre-clinical pharmacokinetics studies are an essential tool to weed out failures early on in the discovery process. Part I of this two-part series described the fundamentals of drug metabolism. This article, Part II of the series introduces the analytical chemist to the pharmacokinetics aspects and prediction of pharmacokinetic parameters from in vitro drug metabolism data.

The first in this series of articles examined general aspects of drug metabolism (1). In Part II we will discuss the general pharmacokinetics principles and parameters and how some of those parameters can be estimated from the in vitro drug metabolism data we discussed in Part I.

Pharmacokinetics (PK) is the quantitative study of the time course of drug absorption, distribution, metabolism and elimination (ADME). This also includes the relationship of ADME to the intensity and time course of therapeutic and toxicological effects of drugs or chemicals. Pharmacokinetic data is very useful in optimization of the dosage form design and establishing the dosage interval. Pharmacodynamics (PD) of a drug describes the relationship between the dose and the pharmacological effect at the site of action.

When a drug is administered, it distributes rapidly from its administration site into the systemic blood circulation. The drug is distributed between plasma and red blood cells in the blood. Low molecular weight drugs can easily reach the extracellular fluid of every organ in the body rather quickly. Most lipophilic drugs cross cell membranes into the intracellular fluid and distribute in various tissues. Since distribution of a drug is rapid and reversible, changes in the concentration of a drug in plasma reflect the changes in the concentration of the drug in other tissues.

Pharmacokinetic Profile

Intravenously administered drugs distribute very rapidly throughout the body without an absorption step. For other routes of administration, absorption needs to be considered. When the drug reaches the systemic circulation it can undergo both elimination and distribution. Elimination can be by metabolism or by excretion. Elimination processes are irreversible and responsible for the removal of the drug from the body. Each of these processes is associated with a rate constant. Rate constants associated with distribution are much greater than the elimination rate constants. Rates of these processes govern the plasma drug concentrations at any given time. A plot of plasma drug concentration versus time is known as a pharmacokinetic profile. An example is shown in **F1**.
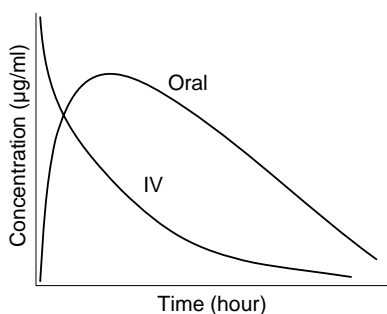
Elimination of a drug at equilibrium generally follows first order kinetics. The elimination rate decreases as the drug concentration decreases and this relationship is given by **Eq.1**, where K is the first order rate constant.

$$dC_p/dt = -KC_p \qquad (1)$$

Integration of **Eq.1** gives

F1

Schematic representation of pharmacokinetic profiles for intravenous and oral doses.
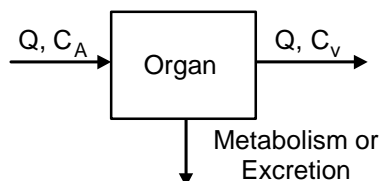
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Estimation of AUC for a first order PK profile.
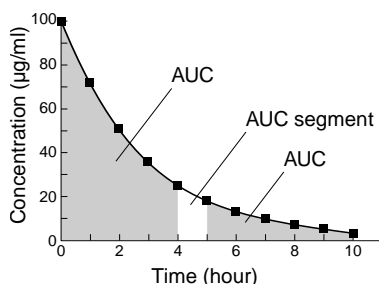
F3

Schematic illustration of organ clearance. C_A - Arterial drug concentration, C_V - venous drug concentration.

$$C_p(t) = C_p(0)*e^{-Kt} \quad (2)$$
$$\log C_p(t) = \log C_p(0) - Kt/2.303 \quad (3)$$

A semi-logarithmic plot of $\log C_p(t)$ vs. time will be linear. Extrapolation to time, t=0 will give the initial plasma concentration $C_p(0)$ and the slope will correspond to the elimination rate constant.

From the PK profile several important pharmacokinetic parameters such as area under the curve (AUC), bioavailability, clearance, and apparent volume of distribution can be estimated.

Pharmacokinetic Parameters

Area under the curve (AUC)

Estimation of AUC is required to determine some pharmacokinetic parameters. AUC is the area under a plot of drug concentration vs. time and given by **Eq. 4** as follows.

$$AUC = \int_0^\infty C dt \quad (4)$$

The most common method of estimating AUC is to use the trapezoidal rule where the concentration-time curve is considered as a series of trapezoids and estimate the total area of all the trapezoids (**F2**).

Area under the first moment curve (AUMC)

AUMC is the total area under the first moment curve. First moment curve is obtained by plotting concentration-time versus time. AUMC can be expressed as

$$AUMC = \int_0^\infty tC \, dt \quad (5)$$

Half-life

Half-life ($t_{1/2}$) of a drug is the time required to reduce the drug concentration by half. This can be estimated from **Eq. 3** for a first-order process and can be expressed as

$$t_{1/2} = 0.693/K \quad (6)$$

Since half-life is a quantitative parameter of the presence of the drug in the body, it is generally used as a guide to determine the dosing interval of a drug. The common practice is to administer a drug once every half-life.

Volume of Distribution

The drug distribution is certainly not even throughout the body at equilibrium. Some sites such as central nervous system or brain are weakly accessible to a certain drug, whereas some tissues may have strong affinity for the drug. Regardless of this uneven distribution, at equilibrium the drug concentration in plasma (C_p) is proportional to the amount of the drug administered (dose) intravenously. When plasma concentration changes linearly with the dose, the drug is known to have linear pharmacokinetics. The relationship between the dose and the plasma concentration can be expressed by the simple equation, **Eq. 7**.

$$V = IV \, dose/C_p(0) \quad (7)$$

The proportionality constant V is called the apparent volume of distribution. It is independent of the drug concentration and is a characteristic of the drug. The parameter V is not a true physiological volume but is a measure of protein binding of the drug. When drugs are highly bound to plasma proteins, V is small and results in high plasma concentrations of the drug. For basic drugs that are preferentially bound to extravascular sites, V is large and plasma concentrations are low, making quantitative examination of the drug more difficult. When drug binding to plasma proteins or tissues is negligible, V approximates the true volume of distribution which is related to the amount of body water.

Bioavailability

Bioavailability (F) of a drug refers to the rate and extent of absorption. Essentially it is the fraction of a dose that reaches the systemic circulation. Bioavailability of an intravenously administered drug is considered as unity because there is no absorption. For a drug administered by any other route, for example orally, bioavailability is given by **Eq. 8**.

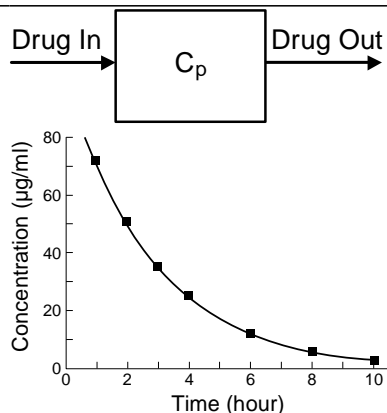$$F = \frac{Dose_{IV} * AUC_{oral}}{Dose_{oral} * AUC_{IV}} \quad (8)$$

When drugs are administered repetitively, the average plasma concentration depends on the amount absorbed from each dose and is independent of the rate of absorption. However, the rate affects the time course of plasma concentration at steady state. Drugs that are used for acute conditions such as pain must be absorbed rapidly to reach the systemic circulation and be effective immediately. Extent of absorption is a more important factor for drugs that are used for chronic conditions.

Clearance

Clearance (Cl) is one of the most useful pharmacokinetic parameters. Clearance of a drug from the body depends on the intrinsic ability of the organs such as the liver and kidneys to metabolize or excrete. Also, clearance is a function of the blood flow rate (Q) to these organs. Clearance by definition represents the volume of blood cleared through an organ in a unit time and can be expressed as
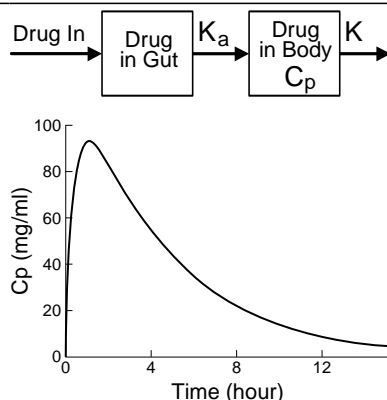
F4

One-compartment model after an intravenous administration.

To estimate clearance, the drug is given intravenously. Clearance cannot be estimated after an oral dose since the total dose doesn't reach the systemic circulation.

For certain drugs that get metabolized completely, renal clearance is negligible. In this case systemic clearance can be approximated to hepatic clearance (Cl_H) by liver. Hepatic clearance is given by:

$$Cl_H = Q_H * ER_H \quad (13)$$

where Q_H is the hepatic blood flow rate and ER_H is the hepatic extraction ratio. Hepatic clearance can be estimated approximately from in vitro metabolism kinetics data. This process of estimating in vivo PK parameters from in vivo data is discussed in detail in a later section.

The basic equations described so far to estimate PK parameters cannot be applied to all drugs. When a drug absorbs slowly, a significant fraction could be eliminated before reaching distribution equilibrium. Then the data cannot be expressed by a simple exponential relationship. In these situations complex mathematical models are required to express the PK profiles. PK data analysis using mathematical models is known as compartmental pharmacokinetics. In compartmental analysis the body is represented as a system of compartments. These compartments have no anatomical meaning. The rate of transfer between these compartments and the rate of elimination are assumed to be following first-order kinetics. The simplest PK models are briefly explained below.

time can be expressed by **Eq. 2**. All the PK parameters can be obtained as described before using the appropriate equations.

Extravascular Administration

Administration of a drug by all routes other than the IV route will involve an absorption step. The change in plasma drug concentration will now be a function of both the absorption rate and elimination rate. **(F5)** The relationship between plasma concentration C_p and time is given by **Eq. 14**

$$C_P = \frac{K_a FD}{V(K_a - K)}(e^{-Kt} - e^{-K_a t}) \quad (14)$$

where F is the fraction of the administered dose, D, that is absorbed after extravascular administration, K_a is the absorption rate constant and K is the elimination rate constant.

The rate constants can be estimated by fitting concentration-time data to **Eq. 14** using a non-linear least squares regression program. Several compartmental as well as non-compartmental pharmacokinetic software packages that fit the concentration-time data to various models are commercially available.

For most drugs the absorption constant is considerably larger than the elimination constant. When $K_a \gg K$, $e^{-k_a t}$ approaches zero and **Eq. 14** becomes,

$$C_P = \frac{K_a FD}{V(K_a - K)}(e^{-Kt}) \quad (15)$$

$$\log C_p = \log \frac{K_a FD}{V(K_a - K)} - \frac{Kt}{2.303} \quad (16)$$

This equation describes the elimination phase of the PK profile. A plot of Log C_p versus time is linear and elimination rate constant can be obtained from the slope.

F5

Schematic representation of one compartment model with absorption step after oral administration.

$$Cl = \text{Elimination rate} / C_A \quad (9)$$

where, C_A is the input concentration of the drug. Elimination rate is a function of the blood flow and the difference between input (arterial) and output (venous) concentrations of the drug. **(F3)** Elimination rate can be expressed as

$$\text{Elimination rate} = Q(C_A - C_V) \quad (10)$$
$$\text{Therefore, Cl} = Q(C_A - C_V)/C_A \quad (11)$$

The quantity, $(C_A - C_V)/C_A$ is defined as the extraction ratio (ER) of the organ. The single organ clearance can be extended to the total body clearance. Total or systemic clearance is the sum of all individual organ clearances that contribute to the overall elimination of the drug. The only organ clearance that can be measured experimentally is the renal clearance, clearance through the kidneys. Therefore, total clearance is evaluated from the PK data as shown in **Eq. 12**.

$$Cl = \text{Dose/AUC} \quad (12)$$

One-Compartment Model

Intravenous administration

The one-compartment model is the simplest and it represents the body as a single unit (**F4**). This model is applicable to drugs that distribute through the body rapidly.

According to this model, after an IV administration the drug is eliminated by first-order kinetics and the plasma concentration at any given

Two-Compartment Model

Intravenous administration

Following an intravenous injection, a drug will distribute rapidly into the peripheral tissues. During this distributive phase the drug concentration in tissues will decline
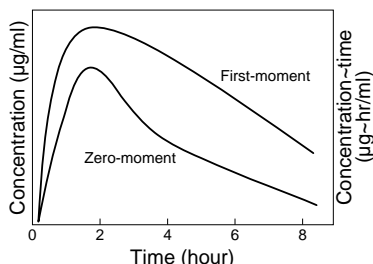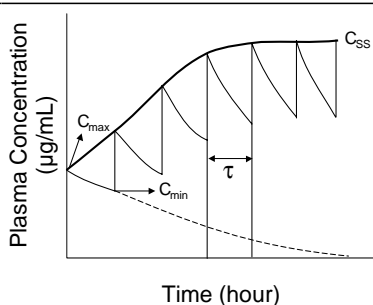
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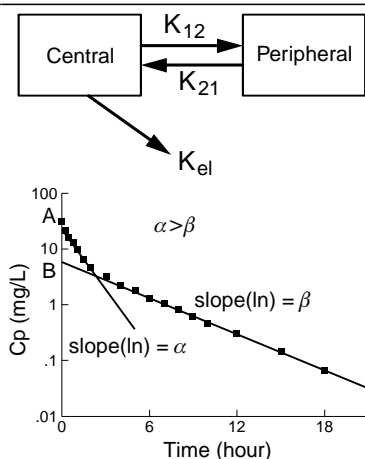
F7

AUC and AUMC are obtained from the concentration versus time curve and the concentration-time versus time curve.

F8

Drug accumulation after multiple IV dosing.

more rapidly than in the postdistributive phase. A bi-exponential decline can be associated with a two-compartmental model as shown in **F6**. Plasma concentration in this case is represented by the sum of the two exponential terms as in **Eq. 17**.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (17)$$

The constants α and β are obtained from the slopes. The constants A and B are intercepts obtained by extrapolating the linear portions to time t=0.

The relationships of A, B, α and β to the rate constants are shown below.

$$K_{21} = \frac{A\beta + B\alpha}{A + B} \quad (18)$$

$$K_{12} = \alpha + \beta - K_{21} - K_{el} \quad (19)$$

$$K_{el} = \frac{\alpha\beta}{K_{21}} \quad (20)$$

Also from these constants, PK parameters AUC, Cl and V_{ss} (volume of distribution at steady state) can be estimated as shown.

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad (21)$$

$$Cl = \frac{Dose_{iv}}{\dfrac{A}{\alpha} + \dfrac{B}{\beta}} = \frac{Dose_{iv}}{AUC} \quad (22)$$

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} \quad (23)$$

The models described above are the simplest pharmacokinetic models and are for single dose administrations.

The most common method of maintaining therapeutic plasma concentration of drugs is through the oral administration of multiple doses. Many drugs like antibiotics are given in multiple doses, although one important goal is to reduce that number to a minimum. Discussion of the complex pharmacokinetic models applicable for these cases would be beyond the scope of this article. More advanced mathematical treatment of PK data can be found in pharmacokinetic texts (2,3).

Non-Compartmental Analysis

Non-compartmental methods can be used to determine pharmacokinetic parameters without fitting the PK data to any specific compartmental model, assuming the data follow linear pharmacokinetics. Non-compartmental methods are based on the theory of statistical moments. AUC, or the total area under the curve of a drug concentration, versus time curve from time zero to infinity is called the zero moment. As mentioned before, AUMC, or the first moment, is the area under the curve of concentration-time (C_p*t) versus time curve from time zero to infinity (**F7**).

The non-compartmental pharmacokinetic parameters are represented in the following equations.

$$MRT = \frac{AUMC}{AUC} \quad (24)$$

MRT is the mean residence time. AUC and AUMC respectively can be obtained from **Eq. 4** and **Eq. 5**. The first order rate constant and half-life can be estimated from MRT as shown below.

$$K = 1/MRT_{iv} \quad (25)$$
$$t_{1/2} = 0.693 \, MRT_{iv} \quad (26)$$

Apparent volume of distribution at steady state, Vss is one of the important PK parameters and is given as

$$Vss = Cl \cdot MRT = \frac{Dose_{iv} * AUMC}{AUC^2} \quad (27)$$

Other PK parameters, volume of distribution, clearance and bioavailability etc. can be estimated from AUC and AUMC as same as for the compartmental treatment of data.

Non-compartmental analysis of the data allows the estimation of most of the basic kinetic parameters for characterizing the disposition of the drug. However, non-compartmental methods do not adequately treat non-linear cases. They do not describe the time course of drug in the blood (different half-lives, rate constants, etc). Compartmental methods partly depend on the experimental design. They provide an insight into understanding the mechanism of drug kinetics and dynamics. Both methods have their own merits.

During multiple dosing the drug concentration in plasma will increase during the dosing interval (τ) and reach a constant level as the number of doses increases. The plasma drug concentration during a dosing interval in the steady state is known as steady-state concentration (C_{ss}) as shown in **F8**.

Area under the curve and C_{ss} directly affect the pharmacological properties or side effects of a drug. Since body clearance (Cl) and hepatic bioavailability (F_H) control AUC and C_{ss}, it is important to know Cl and F_H values. Total body clearance is the sum of clearances from all organs that are connected through blood flow. The two major clearance terms are renal clearance and hepatic

clearance (Cl_H). The only organ clearance that can be determined directly in humans is the renal clearance. The hepatic clearance has to be predicted from animal or in vitro data. Scaling up of animal data to predict human hepatic clearance has not been very successful because of the inherent species differences. The alternative is to use in vitro drug metabolism data to predict the hepatic clearance.

The process of obtaining in vivo pharmacokinetic data from in vitro data is known as **in vitro-in vivo correlation**. Hepatic microsomes and hepatocytes are the main in vitro systems used for this purpose.

Enzyme Kinetics

The basis for the extrapolation of in vitro data to the in vivo case is the parameter intrinsic clearance (CL_{int}). Intrinsic clearance is solely a measure of enzyme activity and is

F9

Dependence of reaction velocity on the substrate concentration.

F10

Linweaver-Burk plot for enzyme kinetics.

F11

Schematic illustration of in vitro-in vivo correlation process.

independent of other physiological factors such as the liver blood flow or drug binding in the blood. CL_{int}, is the proportionality constant between rate of metabolism and the drug substrate concentration at the enzyme site (C_s) as shown in *Eq. 28*.

$$\text{Rate of metabolism } (V_0) = CL_{int}\, C_s \quad (28)$$

Rate of metabolism is generally defined by the Michaelis-Menten enzyme kinetics relationship, as in *Eq. 31* where K_m is the Michaelis-Menten constant and V_{max} is the maximum rate of metabolism. K_m is the substrate concentration at half the maximum velocity (V_{max}).

$$V_0 = \frac{V_{max} * C_s}{K_m + C_s} \quad (29)$$

When the drug concentration is much smaller than K_m ($K_m >> C_s$) under linear conditions CL_{int} can be expressed as,

$$CL_{int} = V_0 / C_s = V_{max}/K_m \quad (30)$$

In this case CL_{int} is a constant and independent of the drug concentration.

To calculate CL_{int}, enzyme activity should be measured at time points over the time period that V_0 is linearly changing with concentration (*F9*).

Enzyme activity is measured by measuring the increase in metabolite and/or the decrease in parent drug.

Michaelis-Menten equation (*Eq. 29*) can be rearranged to give Lineweaver-Burk equation as shown in *Eq. 31*.

$$\frac{1}{V} = \frac{K_{max}}{V_{max}} \frac{1}{C_s} + \frac{1}{V_{max}} \quad (31)$$

The parameters K_m and V_{max} are obtained from a plot of $1/V$ vs. $1/C_s$ as shown below in *F10*.

In Vitro-In Vivo Correlation

Systemic clearance of a drug that is eliminated by metabolism in the liver is a function of the hepatic blood flow and the intrinsic clearance of the liver. Disease conditions

of the liver and administration of drugs that are cytochrome P450 enzyme inducers or inhibitors can therefore influence the systemic clearance.

Once the CL_{int} is obtained from Michaelis-Menten constants, CL_{int} can be scaled to in vivo clearance, $CL_{in\ vivo}$ using scaling factors. The scaling factors depend on the in vitro system used. For liver microsomes and hepatocytes, the scaling factors include microsomal protein content, number of hepatocytes per gram of liver, liver weight and liver blood flow.

The relationship between hepatic clearance, hepatic blood flow and unbound fraction of the drug cannot be determined without knowing the actual circulating drug concentration and C_s. Since these cannot be quantitatively measured, a liver model is needed to estimate the intrinsic in vivo clearance, $CL_{in\ vivo}$. From $CL_{in\ vivo}$ the hepatic clearance of the drug for a particular pathway can be estimated. Essentially, the purpose of the liver model is to convert the scaled kinetic data from liver concentrations to plasma/blood concentrations. The extrapolation of in vitro data to obtain in vivo data is schematically outlined in *F11*.

Several liver models have been proposed (4). However, a discussion on liver models would be beyond the scope of this article. According to the most commonly used venous equilibration model, the hepatic clearance is given as

$$Cl_H = \frac{Q_H * f_u * Cl_{int,invivo}}{Q_H + f_u * Cl_{int,invivo}} \quad (32)$$

where f_u is the fraction of unbound drug in the blood (5).

To obtain useful in vivo clearance values, the in vitro data must be obtained under proper conditions. For example, the drug concentrations used for in vitro incubations must be physiologically relevant. Enzyme kinetics data must be obtained under linear conditions with respect to the enzyme concentration and incubation time.
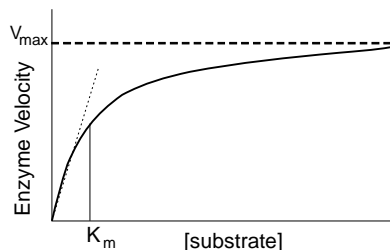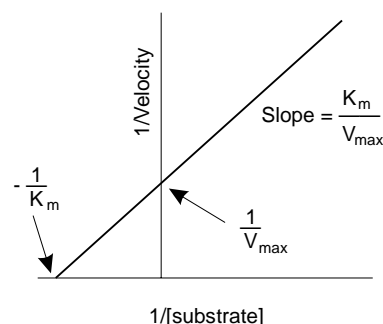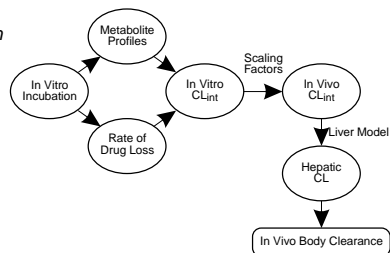
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Using in vitro models to study human drug metabolism as a part of the drug discovery and drug development process is expanding. The prediction of in vivo hepatic or metabolic clearance based on drug metabolism data using human recombinant cytochrome P450 isozymes seems to be possible. The usefulness of in vitro systems will, however, depend largely on the availability and quality of the human in vitro systems. Cell culture systems with single human drug-metabolizing enzymes expressed are commercially available and already in wide use.

As a massive amount of information on the human genome becomes available, and drug discovery process continues to evolve due to the technological advances in synthesis and screening of hundreds of thousands of new drug candidates, the analytical chemist will be challenged to keep up with the accelerated pace of drug discovery. Familiarization with the pharmacokinetics and drug metabolism principles will help prepare the analytical chemist to face this challenge. The goal will continue to be to determine the least desirable compounds and cast them aside as soon as possible to reduce R&D expenditure, allowing more funding to support the most satisfactory candidates through to the market.

References

1. C. Gunaratna, Current Separations, 19, (2000) 17-23.

2. Pharmacokinetics, M. Gibaldi, D. Perrier, Eds. 2nd edition, Marcel Dekker, Inc., New York, (1982).

3. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, J. Gabbrielsson, D. Weiner, Eds. Swedish Pharmaceutical Press, Stockholm, Sweden, (1994).

4. G. R. Wilkinson, Pharmacol. Rev. 39 (1987) 1-47.

5. J. B. Houston, Biochemical Pharmacology 47 (1994) 1469-1479.