Transit Compartments versus Gamma Distribution Function To Model Signal Transduction Processes in Pharmacodynamics

YU-NIEN SUN AND WILLIAM J. JUSKO*

Contribution from the Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.

Received October 28, 1997. Final revised manuscript received March 10, 1998. Accepted for publication March 12, 1998.

Abstract  □ Delayed effects for pharmacodynamic responses can be observed for many signal transduction processes. Three approaches are summarized in this report to describe such effects caused by cascading steps: stochastic process model, gamma distribution function, and transit compartment model. The gamma distribution function, a probability density function of the waiting time for the final step in a stochastic process model, is a function of time with two variables: number of compartments $N$ and the expected number of compartments occurring per unit time $k$. The parameter $k$ is equal to $1/\tau$, where $\tau$ is the mean transit time in the stochastic process model. Effects of $N$ and $k$ on the gamma distribution function were examined.

The transit compartment model can link the pharmacokinetic profile of the tested compound, receptor occupancy, and cascade steps for the signal transduction process. Time delays are described by numbers of steps, the mean transit time $\tau$, and the amplification or suppression of the process as characterized by a power coefficient $y$. The effects of $N$, $\tau$, and $y$ on signal transduction profiles are shown. The gamma distribution function can be utilized to estimate $N$ and $k$ values when the final response profile is available, but it is less flexible than transit compartments when dose–response relationships, receptor dynamics, and efficiency of the transduction process are of concern. The transit compartment model is useful in pharmacokinetic/pharmacodynamic modeling to describe precursor/product relationships in signal transduction process.

Introduction

Pharmacological effects of endogenous compounds (hormones) and exogenous substances (therapeutic drugs) are often produced via signal transduction processes. These cascade responses are initiated by the interactions between hormone or drug molecules and their specific receptors. For some transduction pathways, second messengers (such as cyclic AMP (cAMP), calcium ion, etc.) are involved in the processes. These messengers play important roles in regulating the cascade steps in multiple processes leading to their pharmacological end points.

There are two major classes of receptors involved in signal transduction processes: cell membrane receptors and cytosolic/nuclear receptors. For example, insulin interacts with the insulin receptor located on the membrane of its target cells. After the activation of the receptor tyrosine kinase, second messengers such as phospholipases (1,4,5-inositol trisphosphate (IP$_3$), diacylglycerol (DAG), inositol glycan, etc.) and nucleotide cyclases (cAMP, cGMP) are induced and subsequently regulate the calcium carrier, the calcium ion channel, Na–K-ATPase, and other physiological functions on the affected cell membrane. There are membrane receptors regulated by GTP–binding protein activation including epinephrine, glucagon, and serotonin receptors.

On the other hand, steroid receptors including those for glucocorticoids (GR), sex hormones, thyroid hormone, and vitamin D are cytosolic/nuclear receptors. For example, free corticosteroids diffuse into their target cells and bind to the receptor located in the cytoplasm. The steroid–receptor complex is translocated into the nucleus and then interacts with the glucocorticoid responsive elements (GRE) on DNA. As a result, transcription/translation processes for certain proteins and enzymes are controlled by a receptor/gene-mediated mechanism of corticosteroid action.

Since multiple processes and cascade steps are involved in signal transduction, theoretically there should be a time constant or delay between each cascade step. In fact, such delay effects for pharmacodynamic end points have been seen in different systems. For example, the distribution of lag times for the calcium signaling response to f-Met-Leu-Phe observed in human neutrophils is about 531 ms. Much larger delayed effects on induction of hepatic tyrosine aminotransferase (TAT) mRNA and TAT activity by corticosteroids have been shown in rats given an iv bolus dose of methylprednisolone. The TAT mRNA profile is an intermediate step in the pharmacokinetic/pharmacodynamic (PK/PD) model for the receptor/gene-mediated corticosteroid actions.

Owing to technical difficulties and limitations for research on the molecular/cellular level, pharmacodynamic response vs time profiles of intermediate steps in signal transduction are often difficult to obtain. Similar situations can be encountered in a pharmacokinetic analysis when different steps of drug distribution and biotransformation occur. To characterize such delayed effects, “stochastic” models with transit compartments and transit times are often employed. For example, the kinetic profiles of bromsulfalein in plasma and bile in rabbits were described by a four-compartment model using liver as the transit compartment between two sampling sites. Berman et al. applied a compartmental model to describe iodine kinetics in humans including different release phases of thyroid hormones and the storage.

In addition to the stochastic models, waiting times in a Poisson process/gamma distribution have been applied in pharmacokinetic and pharmacodynamic analyses. Our modeling of corticosteroid effects have employed a transit compartment approach. These models were also applied to account for movement of methotrexate through the biliary system and for the kinetics of Cr$^{51}$- or In$^{111}$-labeled human platelets.

Although these modeling techniques have been applied for about three decades, in depth descriptions and their interrelationships are needed to better understand the advantages and disadvantages when these models are employed. In this report, we summarize the theoretical background for three approaches to model transduction in...
Figure 1—Top: Stochastic process model with N compartments with a mean transit time $\tau$. Bottom: Transit compartment model for signal transduction processes. Symbols are defined in the text.

PK/PD analyses: stochastic process model, gamma distribution function, and transit compartment model. Simulations for each method are presented. The similarities and differences of time delays in these processes are discussed.

Theoretical Section

**Stochastic Process Model**—A typical stochastic process model for precursor/product relationships is shown in Figure 1. Considering that N compartments or events are involved in the transaction processes, and each transaction between two compartments has a same mean transit time $\tau$, then amount vs time profiles in each compartment can be described as follows:

\[
\frac{dA_1}{dt} = -\frac{1}{\tau} A_1 \tag{1.1}
\]

\[
\frac{dA_2}{dt} = \frac{1}{\tau} A_1 - \frac{1}{\tau} A_2 \tag{1.2}
\]

\[\vdots\]

\[
\frac{dA_N}{dt} = \frac{1}{\tau} A_{N-1} - \frac{1}{\tau} A_N \tag{1.N}
\]

where $A_N$ is the amount of drug inside the Nth compartment and $1/\tau$ is equal to a exiting rate constant from each compartment.

**Poisson/Gamma Distribution Function**—Under the conditions for the stochastic process model as described above, consider the following two random variables:

- $X_i$: number of compartments in the transaction within a time interval of length $t$.
- $T_N$: waiting time for the Nth compartment to occur.

The probability of $X_i$ can be described by a Poisson distribution function as follows:

\[
f_i(N) = P(X_i = N) = \frac{(kt)^N e^{-(kt)}}{N!} \tag{2}
\]

where $k$ is the expected number of compartments occurring per unit time and equal to $1/\tau$. Considering the probability of “the waiting time for the Nth compartment to occur is greater than $t$” is equivalent to the probability of “the transaction process has less than $N$ compartments involved within the period of length $t$”:

\[
P(T_N > t) = P(X_i < N) \tag{3}
\]

The cumulative distribution function of the waiting time $T_N$ within the time interval 0 to $t$ ($G_N(t)$) can be described as follows:

\[
G_N(t) = P(T_N \leq t) = 1 - P(T_N > t) = 1 - P(X_i < N) = 1 - \left[ f_i(0) + f_i(1) + \ldots + f_i(N - 1) \right] = 1 - \left[ 1 + kt + \frac{(kt)^2}{2!} + \ldots + \frac{(kt)^{N-1}}{(N-1)!} \right] e^{-(kt)} \tag{4}
\]

the probability density function of the waiting time $T_N$ ($g_N(t)$) is a gamma distribution function:

\[
g_N(t) = \frac{dG_N(t)}{dt} = \frac{k^N t^{N-1}}{(N-1)!} e^{-(kt)} \text{ for } t > 0 \tag{5}
\]

where

\[
\int_0^\infty g_N(t) \, dt = 1 \tag{6}
\]

$G_N(t)$ is a cumulative probability function with the range from 0 to 1, while $g_N(t)$ has a unit of 1/time.

Equation 5 has been applied in both pharmacokinetic and pharmacodynamic analyses. A single gamma distribution function vs parallel models were utilized to characterize the time delay for pharmacokinetic profiles of several markers between the administration site (proximal pulmonary artery) and sampling site (femoral artery) in dogs.\(^\text{11}\) Parameters $N$ and $k$ were estimated when the following equation was applied to describe marker concentration vs time from the sampling site:

\[
C(t) = \text{AUC} \times g_N(t) \tag{7}
\]

where AUC is the area under the concentration curve of the tested marker.

A similar approach was applied to characterize the calcium ion signaling response stimulated by f-Met-Leu-Phe in neutrophils.\(^\text{12}\) Assuming the signal transduction processes in each step has a same transittime $\tau$, the probability density function of the delay time for the response. Using the experimentally measured mean lag time as 531 ms, Hallett and Pettit determined the optimized “number of transduction steps $N$” is 6 by minimizing the $\chi^2$ value for the gamma distribution function.

**Transit Compartment Model for Signal Transduction**—A linkage between pharmacokinetics and pharmacodynamics can be built in the transit compartment model for the signal transduction process as shown in Figure 1. Assuming the concentration of the tested compound has a monoexponential disposition function, the concentration vs time profile after an iv bolus dose can be described as follows:

\[
D = D_0 e^{-\lambda_2 t} \tag{8}
\]

where $D$ is the drug concentration at time $t$, $D_0$ is the initial drug concentration, and $\lambda_2$ is a first-order elimination rate constant. Assuming that signal transduction is initiated by a reversible ligand–receptor binding, the drug concentration vs time profile (eq 8) provides the driving force for signal transduction processes as follows:

\[
\frac{dR}{dt} = -k_{on}(D)(R) + k_{off}(DR) \tag{9}
\]

\[
\frac{dDR}{dt} = k_{on}(D)(R) - k_{off}(DR) \tag{10}
\]
where \( R \) is the free receptor concentration, \( k_a \) and \( k_d \) are the association and dissociation constants for drug and receptor, the dissociation equilibrium constant \( K_D \) is equal to \( k_d/k_a \), and \( DR \) is the drug–receptor complex. Assuming that the stimulation of the response is proportional to the drug–receptor complex, the effect can be described as follows:

\[
effect = \alpha(DR) = \frac{\alpha(R + DR)D}{D + K_D} = \frac{E_{max}D}{D + EC_{50}}
\]  

where \( \alpha \) is an intrinsic efficacy factor, \( E_{max} \) is the maximum effect representing the apparent efficacy, \( EC_{50} \) is an intrinsic potency factor which is equal to the drug concentration (D) required to produce 50% of the maximum effect (\( E_{max} \)). The Hill coefficient (\( \gamma \)) can be incorporated in eq 11 to describe different degrees of steepness for this sigmoid function.

Considering that a series of signal transduction processes are initiated by the drug–receptor complex (DR) as a biosignal, the cascade steps for signal transduction can be described using a transit compartment model as follows:

\[
\frac{dM_1}{d\tau} = \frac{1}{\tau_1} M_1 - \frac{1}{\tau_1} M_1
\]

\[
\frac{dM_2}{d\tau} = \frac{1}{\tau_2} M_2 - \frac{1}{\tau_2} M_2
\]

\[
\frac{dM_N}{d\tau} = \frac{1}{\tau_N} M_N - \frac{1}{\tau_N} M_N
\]

where \( N \) is the number of transit compartments involved in the transduction, \( DR \) is the drug–receptor complex described in eq 10, and \( \tau_i \) is the mean transit time for the transduction between transit compartment \( N = 1 \) and \( N \). These functions resemble that of the stochastic process model except for their starting points. For the stochastic model, compartment 1 is described by a monotonic decreasing function (eq 1.1). For the transit compartment model, \( M_1 \) is a function of \( DR \) (eq 12.1). To describe the amplification or diminution of the transduction process, a power coefficient \( \gamma \) can be included in any or any of the steps. The receptor/gene-mediated effects on TAT induction by corticosteroids has been modeled using this approach. A simplified signal transduction process involving three transit compartments can be described as follows:

\[
\frac{dM_1}{d\tau} = \frac{1}{\tau} DR - \frac{1}{\tau} M_1
\]

\[
\frac{dM_2}{d\tau} = \frac{1}{\tau} M_1 - \frac{1}{\tau} M_2
\]

\[
\frac{dM_3}{d\tau} = \frac{1}{\tau} M_2 - \frac{1}{\tau} M_3
\]

where \( \tau \) is a mean transit time which is identical for each of three steps, \( \gamma \) is the power coefficient, \( M_1 \) and \( M_2 \) are considered as second messengers in the transduction, and \( M_3 \) is a pharmacological end point initiated by DR and regulated by the intermediary processes.

### Methods

Simulations for the stochastic process model, gamma distribution function, and transit compartment model for signal transduction processes were performed using the ADAPT II program. To demonstrate the stochastic model, a 10-step transduction process (\( N = 10 \)) was assigned. Simulations for the stochastic process model (eqs 1.1–1.10), gamma distribution function (eq 6), and transit compartment model (eq 12.1) were performed using the ADAPT II program. The probability density function for the waiting time \( T_N \) (eq 5) was assigned. To study the effects of the numbers of transit compartments (\( N \)) and transistion time (\( \tau \)) on the gamma distribution function, four different \( N \) values (\( N = 2, 3, 5, \) and \( 10 \)) and two different transit times (\( \tau = 1 \) and \( 2 \) h) are simulated. The cumulative distribution function of the waiting \( T_N \) within the time interval 0 to \( t_i \) (\( G(t_i) \)) was obtained using eq 6.

### Results

**Stochastic Process Model**—Simulations for eqs 1.1–1.10 are shown in Figure 2. When the number of compartments is low (\( N = 2–5 \)), the amount vs time profile is asymmetric and skewed to the right. When \( N \) is increased, the maximum amount in the \( N \)th compartment (\( A_{N,max} \)) is decreased, and the curve moves towards the right. The AUC (area under the curve) values are identical for each of the \( N \) compartments (equal to 100 units, which is the starting value in compartment 1). The curve becomes more symmetric when \( N \) is increased.

**Gamma Distribution Function**—Simulations for the gamma distribution function (eq 5) are shown in Figure 3. Since this is a probability density function for a specific N...
and \( r \) in the stochastic process, the profile is identical to the amount vs time relationship in the stochastic process model after it is normalized by the AUC value. For example, when \( k = 1 \) h\(^{-1}\) (or \( r = 1 \) h), the gamma distribution function for the \( N \)th compartment is equal to the corresponding profile in Figure 2 normalized by 100 (AUC value). The gamma distribution function has the same properties as the stochastic process model: when \( N \) is small, the curve is asymmetric and skewed to the right; when \( N \) is increased, the curve becomes more symmetric, but the maximum density value becomes lower. However, the gamma distribution function (eq 5) can only describe the probability density as a function of time for the final step in the process.

The gamma distribution profiles for \( N = 2, 3, 5, \) and 10 when \( k = 1.0 \) and 0.5 h\(^{-1}\) (or \( r = 1 \) and 2 h) are shown in Figure 3. Their cumulative distribution profiles (eq 6), ranging from 0 to 1.0, are also shown as inserts. When the \( k \) value is decreased (or \( r \) is increased), all curves move toward the right, and the maximum density values become lower. The reason is that when "the expected number of compartments occurring per hour" is decreased, the waiting time for a specific compartment to occur will be increased. Since the maximum cumulative probability density is always equal to 1.0 and the distribution profile has been stretched to a longer period of time, the maximum density will be lower.

Transit Compartment Model for Signal Transduction Process—Simulations for the receptor-mediated signal transduction processes using transit compartments are shown in Figure 4–6. The pharmacokinetic profile has a monoexponential decline as described by eq 8 (Figure 4). For the receptor dynamics, the free receptor level \( R \) (eq 9) decreased to 0 immediately after the dose. Since the drug concentration is much higher than the free receptor, then almost all of the free receptors are bound to the drug molecules in the first 3 h. Free receptor regains its original value after the drug concentration declines to an insignificant level. Meanwhile, the drug–receptor complex level \( DR \) (eq 10) is increased to its maximum level soon after the dose and slowly returns to 0.

We assume that signal transduction is initiated by DR and is followed by inductions of second messengers \( M_1 \) and \( M_2 \). The final cascade step between \( M_2 \) and end point \( M_3 \) has an amplification/diminution factor \( \gamma \) involved. Figure 5 shows the effect of transit time \( r \) on the signal transduction profile when \( \gamma = 1 \). Similar to the results for the stochastic process model, the profiles for the early transit compartments (\( M_1 \) and \( M_2 \) in this case) are asymmetric and skewed to the right. \( M_3 \) is more symmetric than \( M_1 \) and \( M_2 \), but the maximum value is lower. When \( r \) is increased, all three curves (\( M_1 \), \( M_2 \), and \( M_3 \)) move toward the right.
since the waiting time becomes longer. However, the maximum values are decreased when the $\tau$ is increased.

The effect of the power coefficient $\gamma$ on the signal transduction profile when $\tau$ is equal to 2 h is shown in Figure 6. When $\gamma$ is equal to 0.8, induction of $M_3$ is lower than the unity condition ($\gamma = 1.0$), indicating that a suppression mechanism is involved between cascade step $M_2$ and $M_3$. When $\gamma$ is equal to 1.2, an amplification effect between cascade step $M_2$ and $M_1$ results in a higher $M_3$ induction profile. It should be noted that alteration of $\gamma$ does not change the degree of symmetry or the centroid of the $M_3$ curves. The $\gamma$ term functions similarly to the Hill coefficient in controlling the steepness of the response curves.

**Discussion**

Delays of pharmacodynamic responses occur because of different mechanisms. Sheiner et al. proposed the “link model” to account for distribution of drug between the plasma compartment and a hypothetical effect site. Four basic models of indirect pharmacodynamic responses were proposed using the sigmoid model (eq 11) as either a simulation or inhibition function affecting the production ($k_{in}$) or removal ($k_{out}$) of a mediator biosignal. Such models account for delays caused by the time needed for changes in $k_{in}$ or $k_{out}$ to be fully realized in the measured response. The transit compartment model is a legitimate tool to describe delays caused by the transduction processes between the biosignal and dynamic response.

We have summarized three approaches to characterize delays which may be encountered in kinetics or dynamic responses caused by cascading steps. The derivation of the gamma distribution function is based on a basic structure of a stochastic process model (Figure 1): a probability density function for the waiting time of $N$th compartment to occur assuming the time delay ($\tau$ value) between each compartment is identical. Once the time profile of the final step in the signal transduction processes can be determined experimentally, the gamma distribution function can be utilized to estimate how many cascade steps may be involved ($N$ value) and the waiting time between ($\tau = 1/k$) by nonlinear least-squares fitting. There are three assumptions to describe random effects in time for a Poisson process (eq 2): Independence (the numbers of events in nonoverlapping time intervals are independent), individuality (events occur singly rather than in pairs or groups), and homogeneity (events occur at a uniform rate over the entire time period). For signal transduction processes, the independence and homogeneity of nonoverlapping events may not always be true. For example, drug–receptor binding could be a rate-limiting step since the quantity of free receptor is limited compared to drug concentration. Therefore, the drug–receptor complex level is controlled by the drug kinetics, binding properties, and receptor dynamics in a nonlinear fashion. In addition, the maximum response can occur with partial receptor occupancy. The second messenger system becomes saturated before all receptors are occupied. When regulation of transcription/translation of enzymes or proteins is involved in signal transduction, the efficiency for the process can be limited by the availability of responsive elements on the target DNA or by the translation factory ribosome RNA. Since there is no linkage between drug concentrations and the gamma distribution function, it is difficult to use this approach to describe dose–response relationships in PK/PD modeling.

Some additional problems may be encountered when $N$ and $\tau$ values are estimated using the gamma distribution function. The AUC value has to be obtained before eq 7 can be applied in pharmacokinetic or pharmacodynamic data fitting. An extra error term in AUC estimation may be involved. Similar error will be introduced when the probability density function (eq 5) is converted from the raw data as an alternative approach. Additional error will be introduced in nonlinear least-squares fitting when $N$ is manipulated as an integer rather than a real number.

Although the starting points of the processes are different, functions for the transit compartment model resemble those of the stochastic process model. For the stochastic model, compartment 1 is described by a monotonically decreasing function (eq 1.1) as shown in Figure 2. For the transit compartment model, $M_1$ is a function of DR (eq 12.1). However, it is readily possible to use other kinetic or receptor processes as the initial steps in this type of model. The approach works well in a traditional model-building process. Although the numbers of transit compartments ($N$) have to be assigned arbitrarily for a specific fitting, one can test different $N$ values and select the best model by usual criteria for goodness-of-fitting such as visual inspection, residual sum-of-squares, and the estimator criterion value generated by the nonlinear regression.

For the transit compartment model, additional elements can be included if more cascade steps can be quantified. For example, in our second-generation model for the corticosteroid effects on TAT induction, a receptor-mediated mechanism followed by transit compartments were described in the PK/PD model. After quantitative Northern hybridization was available for measurement of mRNA levels, the TAT mRNA vs time profile was added as an intermediate step in TAT induction leading to our third-generation model. Considering the down-regulation of glucocorticoid receptor and its mRNA by corticosteroids, a fourth-generation model was proposed to describe additional determinations of corticosteroid action. Receptor down-regulation or up-regulation is often produced by its agonist or antagonist in receptor-mediated signal trans-
duction processes, and the transit compartment model can serve as a flexible means to deal with these issues. Other systems show similar phenomena. For example, insulin can down-regulate insulin receptor activity and its mRNA concentration.\textsuperscript{25} On the other hand, glucose can up-regulate both components.\textsuperscript{26} Therefore, it is important to include feed-back mechanisms in a optimized PK/PD model in order to describe dose–response relationships. Similar effects have been seen in β-adrenergic receptor dynamics. Isoprotenerol, a β-adrenergic agonist, can down-regulate receptor mRNA and receptor levels;\textsuperscript{27} propranolol, a β-adrenergic antagonist, can up-regulate the receptor density.\textsuperscript{28,29} These complexities can be included in the transit compartment model once the receptor dynamic profile is properly defined, and then sensitization or tolerance effects can be described. The model can be extended as well. Different ρ values for different cascade steps can be assigned in the transit compartment model if necessary. When amplification or suppression effects occur in signal transduction, the power coefficient γ can characterize the sharper or stunted nature of the curve and can be applied at any step. Thus the transit compartments may be empirical at first, but they closely resemble actual steps occurring in signal transduction and may mimic real phenomena.

In conclusion, the use of transit compartments with transit time ρ and power coefficient γ is helpful to describe delays occurring in pharmacodynamic responses. With efforts to better understand the mechanisms of drug actions and the improvement of experimental techniques, the transit compartment model is a useful means of characterizing precursor/product relationships in signal transduction processes.

References and Notes


Acknowledgments

This work was supported by Grant GM 24211 from the National Institute of General Medical Sciences, NIH.

J S970414Z