A Dispersion Model for Cellular Signal Transduction Cascades

Murali Ramanathan[1,2]

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Purpose. The purpose of this study was to evaluate the ability of the dispersion model to describe pharmacokinetic-pharmacodynamic data containing contributions from signal transduction cascades.
Methods. The partial differential equations and appropriate boundary conditions describing the dispersion model for signal transduction were obtained. Explicit analytical solutions to the dispersion equation were not available, and a numerical approach was necessary. Solutions were obtained by numerical inversion of the output Laplace transform. Generalized least square fitting was used to obtain parameter estimates for a variety of experimental data sets.
Results. The parameters of the dispersion model estimate the relative roles of diffusion, convection, and chemical reaction in signal transduction. The model is capable of describing messenger RNA and protein expression kinetics induced by drug action.
Conclusions. The dispersion model may find potential applications in pharmacokinetic-pharmacodynamic models involving delayed drug effects mediated by transcriptional changes.

KEY WORDS: pharmacodynamics; dispersion; pharmacokinetics; receptor.

INTRODUCTION

The tanks-in-series and gamma distribution models have been used to model signal transduction and transfer delays in a variety of systems ranging from calcium ion-mediated signaling in neutrophils (1), platelet dynamics in idiopathic thrombocytic purpura, and methotrexate pharmacokinetics in the liver (2,3). The transit compartment model has been coupled with traditional compartmental pharmacokinetic/pharmacodynamic modeling elements to describe the dynamics of gene expression events induced by corticosteroid treatment (4).

The tanks-in-series model assumes a series of well-stirred compartments with identical residence times (Model A, Fig. 1). Mathematically, the bolus or impulse response of the tanks-in-series model is an Erlang distribution, a special case of the Gamma distribution with shape parameters restricted to positive integer values. Statistically, the Erlang distribution represents the time required to perform a sequence of N tasks whose durations are identical, exponential probability distributions. For this reason, the Erlang/Gamma distribution has also been referred to as the stochastic model. In its simplest form, the tanks-in-series compartment model has only two parameters, N, the number of compartments, and τ, the residence time in each compartment, that can be robustly determined by computer fitting programs. The model readily describes behaviors ranging from mono-exponential decay (single well-stirred compartment) to the parallel tube model. The transit compartment model (Model B, Fig. 1) represents an extension of the tanks-in-series model in which one (or more) of the signaling compartments incorporates nonlinearity via a Hill exponent, h, and the input function is altered through non-equilibrium receptor binding.

Here, we evaluate the dispersion model (Model C, Fig. 1) as an alternative to the tanks-in-series, Gamma distribution, and transit compartment models for modeling signal transduction. Although dispersion models are widely used to measure deviations from ideal behavior in chemical reactors and have been used to examine residence time distributions in the liver and kidney, they have not been examined in the context of modeling signal transduction cascades.

DERIVATIONS AND RESULTS

The Dispersion Model

The governing equations of the dispersion model are obtained using a differential mass balance (Model C, Fig. 1) in which the signal or signaling molecule is present at concentration c, and is assumed to move at velocity u along the spatial direction x while undergoing axial dispersion with axial diffusion coefficient D, and elimination at a rate r. The derivation is available elsewhere (5,6) and the one-dimensional case is presented without extensive derivation as follows:

$$D\frac{\partial^2 c}{\partial z^2} - u\frac{\partial c}{\partial z} - r = \frac{\partial c}{\partial t} \tag{1}$$

The axial diffusional mechanism of signal transmission is represented by the first term, convective signal transmission by the second term and signal elimination (or generation) is represented in the third term. In the case of a signaling molecule eliminated via a first-order process with rate constant k:

$$D\frac{\partial^2 c}{\partial z^2} - u\frac{\partial c}{\partial z} - kc = \frac{\partial c}{\partial t} \tag{2}$$

The spatial dimension z is rendered non-dimensional using $Z = \frac{z}{L}$, where L is the length scale the signal has to travel and time is normalized using $T = \frac{tu}{L} = \frac{t}{\tau}$, where τ is the apparent mean residence time. The concentration c is normalized to a nondimensional concentration C using the dose and volume of distribution of the system.

$$D_N\frac{\partial^2 C}{\partial Z^2} - \frac{\partial C}{\partial Z} - R_N C = \frac{\partial C}{\partial T} \tag{3}$$

The nondimensional axial dispersion number $D_N = \frac{D}{uL}$, measures the rate of axial dispersion relative to the rate of convective signal transfer and correspondingly, the nondimensional number $R_N = \frac{kL}{u}$ measures the rate of elimination relative to the rate of convective signal transfer.

We assumed closed boundary conditions that do not al-

[1] Department of Pharmaceutical Sciences, 543 Cooke Hall, State University of New York at Buffalo, Buffalo, New York 14260-1200.
[2] To whom correspondence should be addressed. (e-mail murali@acsu.buffalo.edu)

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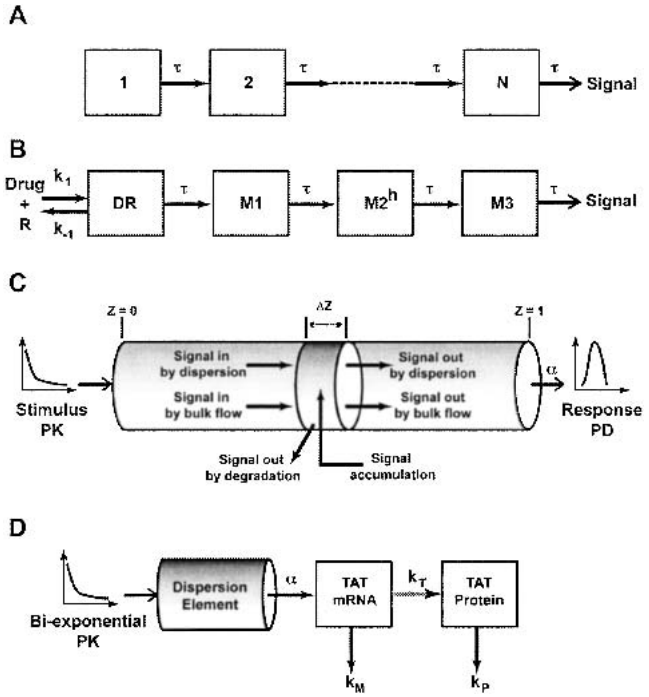

Fig. 1. Models of the tanks-in-series or Gamma distribution (A), transit compartment (B) and dispersion models (C and D). The gray line in D indicates that information not mass is transferred from the TAT mRNA to the TAT protein compartment. In A, N is the number of compartments and τ the residence time in each compartment. In B, *Drug* is the free drug concentration, *R* is the free drug receptor concentration, *DR* is the concentration of the drug-receptor complex, and k_1 and k_{-1} are the rate constants for receptor binding and drug-receptor complex dissociation, respectively. The *M1*, *M2*, and *M3* are concentrations of signaling intermediates, *h* is the Hill exponent and the τ is the mean residence time in each compartment. In Figure 1D, k_T, k_M, and k_P are rate constants for translation, mRNA degradation and protein degradation, respectively, and α is the "signal to transcript" conversion parameter.

low signaling molecules to leave the system. The mathematical description of the closed boundary conditions for $T \geq 0$ is:

$$\text{At } Z = 0,\ C - D_N \frac{\partial C}{\partial Z} = C_{in} \text{ and at } Z = 1,\ \frac{\partial C}{\partial Z} = 0 \qquad (4)$$

For a bolus input, C_{in} can be represented by $\delta(Z)$, the Dirac delta function. These boundary conditions are reasonable approximations for the many signaling cascades initiated by ligand binding to cell surface receptors and whose effects are mediated by the binding transcription factors to DNA. In the remainder of the paper, the linear, non-dimensional form of the dispersion model with closed boundary conditions is assessed.

Analysis of Dispersion Model Behavior

The behavior of the dispersion model is reviewed in (5,6). Notably, in the limit of $D_N = 0$, the signal is convected and behavior corresponding to the parallel tube model is approximated. For very large values of D_N, the behavior corresponds to a single well-mixed compartment.

Numerical Solutions for the Dispersion Model

Analytical solutions to the dispersion model are not available. The transfer function method was used for solving the dispersion model partial differential equation. The Laplace transform $L(s)$ of the bolus response of the dispersion model with closed boundary conditions is (5,6):

$$L(s) = \frac{4a}{[(1+a)^2\, e^{(a-1)/(2D_N)} - (1-a)^2\, e^{-(a+1)/(2D_N)}]} \qquad (5)$$

The *s* in the equation is the Laplace transform variable and $a = \sqrt{1 + 4D_N(s + R_N)}$. For a linear system, the product of Laplace transforms of the bolus response and the pharmacokinetic input represents the Laplace transform of the output. If $L_{Input}(s)$ and $L_{Output}(s)$ are the Laplace transforms of the input and output, respectively, then:

$$L_{Output}(s) = L_{Input}(s) \cdot L(s) \qquad (6)$$

The numerical inverse of the Laplace transform was obtained using a publicly available Fortran implementation of Weeks' method (7–10).

Simulations and Modeling

Model identification and simulation were conducted using Adapt II, a software package for pharmacokinetic and pharmacodynamic modeling on a Sun/Solaris cluster running the Unix operating system. The generalized least squares algorithm was used for parameter estimation. Figure 2A shows the bolus response of the dispersion model 4 values of D_N with $R_N = 0$. Figure 2B shows the effect of $R_N = 0.1$ on the bolus response.

Feasibility of Using the Dispersion Model for Pharmacokinetic and Pharmacodynamic Modeling

We used published data from Xu *et al.* (11) to assess the usefulness of the dispersion to describe *in vivo* gene-mediated drug effects. These authors modeled experimental pharmacokinetic and pharmacodynamic data for tyrosine aminotransferase (TAT) mRNA and protein induced by intravenous administration of 50 mg/kg methylprednisolone in adrenalectomized rats. The TAT mRNA and activity data in Fig. 2 of Reference 11 were obtained by scanning and image analysis.

The bi-exponential pharmacokinetic estimates of methylprednisolone ($C_{MP} = 56.27e^{-9.88t} + 14.49e^{-1.22t}$, where C_{MP} is the drug concentration in μg/mL) reported by Xu *et al.* (11) were used as an input to a combination model, Model D in Fig. 1. The model contained a dispersion element to describe signal transduction and the Hargrove-Schmidt model to describe mRNA and protein dynamics (12,13). A 'signal-to-transcript' conversion parameter, α, was used to linearly convert the dimensionless output signal from the dispersion model into a transcription rate for the Hargrove-Schmidt model. Although the α term is placed at the end of the dispersion element in Fig. 1, it also subsumes the proportionality constant that renders the input drug concentration nondimensional for the dispersion element. Because the dispersion model is linear, individual proportionality constants can be combined without affecting the overall result. The dimensionless reaction parameter, R_N, was set to zero because preliminary system identification runs and the simulation experiments in Fig. 2 also supported the possibility that R_N had a relatively small impact on the output of the dispersion model

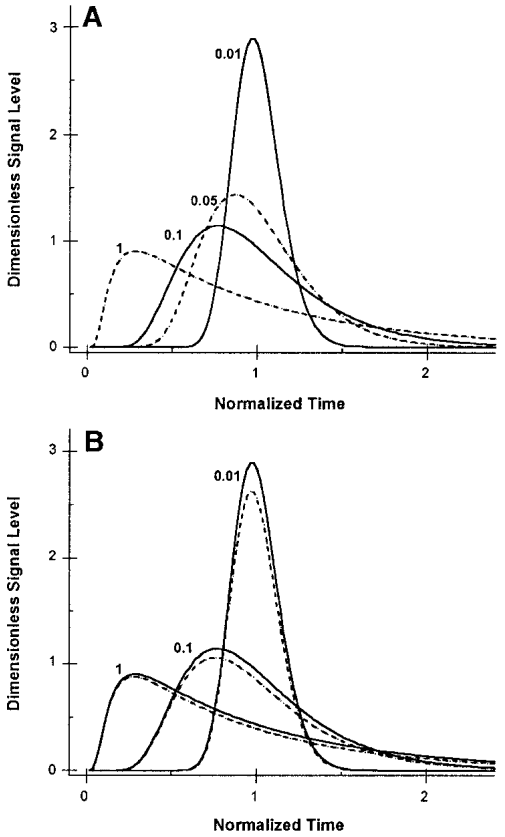

Fig. 2. Simulations of the dispersion model for a bolus or impulse input. A, Results for the 4 values of dimensionless dispersion number, D_N indicated. The dimensionless reaction number, R_N, was set to zero. The dashed lines are used with alternate lines for clarity. B, Effect of the dimensionless reaction number, R_N, on the output from the dispersion model. The D_N values examined are shown. The dashed lines are for $R_N = 0.1$ and the solid lines show the corresponding results for $R_N = 0$.

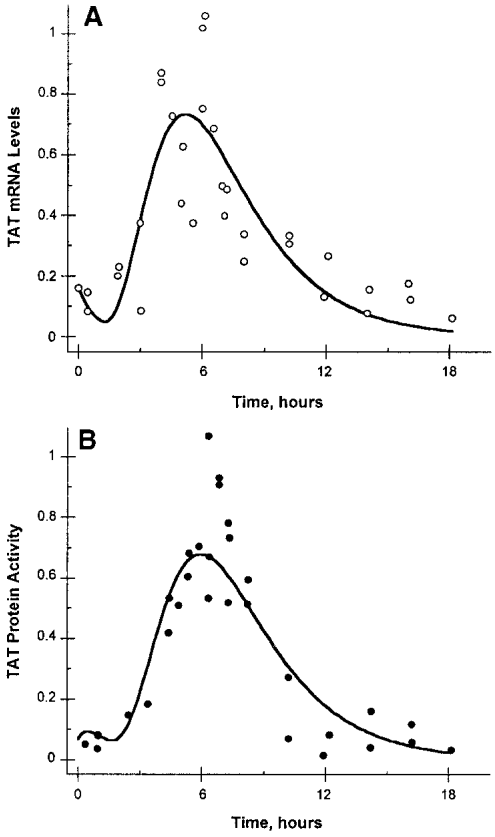

Fig. 3. The fit of the dispersion model combined with the Hargrove Schmidt model to the tyrosine aminotransferase mRNA (in pmol/g) and activity level data (Absorbance at 331 nm/min g) from Xu et al. (11).

over the parameter range of interest. The dispersion element contained three fitted parameters, α, D_N, and τ; the Hargrove-Schmidt model element contained three fitted parameters. The initial values for TAT mRNA and activity level were set to the baseline levels in control animals. The parameter estimates and the associated coefficients of variation are summarized in Table I. Figure 3 A and B, which overlays the fitted curves from the dispersion model (Model D, Fig. 1) on experimental data for TAT mRNA and activity, demonstrate that the dispersion model is capable of representing the pharmacodynamics of gene expression.

Table I. Parameter Estimates for TAT mRNA and Activity from the Dispersion Model

Parameter	Estimate	CV%
D_N	0.21	14
R_N	0	Fixed
τ (h)	5.60	8.3
α	0.305	39
k_M hr^{-1}	1.08	40
k_T hr^{-1}	1.40	39
k_p hr^{-1}	1.45	40
mRNA at t = 0, pmol/g	0.161	Fixed
Activity at t = 0, ΔA_{331}/min g	0.0694	Fixed

Comparison of Dispersion Model Behavior to the Transit Compartment

We obtained simulated data from the transit compartment (Model B, Fig. 1) for compartment *M3* using the parameter values used by Sun and Jusko (first-order pharmacokinetics: first-order elimination rate constant $\lambda_z = 1\ h^{-1}$, initial drug concentration = 10^4 units; pharmacodynamics: receptor levels = 100 units, on-rate $k_1 = 0.1$ units/h^{-1}, off-rate $k_{-1} = 1\ h^{-1}$; 4). The mean time in each of the transit compartment was set to 1 h. The Hill-type power coefficient, h, was set to either 1 or 1.2. The dispersion model (Model C, Fig. 1) was then used to fit the simulated data from the transit compartment model. The dispersion model contained three parameters, α, D_N and τ, that were obtained from fitting with the value of R_N fixed at zero.

Figure 4 visually summarizes the results from fitting the dispersion model to the simulated data from the transit compartment model for compartment *M3* with $h = 1$ and 1.2. Under these conditions, the best-fit dispersion model curves were more peaked and had longer tails than the transit compartment model. However, these differences were relatively modest and would probably be obscured by the experimental error in many pharmacodynamic measurements. These preliminary findings suggest that the dispersion model may also be capable of parsimoniously describing the results of transit compartment models.

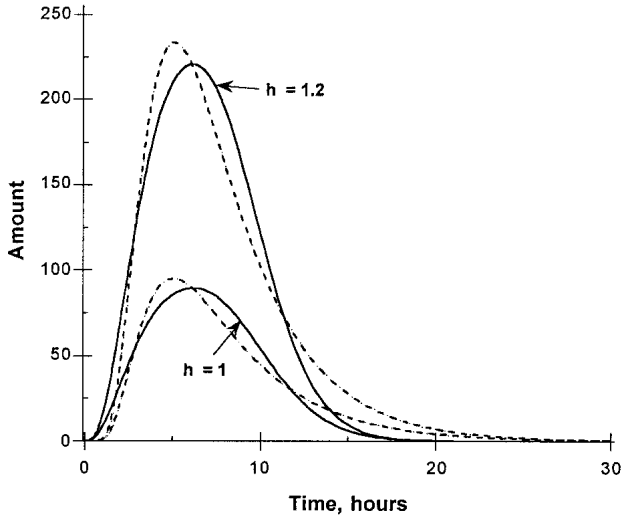

Fig. 4. The fit of the dispersion Model C to the transit compartment Model B (Fig. 1). The transit compartment simulations (using parameter values shown in the text) for compartment *M3* are shown in solid lines and the best fit curves from the dispersion model are shown in the dashed lines. The simulations differed in the values of the Hill exponent, *h*, which was set to the values indicated.

DISCUSSION

In this report, it has been demonstrated that the dispersion model is capable of characterizing biologic signal transduction. The model was assessed using simulations and published *in vivo* pharmacokinetic and pharmacodynamic data. The use of the dispersion model for describing signal transduction cascades is novel although the formalism of the dispersion model itself is not: it has been widely used to describe residence time distributions in chemical reactors, the liver and the kidney. The results demonstrate that it provides succinct mechanistic descriptors of the signal transduction process.

Although the dispersion model is mathematically more demanding than the Gamma distribution, tanks-in-series and transit compartment models, it is very parsimonious, parameter efficient and at its core, conceptually simple. The processes described include signal dispersion, convective transfer, and signal elimination. The dispersion model element does not contain discrete compartmental structure and represents the processes of the transduction cascade with the solution to a partial differential equation. Importantly, the dispersion model identifies the overall characteristics of the signaling process from the data instead of focusing on the structure and relationships between the specific signaling elements. However, in the limit of very large and very small D_N values, the characteristics of the dispersion model are identical to those of the tanks-in-series/Gamma distribution models with a single and an infinite number of compartments, respectively. The Gamma distribution and transit compartment models, in contrast, assume or predict specific compartmental structures for the signaling cascade. Given the known complexity of many biologic signaling cascades and the increasing rate at which additional information is emerging, the assignment of compartmental structure to mechanistic elements in the signaling cascade can be problematic with these models.

Unlike the transit and tank-in-series models, the dispersion model explicitly accounts for both spatial and temporal dimensions in signal transduction. Although the time course of the output of dispersion models can appear qualitatively similar to those from the transit compartment model (e.g., Fig. 4), the "microscopic" fate of a signal in the two models in distinct: in the dispersion model, a bolus signal is dissipated incrementally by axial dispersion as it moves along the spatial dimension and in the transit compartment and tanks-in-series models, the bolus signal is mixed completely and instantaneously upon entry into the first compartment. Because the dispersion model accounts for spatial dimension explicitly, the results depend on the boundary conditions and here, the closed-closed boundary conditions, which assume that signaling molecules do not leave the system, were used. In the chemical reactor engineering literature, a boundary condition at a reactor entrance (or exit) is referred to as closed if the dispersion coefficient is zero and open if the dispersion coefficient is infinite (14). From the mechanistic standpoint, this boundary condition-dependence suggests that signaling can also be dependent on the specific conditions prevailing at the regions where the signal is received (e.g., the cell surface) and transduced (e.g., the interactions between transcription factors and promoter regions of DNA).

The distinctive quantitative differences between the tanks-in-series and the dispersion model become most apparent when the variances of the normalized residence time distributions (in residence time distribution analysis, it is conventional to normalize time using the mean residence time of a non-eliminated bolus input) are examined. For a dispersion model with closed boundary conditions (5), the change in the variance of the normalized residence time of the signal, $\Delta\sigma^2$, where σ^2_{out} and σ^2_{in} are the normalized output and input variances, respectively, is given by $\Delta\sigma^2 = \sigma^2_{out} - \sigma^2_{in} = 2D_N - 2D_N^2 (1 - e^{-1/D_N})$. Thus, the variance of the normalized residence time distribution increases with increased values of the axial dispersion number D_N. In contrast, the change in the variance of the normalized residence distribution of the signal upon passage through a tanks-in-series model is given by $\Delta\sigma^2 = N^{-1}$.

Although indirect effect models have also been used to describe complex pharmacodynamic effects, the underlying principles of indirect effect approaches and dispersion models are not mutually exclusive. In general, indirect effect models describe pharmacodynamics by delineating the non-linear concentration-effect dependence of specific rate constants on drug, metabolites or intermediate compartment concentrations (15). Dispersion models on the other hand describe the time courses of signal processing and represent an alternate modeling element, i.e., they contain a qualitatively different type of compartment.

In this report, the linear form of the dispersion equation and the Laplace transform of its solution were used for modeling. Additional nonlinearities, e.g., the Hill equation to account for saturable receptor binding and rate constants modulated by indirect effects, are readily incorporated but the Laplace transform method cannot be used and alternative numerical approaches such as finite differences must be employed. Oliver *et al.* (16) and Hisaka and Sugiyama (17) have reported results using the finite difference method for more complex dispersion models such as physiologically based whole-body models and multi-element dispersion models for pharmacokinetic modeling of hepatic residence time distributions.

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