Subcellular Pharmacokinetics: One-compartment Model

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Subcellular pharmacokinetics aims at descriptions of the time courses of drug concentrations in individual physically distinct subcellular compartments, as are the extra- and intracellular aqueous phases and membranes. Full descriptions work well for cell suspensions and other small biological systems with a few subcellular compartments. For tissues, organs, and organisms, containing a huge number of subcellular compartments, the modeling principles for large systems (time hierarchy, lumping) are applied to arrive at simplified descriptions that have complexity commensurate with available experimental information. Here, the derivation of a one-compartment model at the subcellular level is described in detail. The resulting description provides the expressions for pharmacokinetic parameters (the distribution volume, clearance, and the elimination rate constant) as related to body composition and drug properties. The principles of subcellular pharmacokinetics have been used in teaching pharmacokinetics in both the PharmD and graduate programs and contributed to a better understanding of drug disposition.

INTRODUCTION

Classical pharmacokinetics(1) deals with phenomenological descriptions of the time courses of drug concentrations in tissues, organs and organisms. The biological system is represented by a variably structured set of kinetically distinct macroscopic compartments, which may correspond to blood plasma or organs, but in general have very loosely defined morphological basis. Drug disposition is expressed in terms of the space-averaged drug concentrations in the non-homogeneous compartments. Though these concentrations are of great value for the practical purposes of chemotherapy, the analysis of the drug effects at the molecular level requires the use of actual drug concentrations in immediate surroundings of the receptors. Classical pharmacokinetics is mainly interested in the fates of a single drug in the body, therefore the aspect of time has been stressed in its development. The phenomenological parameters in the classical pharmacokinetic models may not bear any obvious relation to body composition and drug properties. These relations are sought additionally from partial models for individual pharmacokinetic quantities.

Subcellular pharmacokinetics tackles the problem of drug disposition from the opposite direction. The biological system is represented by a large set of subcellular compartments, as are extra- and intracellular aqueous phases and the bilayer parts of membranes. This representation is physically correct because: (i) drug concentrations usually differ dramatically in flanking subcellular compartments due to different solvation properties of water and nonpolar phases, and (ii) the compartments are small enough for the drug molecules to achieve practically homogeneous distribution in their whole volume within a fraction of a second(2). The detailed models, however, can be mathematically challenging for some scenarios. However, for a mono-exponential concentration/time dependence, the one-compartment model, the derivation is sufficiently simple and, I believe, instructive, to be taught in basic pharmacokinetic courses. The derivation of the one-compartment model at subcellular level is given below. The resulting descriptions of pharmacokinetic parameters are discussed with regard to their dependencies on body composition and drug properties.

MODEL CONSTRUCTION

If the drug concentrations in plasma or other body fluids decrease mono-exponentially in time, absorption is apparently absent or very fast. Since distribution is usually faster than elimination, this situation can be envisaged as follows (Figure 1). Drug molecules are quickly transported into all accessible parts of the biological system and subsequently eliminated by excretion and metabolism. The equilibria for ionization, ionpairing, protein binding, and membrane/water partitioning of the drug molecules (Figure 2) are achieved practically immediately. No kinetics need to be considered for these processes.

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they are completely characterized by the respective equilibrium constants. As drug concentrations in individual subcellular compartments decrease due to elimination, the equilibria are disturbed and the drug molecules are quickly released from the membranes or from complexes with proteins or other body constituents. These releases cause the decrease in drug concentrations to be slower than that caused solely by elimination. The extent of the releases depends on the composition of the biological system (content of proteins, phospholipids, lipids, and ions, pH of aqueous phases) and also on the affinities of drug molecules to body constituents.

**Fast Processes**

**Transport.** The nonionized drug molecules are transported much faster than the ionized species (3). For fast distribution, the concentration of free nonionized drug molecules \( c_A \) will be identical in all aqueous phases. This concentration will be considered as a reference concentration, for which the time course will be analyzed. The concentrations of all other molecular species (ionized, bound to proteins, accumulated in membranes) that are in fast multiple equilibria with the free drug molecules in the same aqueous compartment will be at any moment proportional to \( c_A \) and can be expressed as the product of \( c_A \) and a proportionality factor. The proportionality factors for individual processes are derived in the following sections. Membrane accumulation, although intimately associated with transport, will be treated later because it requires a description of ionization and ion pairing. Although the derivations are made in a comprehensive way, simplified scenarios (e.g., mono-valent acids and bases only considered, ion-pairing neglected) resulting in simpler expressions can be more suitable for teaching.

**Ionization.** The factor \( d_{ij} \) (from dissociation) relates the concentrations of the species ionized (dissociated) to the \( j \)-th degree in the \( i \)-th aqueous compartment to the concentration of the non-ionized molecules. The loss of the first proton of the drug molecule \( D \) (ionization to the first degree) in the \( i \)-th aqueous phase

\[
D^0 \overset{K_{a1}}{\longrightarrow} H^+ + D^-^1
\]

is characterized by the dissociation constant

\[
K_{a1} = \frac{[H^+][D]_{i1}}{c_A}
\]  

(2)

The square brackets denote concentrations. The charge of the drug molecule is expressed by the subscript 1. The proportionality factor \( d_{i1} \) relating the concentration of the uni-valent anions to that of the nonionized species in the \( i \)-th compartment is obtained from the definition of the dissociation constant \( K_{a1} \) (Eq. (2)):

\[
d_{i1} = \frac{[D]_{i1}}{c_A} \frac{K_{a1}}{[H^+]_{i1}}
\]  

(3)

The proton concentration is assumed to be independent of the interaction with the drug molecules, since the intracellular aqueous compartments are buffered sufficiently with regard to low drug concentrations. The bi-valent anions originate in the ionization reaction

\[
D^{i-1} \overset{K_{a2}}{\longrightarrow} H^+ + D^{i-2}
\]  

(4)

and the proportionality factor is

\[
d_{i2} = \frac{[D]_{i2}}{c_A} \frac{K_{a1}[D]_{i1}[H^+]_{i1}}{[H^+]_{i1}^2} = \frac{K_{a1} K_{a2}}{[H^+]_{i1}^2}
\]  

(5)

The second equality comes from the substitution of \( c_A \) from Eq. (3) and the third equality from the definition of the dissociation constant to the second degree characterizing the equilibrium shown in scheme (4). For ionization to the \( j \)-th degree

\[
D^{i+j-1} \overset{K_{aj}}{\longrightarrow} H^+ + D^{i+j}
\]  

(6)

the proportionality factor relating the concentration of the species with the charge \( j \) to that of the non-ionized molecules in the \( i \)-th compartment can be derived by induction as
where \( j = 1, 2, \ldots, D \), with \( D \) being the maximal ionization (dissociation) degree, \( \text{sgn} = -1 \) for anions and \( \text{sgn} = 1 \) for cations (i.e., \( \text{sgn} \) has always the value of the charge sign of the resulting ion). By definition, \( d_0 = 1 \).

**Ion Pairing.** Formation of ion pairs leads to charge neutralization of the ionized species and is important mainly for membrane accumulation. For simplicity, only the 1:1 ion-pairs will be considered. The interaction of drug molecules ionized to the \( j \)-th degree (\( D_i \)) with the \( k \)-th counter-ion of opposite charge \( j \) (\( C_k \))

\[
D^j + C^j_k \xrightarrow{K_{Cjk}} DC_{jk}
\]

is characterized by the association constant \( K_{Cjk} \):

\[
K_{Cjk} = \frac{[DC]_{jk}}{[D]_i [C]_j}
\]

The proportionality factor \( g_{ijk} \) relating the concentration of the \( k \)-th ion pairs \( DC_k \) to the concentration of drug molecules ionized to the \( j \)-th degree in the \( i \)-th compartment is then

\[
g_{ijk} = \frac{[DC]_{ijk}}{[D]_{ij}} = K_{Cjk} [C]_{ijk}
\]

Concentration of the counter-ions \( C \) is assumed to be constant during the process due to low drug concentrations. The ion-pair concentration is then

\[
[DC]_{ijk} = g_{ijk} [D]_{ij} = c_A g_{ijk} d_{ij}
\]

with \( d_{ij} \) defined by Eq. (7).

**Protein Binding.** Binding of the drug ionized to the \( j \)-th degree to the \( k \)-th protein binding site in the \( i \)-th compartment is described by the association constant \( K_{ijk} = [DP]_{ijk} /[P]_{ijk} [D]_i \), where the square brackets denote equilibrium concentrations, \( D \) stands for drug and \( P \) for protein binding sites (total number \( S \)). Since drugs are applied in low concentrations, a negligible fraction of each binding protein will be modified by binding. The concentration of free protein binding sites \( [P]_{ijk} \) can be approximated by the total concentration of the binding sites \( s_{ijk} \). The concentration of the drug that is bound to the \( k \)-th protein is \( [DP]_{ijk} = [D]_{ij} K_{ijk} s_{ijk} \). The bound concentration of the drug molecules in the \( j \)-th ionization degree in the \( i \)-th compartment is simply

\[
[DP]_{ij} = [D]_{ij} \sum_{k=1}^{s} K_{ijk} s_{ijk} = c_A d_{ij} b_{ij}
\]

The \( k \)-summation goes through all protein binding sites that are available to the drug molecules ionized to the \( j \)-th degree in the \( i \)-th compartment. The concentration \( [D]_{ij} \) is expressed as the product of the free nonionized drug concentration \( c_A \) and the proportionality factor \( D_i \) as defined in Eq. (7). The proportionality factor \( b_{ij} \) is equal to the \( k \)-summation in the second equality of Eq. (12) and relates the free and bound concentrations of the drug molecules in the \( j \)-th ionization degree that are present in the \( i \)-th compartment.

**Membrane Accumulation.** The lipid core of the bilayer seems to have practically identical solvation properties in all membranes. Fatty acyl chains of phospholipids, although containing varying fraction of double bonds, probably do not create a substantial degree of physical variation in individual membranes. For drugs that do not bind to the bilayer/water interface and only accumulate in the core of the bilayer, the partition equilibrium is described by the apparent core/water partition coefficient \( P_i \) that accounts for partitioning of both nonionized and ionized molecules (subscript \( j \) indicates the \( j \)-th ionization degree) as well as for partitioning of ion pairs (subscript \( k \) indicates the \( k \)-th counter ion):

\[
P_i = \frac{c_A + \sum_{j=1}^{D} \left( [D]_{Aij} + \sum_{k=1}^{C} [DC]_{Aijk} \right)}{\sum_{j=1}^{D} \left( [D]_{Mij} + \sum_{k=1}^{C} [DC]_{Mijk} \right)}
\]

Subscripts indicate the place for the concentrations (\( M \) the membrane core, \( A \) the aqueous phase). Each of the present molecular species has its own partition coefficient that is given as the ratio of the species' concentrations in the core and in the water phase surrounded by the membrane. The membrane concentration of each species can be expressed as the product of the partition coefficient and the aqueous concentration of that species:

\[
P_i = \frac{c_A + \sum_{j=1}^{D} \left( [D]_{Aij} + \sum_{k=1}^{C} [DC]_{Aijk} \right)}{\sum_{j=1}^{D} \left( [D]_{Mij} + \sum_{k=1}^{C} [DC]_{Mijk} \right)}
\]

The proportionality factors relating the free nonionized drug concentration to the concentrations of ionized species (\( d_{ij} \)) and ion pairs (\( g_{ijk} \)) were defined above. Their use leads to

\[
P_i = \frac{c_A + \sum_{j=1}^{D} \left( c_A d_{ij} + \sum_{k=1}^{C} c_A g_{ijk} d_{ij} \right)}{\sum_{j=1}^{D} \left( c_A d_{ij} + \sum_{k=1}^{C} c_A g_{ijk} d_{ij} \right)}
\]

The \( k \)-summation goes through all protein binding sites that are available to the drug molecules ionized to the \( j \)-th degree in the
The core/water partition coefficient of nonionized molecules (P) is identical for all membranes. The partition coefficients of drug molecules ionized to the j-th degree (P_ij) and ion pairs with the k-th counter-ion (P_ijk) depend on the composition (pH, concentration of counter-ions) of the aqueous phase (subscript i) that is surrounded by the respective membrane. Eq. (15) can frequently be simplified: (i) the partition coefficients of the ionized species P_ij will be much lower than the partition coefficients of the ion pairs (P_ijk) and can be neglected, and (ii) ion pairing in water can be neglected (all g_ijk = 0 in the denominator).

The partition coefficient is an additive-constitutive property that can be expressed as the product of the hypothetical partition coefficients of non-interacting substructures(4). Applying this principle, the partition coefficients of ionized molecules and ion pairs can be expressed as the product of the partition coefficient of nonionized molecules (P) and a factor R that has specific values for ionizable groups and counter-ions:

\[
P_i = P_0 \prod_{j=1}^{D} \left[ 1 + \sum_{k=1}^{C} \left( R_{ij} + \sum_{k=1}^{C} R_{ijk} g_{ijk} \right) \right]
\]

The value of the apparent partition coefficient P_i depends on the composition (pH, concentration of counter-ions) of the aqueous phase that is surrounded by the respective membrane and also on the nature of the ionizable group. This dependence is described by the accumulation factor a_i. The value of a_i is at least one and grows as more ionized molecules and ion pairs partition into the membrane.

Partitioning of amphiphilic drugs is more complicated and requires the use of the interface/water partition coefficient. These drugs, however, will be transported slowly(5) and their behavior will seldom conform to the one-compartment model.

Elimination Rate

The overall loss of the drug amount A due to elimination is the summation of the losses by metabolism and excretion. Let us focus first on metabolism. We need to summarize the drug losses in all metabolizing cells. In each cell, the free drug concentration decreases due to spontaneous and enzymatic reactions that may have different rate constants (r and e, respectively) for nonionized and ionized molecules:

\[
\frac{dc_i}{dt} = \sum_{j=0}^{D} c_{ij} (r_{ij} + e_{ij}) = c_A \sum_{j=0}^{D} d_{ij} (r_{ij} + e_{ij})
\]

The j-summation runs through all ionization degrees starting with 0 for the neutral molecules (d_0 = 0). The enzymatic reactions are assumed to be of first order (i.e. far from saturation) due to low drug concentrations. The term e = c_E v_m / K_m, where c_E is the enzyme concentration, v_m is the maximum rate, and K_m is the Michaelis-Menten constant. The term r is equal to the product of the rate constant and the concentration of the reactant (water, free amino acids, glutathion, etc.) that is assumed to be constant during the metabolic reaction. The total metabolic rate is the i-summation of metabolic rates for all W aqueous phases:

\[
\frac{dA_m}{dt} = c_A \sum_{j=1}^{W} V_{Ai} \sum_{j=0}^{D} d_{ij} (r_{ij} + e_{ij}) = C_{l_m} c_A
\]

Here V_{Ai} is the volume of the i-th aqueous phase and C_{l_m} is metabolic clearance.

Excretion clearance C_l is difficult to analyze at the subcellular level because of reabsorption that proceeds from the filtrate, the volume of which is being greatly reduced in the process. The overall elimination rate is then

\[
\frac{dA}{dt} = (C_l + C_{l_m}) c_A = C_l c_A
\]

For integration of Eq. (19), the same variable is needed on its left-hand and right-hand sides.

Distribution

For fast distribution, the concentration of free nonionized molecules is equal to c_A in all aqueous compartments and equal to c_M in all membranes. The definition of the partition coefficient P can be used to find the relation between the total drug amount A and the free nonionized aqueous drug concentration c_A:

\[
P = \frac{c_M}{c_A} =
\]

The nonionized drug amount in the membranes was expressed as the difference between the actual drug amount, drug amount in membranes in the form of ions and ion pairs and drug amount in water. The volumes V of the aqueous phases are indicated by A in the subscript; the volumes of the membranes by M in the subscript. Separating c_A, we get

\[
c_A = \frac{A}{V_{Df}}
\]

The denominator is equal to the volume of distribution defined with regard to the free nonionized drug molecules (V_{Df}), a.k.a. unbound volume of distribution. The first two terms in the denominator represent the volume of membranes corrected for accumulation and the third term represents the volume of the aqueous phases corrected for ionization and protein binding (see below). If Eq. (21) is applied to tissues, the drug amount in tissues can be expressed as the product of V_{Df} and c_A or the product of the volume of tissues V_T and the average drug concentration.
in tissues \((c_T)\). Since the fraction unbound in tissues is \(f_{UT} = c_A/c_T\), the unbound volume of distribution is \(V_{DF} = V_T/f_{UT}\).

**Integration**

Division of Eq. (19) by \(V_{DF}\) provides

\[
\frac{dc_A}{dt} = Cl \frac{c_A}{V_{DF}} = k_e c_A
\]

where \(k_e\) is the elimination rate constant. Integration of Eq. (22) results in the description of the time course of the free nonionized aqueous concentration \(c_A\) corresponding to the one-compartment model:

\[
c_A = \frac{A(0)}{V_{DF}} e^{-kt}
\]

The concentration \(c_A\) is, at any moment, identical in all aqueous phases. The total drug concentrations in individual phases are different, due to ionization and protein binding in the aqueous phases and to partitioning of ions and ion pairs in membranes. The actual concentration of any molecular species in the given compartment can be easily calculated as the product of \(c_A\) and the respective proportionality factor.

**MODEL APPLICATION**

**Distribution Volume**

The distribution volume in Eq. (23) or a similar description of the one-compartment model is related to the concentration on the left side. In practice, the distribution volume \(V_D\) is related to plasma drug concentration \(c_P\) that comprises free and protein-bound drug molecules present in all ionization degrees:

\[c_P = c_A \sum_{i=0}^{D} d_{ij} (1 + b_{ij}) = c_A / f_{UP} \]

Eq. (24) was derived using Eqs. (7) and (12). Subscript \(P\) indicates plasma. Fraction unbound in plasma \((f_{UP})\) will be used later. If Eq. (23) is multiplied by \(V_{DF}\), the product on the left side \((c_AV_{DF})\) is equal to the drug amount that can also be expressed using the product of the plasma concentration and \(V_D\) resulting from Eq. (24). Then \(V_D\) can be calculated as \(V_{DF}c_A/c_P\) using Eqs. (16), (21) and (24):

\[V_D = \frac{D}{\sum_{j=0}^{D} d_{ij} (1+b_{ij})} \]

The rightmost i-summation in the numerator contains also the term for plasma as one of the aqueous phases. Let us number plasma as the aqueous phase number one \((i = 1)\). If the term for plasma is separated, Eq. (25) can be rewritten as:

\[V_D = V_P + \sum_{i=1}^{M} V_{Mi} + \sum_{i=2}^{W} V_{Ai} \]

Eq. (26) can be rearranged into a well-known expression \(V_D = V_P + V_{DF}f_{UP}/f_{UT}\) that results from mass balance for the drug amount. For this purpose, Eqs. (21), (24), and the
accompanying texts can be used.

For fast distribution, body fat has the same role as membranes: it serves as depot in which the drug molecules accumulate. Therefore, the fat volume can be added to the volumes of membranes \( V_{ij} \) in Eq. (26). This substitution provides a model-based relation for adjustment of the distribution volume in fat content, interspecies scaling, and population pharmaco-kinetics.

Eq. (26) can also be used to obtain a model-based relation for correlation of the distribution volumes with drug properties. The ionization factors \( d_{ij} \) contain the ionization constants. The accumulation factors \( a_i \) contain the ion-pairing association constants comprised in the factors \( g_{ik} \). The core/water partition coefficient \( P \) and the protein association constants in terms \( b_{ij} \) can be substituted by relations to the 1-octanol/water (or other reference) partition coefficient \( P_{ow} \). The relations usually have the form \( Y=\alpha P_{ow}^\beta \) where \( Y \) is either \( P \) or \( b_{ij} \). Hypothetical dependences of the distribution volume on drug lipophilicity (\( \log P_{ow} \)) for varying fat content as described by Eq. (26) with specified values of parameters are shown in Fig. 3. With increasing lipophilicity, the distribution volume initially increases due to increased membrane accumulation and later decrease because the plasma protein binding prevails for very lipophilic drugs. The magnitude of the changes in the distribution volume is proportional to the fat content. In this example, it was assumed that the coefficient \( \beta \) in the above relation increases in the order \( b_0 < P < b_0 \). The shape of the curves will change if these proportions are altered. In similar way, the dependence of the distribution volume on many other factors can be quantitatively analyzed. Eq. (26) has been used to correlate the distribution volumes with the reference partition coefficients(6) and for formulation of quantitative structure-time-activity relationships(7).

**Clearance**

As Eq. (18) shows, metabolic clearance can be regarded as a weighed sum of the first order rate constants of metabolism. The weighing factors correspond to the volumes of individual phases where the metabolic reactions take place that are multiplied by the dissociation factors \( d_{ij} \) if the reaction affects ionized molecules. It is also obvious that the overall clearance is the sum of clearances of individual processes. Eq. (18) can also be used as a basis for interspecies scaling and population pharmaco-kinetics.

**Elimination Rate Constant**

The elimination rate constant in the one-compartment model is the ratio of clearance and the volume of distribution. Inspection of Eq. (22), with Eqs. (18) and (19) defining clearance, shows that the overall elimination rate constant is a weighed sum of individual rate constants. The weights are the products of the individual volumes and the dissociation factors \( d_{ij} \), divided by the distribution volume. The rate constants can directly be summed up only if the metabolic processes take place in the same phase. Otherwise, the rate constants of metabolic processes need to be scaled before the summation is performed.

**TEACHING APPLICATION**

The one-compartment model treated at subcellular level has been taught in pharmacokinetics courses in both the PharmD and graduate programs. Its use seemed to contribute to the students' understanding of drug disposition, as reflected in 3-5 percent improvement in the grades of the tests of similar quality. The more difficult pharmacokinetic situations were explained with the help of a web-based simulator. The students liked the teaching innovation as evidenced by a significant increase in the students' rating of the course. The rating increased in most categories by 0.8-0.9 points on the five-point scale what is more than would be expected on the basis of improved grades.

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**References**