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Stochastic Models for Prodrug Targeting. 1. Diffusion of the Efflux Drug

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Abstract: In modeling prodrug targeting using the stochastic approach, we first modeled diffusion of the efflux drug. Drug efflux is one of the major reasons for the failure of prodrug strategy: the active agent is pumped out the membrane ("efflux"), causing an insufficient amount to be delivered to the targeted sites and thus diminishing the efficacy of chemotherapy. Because the biological body is a nonlinear nonequilibrium complex system, the molecular transport taking place in vivo often showed stochasticity. The model described here for diffusion of the efflux drug is basically a diffusion process with reflecting/absorbing boundary conditions, divided into two distinctive regions with one allowing the particles to jump to the origin as a result of efflux pumping. We study discrete time birth−death Markov chain and compute the time-dependent spatial probability density function (PDF) of particles. The results showed that the jumping probability, although small, has a significant impact on the evolution of PDF of the efflux drug. The implications of this model were discussed.

Keywords: Prodrug targeting; efflux; transporter; Fokker−Planck equation; stochastic model; transport phenomena; partial differential equations

Introduction

The prodrug approach, a chemical approach using reversible derivatives, can be useful in the optimization of the clinical application of a drug. The prodrug approach gained attention as a technique for improving drug therapy in the early 1970s. Numerous prodrugs have been designed and developed since then to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application, such as low oral drug absorption, lack of site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at injection site, etc.).[1,2] However, relatively few "oral" prodrugs have been marketed to date.[3] One of the major reasons for the failure of prodrug strategy is drug efflux, that is, the active agents being pumped out through the membrane by efflux pumping, causing an insufficient amount to be delivered to the targeted site and thus diminishing the efficacy of chemotherapy.

The transport process taking place in biological systems is an extremely complex process. The most fundamental function of the biological membrane is to serve as a general permeation barrier and at the same time to allow the selective permeation of certain types of molecules. Li and Nikaido[4] discovered that an effective permeability barrier on the cell surface is the cell wall of mycobacteria, a bilayer of very unusual composition, with the parallel arrangement of extremely long fatty acid chains producing a structure of exceptionally low fluidity, which prevents the rapid influx of antibiotics and chemotherapeutic agents and thus contributes to the intrinsic resistance of these bacteria.

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The permeability barriers by themselves are not sufficient to produce high levels of drug resistance. Most bacteria produce active efflux pumps which display an extremely wide range of substrate specificity, slowing down the entry of drugs through the outer membrane. For example, P-glycoprotein (Pgp), a 170 kDa plasma membrane ABC transporter, was found to function as an ATP-driven efflux pump with broad specificity for hydrophobic drugs, peptides, and natural products. To face this issue, efflux pump inhibitors or modifiers, expected to completely or partially block the pump, draw particular interest.[5−7] Mankhetkorn and Garnier-Suillero[6] found that verapamil is unable to completely block the Pgp-mediated efflux of anthracyclines and that 10% of its functionality remains even with high verapamil concentrations. They also found that the ability of verapamil to restore intracellular accumulation of anthracyclines in multiple drug resistance (MDR) cells depends on the kinetics of their uptake. As a comparison, cyclosporin A is a more potent modulator and is able to fully restore daunorubicin accumulation in MDR. Aszalos et al.[7] investigated the effects of three prototype Pgp modifiers on the function of the multidrug resistance associated protein (MRP) in two MRP-overexpressing cell lines: UMCC/VP lung and MCF-7/VP breast cancer cells. The results showed that cremophor completely blocked the function of Pgp in Pgp overexpressing cells, but only partially blocked the function of MRP.

To model the complex biological transport process, deterministic differential equations originate as a general approach, whereby internal fluctuations are ignored. It has been recognized that there is internal and external variability affecting the function of the transporters. For example, the rate of Pgp activation depends on the metabolic state of the cell;[8] enzymatic molecules are inhomogeneously distributed;[9] the substrate induced conformational changes in the binding site;[10] substrate binding in the transporter is a thermodynamic process, and the molecule has to cross the potential barrier height.[11] Furthermore, variability issues in the pharmacokinetics and pahramcodynamics appear in most of drugs.[12,13]

A combination of the deterministic approach and the stochastic approach could assist us in understanding the physical nature of complex biological systems.[14,15] Compared to the deterministic approach, stochastic modeling is probabilistic thinking.[16−18] Probabilistic thinking is also applicable to model prodrug targeting and diffusion of the efflux drug. Macroscopically, prodrug targeting and diffusion of the efflux drug involves evolution of the densities of a pulse of particles. Microscopically, these two processes entail a strong element of random motion of the particles around the potential barrier height.

As a first step on modeling prodrug targeting using the stochastic approach, we first present a theoretical work on diffusion of the efflux drug. In the next paper, we will mainly be working on the survival probability of a prodrug during its journey to the destination, the first passage time problem.

The stochastic model for diffusion of the efflux drug presented here essentially is the jump−diffusion model. Use of the jump−diffusion model for biological transport systems dated back 1970s.[19] Frehland[19] studied a transport system given by a number of binding sites separated by energy barriers. He suggested that the jump−diffusion approach may be applied to a number of different transport systems in biology and physics (ion transport through porous channels in membranes; carrier-mediated ion transport through membranes). Recently, Goychuk[20] used jump−diffusion model to study rate processes with dynamical disorder within a framework provided by unidirectional electron transfer with a fluctuating transfer rate. The stochastic jump process reflected conformational dynamics of an electron transferring

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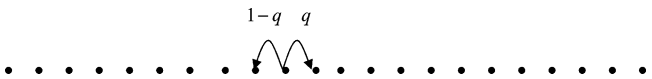

Figure 1. The stochastic model for one-dimensional nearest-neighbor random walk defined by eq 1.

donor−acceptor molecular complex. Ramanathan[21] used the jump−diffusion model to study the pharmacokinetic risks associated with drug interactions in populations. The model accounts for the variability inherent in the pharmacokinetics, the nature of the possibility of drug interactions, and the variability inherent in the extent of drug interaction. His study provides useful mechanistic insights into the stochastic processes that "drive" drug interactions in populations.

The work in this paper comprises two main sections: The first section begins with an introduction of a stochastic model for the transport of individual particles, with the implication that the stochastic approach achieves the same result that was obtained by macroscopic observation. The second section presents the stochastic model and discusses the implications of the simulation results.

An Introduction of Random Walk and the Fokker−Planck Equation

A stochastic model for the transport of individual particles leads to the same partial differential equation for the particle density that can be obtained by macroscopic considerations.

Consider only a particle that hops at discrete times between neighboring sites on a one-dimensional chain with unit spacing, as in Figure 1. Let $p(m,N)$ be the probability that the particle is at site m at the Nth time step. The particle jumps to the right with the probability q or to the left with probability $1 - q$ at each time interval, Δt, with a fixed jump distance, Δx. The evolution of this occupation probability is described as[16−18]

$$p(m,N+1) = qp(m-1,N) + (1-q)p(m+1,N) \qquad (1)$$

Define the drift velocity, v, and the diffusion coefficient, D:

$$v = (2q-1)\frac{\Delta x}{\Delta t} \qquad D = 2q(1-q)\frac{\Delta x^2}{\Delta t} \qquad (2)$$

After eliminating the vanishing term, the equation becomes

$$\frac{\partial p(x,t)}{\partial t} = -\frac{\partial}{\partial x}[v(x)\,p(x,t)] + \frac{\partial^2}{\partial x^2}[D(x)\,p(x,t)] \qquad (3)$$

This is the Fokker−Planck equation for the probability distribution of the particles.[18] For symmetric random walk, $q = 0.5$ (so $v = 0$), and the probability distribution obeys Fick's second law.

For the particles diffusing in the potential, $U(x)$, and driven by the corresponding force, $F(x) = \partial U(x)/\partial x$, the Fokker−Planck equation describing the PDF for particle position can be expressed as Smoluchowski equation,[17−18]

$$\frac{\partial p(x,t)}{\partial t} = -\frac{\partial}{\partial x}\left[\frac{D(x)\,F(x)}{k_B T}p(x,t)\right] + \frac{\partial^2}{\partial x^2}[D(x)\,p(x,t)] \qquad (4)$$

where k_B is the Boltzmann constant and T is the temperature.

The Stochastic Model for the Diffusion of Efflux Drug

Following the presentation of Dehling et al.,[22] we applied the physical principles to the problem of drug efflux. The model for diffusion of the efflux drug is shown in Figure 2. As the transporters interact with the substrates within the membrane environment, in order to have biological meaning, pumping is allowed only in the region where the efflux transporters are located. Recently, Lugo and Sharom[24] used fluorescence approaches to characterize the molecular interaction of purified Pgp with the dye LDS-751, which is proposed to bind to the R site. A 50-fold enhancement of LDS-751 fluorescence indicated that the protein binding site was located in a hydrophobic environment, with a polarity lower than that of chloroform. LDS-751 bound with submicromolar affinity and quenched Pgp intrinsic Trp fluorescence by 40%, suggesting that Trp emitters are probably located close to the drug-binding regions of the transporter and may interact directly with the dye. Using a FRET approach, they mapped the possible locations of the LDS-751 binding site relative to the NB domain active sites. The R site appeared to be positioned close to the membrane boundary of the cytoplasmic leaflet. Their study is in agreement with the idea that Pgp may operate as a drug flippase, moving substrates from the inner leaflet to the outer leaflet of the plasma membrane.

Denoting the distance of the particle from the original place at time t, $X(t)$, we model the motion by a stochastic process (X_t) and divide the path from left to right into individual cells with equal width. There is an extra cell with index $N + 1$ denoting the absorption site. Particles that have entered state $N + 1$ cannot return to the diffusion region. We can view it as the particle entering the circulatory system or being eliminated. The dynamics of the diffusion process for the efflux drug is described by a Markov chain $(X_n)_{n\geq 0}$ with state space as follows:

$$\{1, 2, ..., m-1, m \text{ (region 1, } 0 \leq X \leq L_1), m+1, ..., N, N+1\}$$

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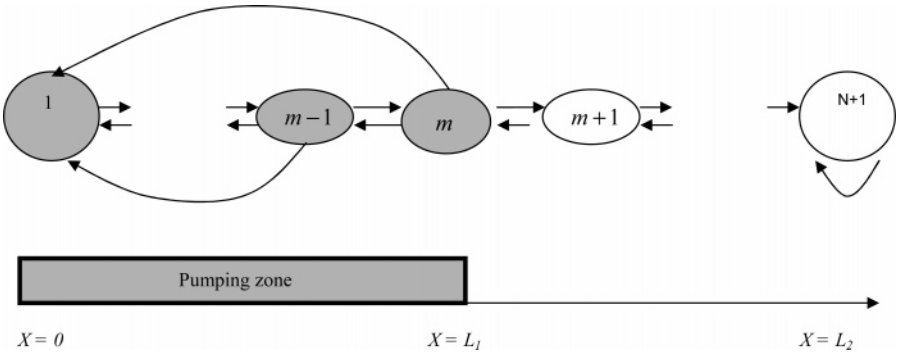

Figure 2. The stochastic model for diffusion of the efflux drug. The diffusion of the efflux drug is partitioned into N cells of equal width, with cell $N + 1$ symbolizing the absorption site and region 1 ($X = 0 \sim L_1$) symbolizing the pumping zone. The possible transitions are that a particle can move one cell left, stay in the same cell, or move one cell right. If the particle is in region 1, it has a possibility of jumping to the starting point ($X = 0$).

and an absorbing boundary at $N + 1$ and pumping region from the original place to L_1. Time is discretized by considering the particle position at integer times only. As you will find, our Markov chain is basically a birth−death process, modified to allow for instant jump only in one region to the first cell, thus modeling the probability that a particle gets efflux by the transporter in the membrane.

In the pumping zone, a particle positioned at i can move one step left toward the jumping zone with probability β_i, move one step toward the targeting site with probability δ_i, or stay in the present location with the probability $\alpha_i = 1 - \beta_i - \delta_i$. In addition, the particle has a probability of pumping to the original size with probability λ_i. Pumping is not allowed after the particles have left the pumping zone.

In reality, the transport process occurs in continuous space and time. Dehling et al.[22] used a discrete birth−death Markov chain to model transport of particles in a fluidized bed, in which the particles are allowed to jump to the first cell from all cells. They gave a rigorous mathematical analysis and proved that the discrete model converged to the ordinary diffusion process with reflecting/absorbing boundary conditions. As we assumed the pumping probability, λ_i, to be a spatially dependent parameter, our model is the same as the one they used as we just put $\lambda_i = 0$ when the particle left the pumping zone.

The Markov chain is specified once we know (1) the initial position probability vector, $p(0) = (p(0,1), ..., p(0,N+1))$, of the particle's initial position, where $p(0,i) = P(X_0=i)$, where the probability function X_n is defined by $p(n,i) = P(X_n=i)$; (2) the transition matrix $P = (p_{ij})_{1\le i,j\le N+1}$, where p_{ij} gives the conditional probability that the particle is in cell j at time $n + 1$, given it was in cell i at time n, i.e., $p_{ij} = P(X_{n+1}=j|X_n=i)$.

A recursion formula for $p(n,j)$ can be expressed as

$$p(n,j) = \sum_{i=1}^{N+1} p(n-1,i)p_{ij} \tag{5}$$

Iterating this identity, we obtain the explicit formula $p(n) = p(0)P^n$ for the PDF of the particle at time n. From a macroscopic point of view, the probability vector $p(n)$ gives the distribution of particles over the cells if at time $n = 0$ a large number of particles were placed in the first cell. In such a setup, eq 5 can be interpreted as a mass-balance equation.[22]

The transition probability matrix can be constructed as

$$p_{i,1} = \lambda_i \tag{6a}$$

$$p_{i,i-1} = \delta_i(1-\lambda_i), \quad p_{i,i} = \alpha_i(1-\lambda_i), \quad p_{i,i+1} = \beta_i(1-\lambda_i), \quad \lambda_i = 0 \text{ if } i \ge m+1 \tag{6b}$$

with the initial condition and the boundary conditions

$$p(0) = (1, 0, ..., 0) \tag{6c}$$

$$p_{1,1} = 1 - \beta_i(1-\lambda_i),\ p_{1,2} = \beta_i(1-\lambda_i),\ p_{N+1,N+1} = 1 \tag{6d}$$

The last cell ($i = N + 1$) is the absorption site where the particle has no chance to return. The transition matrix for this model is tabulated in Figure 3.

Clearly, if the corresponding Markov chain does not return to the first cell, then it is an ordinary birth−death process with transition probability δ_i, α_i, and β_i and reflecting/absorbing boundary conditions.[17] The return probability is, in general, state dependent. This makes sense as the distribution of the transporters and the substrate binding are distributed inhomogeneously.[24−27]

Dehling and co-workers[22,23] proved the discrete birth−death process can converge to the diffusion limit by defining $\Delta,\epsilon\rightarrow 0$.

$$\beta_i = \frac{\epsilon}{2\Delta^2}D(i\Delta) + \frac{\epsilon}{2\Delta}v(i\Delta) \tag{7a}$$

$$\delta_i = \frac{\epsilon}{2\Delta^2}D(i\Delta) - \frac{\epsilon}{2\Delta}v(i\Delta) \tag{7b}$$

$D(x)$ and $v(x)$ are diffusion coefficient and particle velocity, respectively. $D(x)$ and $v(x)$ are continuous functions with

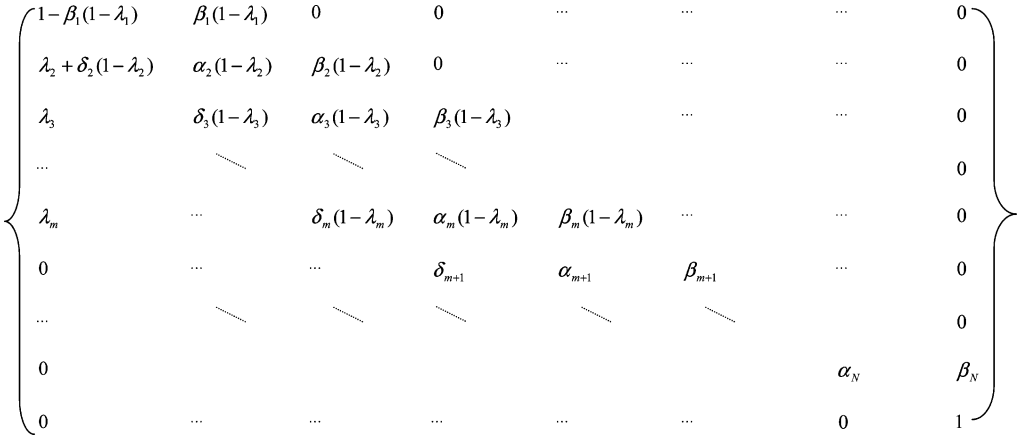

Figure 3. The full transition matrix for the stochastic model for diffusion of the efflux drug.

values in (0, ∞). The corresponding Fokker−Planck equation for the limit density is expressed as

$$\frac{\partial p(x,t)}{\partial t} = -\frac{\partial}{\partial x}[v(x)\,p(x,t)] + \frac{\partial^2}{\partial x^2}[D(x)\,p(x,t)] - \lambda(x)\,p(x,t) \tag{8a}$$

with the boundary condition expressed as

$$\int_0^1 \lambda(x)\,p(x,t)\,dx + \frac{\partial}{\partial x}[D(x)\,p(x,t)]_{x=0} - v(0)\,p(0,t) = 0 \tag{8b}$$

When no jump occurs, eq 8a reduces to eq 3.

The explanation for the fact that we obtain the same partial differential equation for the particle density, a macroscopic quantity, and the probability density, a microscopic quantity, is the law of large numbers. The empirical distribution of a very large number of particles namely follows the probability distribution.

Results

The numerical computations were carried out in MATLAB ((MATLAB Version 6.5, The Mathworks Inc, Natick, MA), a matrix-oriented package. We first presented three runs of the transport process for three different choices of the return probability. In all cases we have $L_1 = 16$ (the pumping zone), $L_2 = 32$ (no pumping zone), $\beta_i = 0.3$, $\delta_i = 0.2$, $\alpha_i = 0.5$. We have the return probability $\lambda_i = 0$ for all regions (Figure 4a), $\lambda_i = 0.01$ (Figure 4b) and $\lambda_i = 0.05$ (Figure 4c) for the pumping zone, respectively. We set $\beta_i > \delta_i$ as we worked on a particle with a problem to cross the biological membrane.

Figure 4 shows how the jump probability, λ_i, influences the evolution of the diffusion path. When the pumping probability is zero (Figure 4a), the diffusion path is a typical biased random walk.[17] As the pumping probability increases, the particles mostly like to stay in the pumping zone (Figure 4b) or the first cell (Figure 4c). In other words, the particles will take a longer time to reach their destinations when $\lambda_i = 0.05$ compared to when λ_i approaches zero. A value of 0.01 was intentionally set in Figure 4b because, when λ_i is less than 0.01, the particle has less of a chance to return to 0 whereas, when λ_i is greater than 0.01, the particle is more likely to return to 0. It is unknown why 0.01 is a transitional point.

Figure 5 shows the evolution of the probability distribution of the particle after time points $n = 25$, 50, and 100. The shapes of Figure 5b and Figure 5c are completely different from that of Figure 5a, due to the returns to the first cell. Figure 5a displays a typical biased distribution after a long time (time point $n > 100$ is not plotted as there is no visible difference from $n = 100$). Similar to the results from Figure 4, a higher probability density was found in the pumping zone ($\lambda_i = 0.01$) or the first cell ($\lambda_i = 0.05$) compared to that when $\lambda_i = 0$. The result suggests that the pumping probability, although small, has a dramatic impact on the evolution of PDF. As we can view $\lambda_i = 0$ as the efflux transporters are completely blocked, and $\lambda_i \neq 0$ as the efflux transporters are partially or not blocked, this study emphasizes the importance of the cellular-based studies that elucidated the functions and identified the locations of the efflux pumps.[8,11]

The simulation study not only shows the role of the pumping in the transport of the efflux drug to the targeting site but also suggests that an experiment that not only separates pumping from transport (i.e., blocks the efflux transporters) but also gathers the temporal and spatial distribution of the efflux drug in the membrane is necessary and crucial for drug discovery. Unfortunately, to my best knowledge, by the time of the preparation of this manuscript, no temporal and spatial distribution of the efflux drug is

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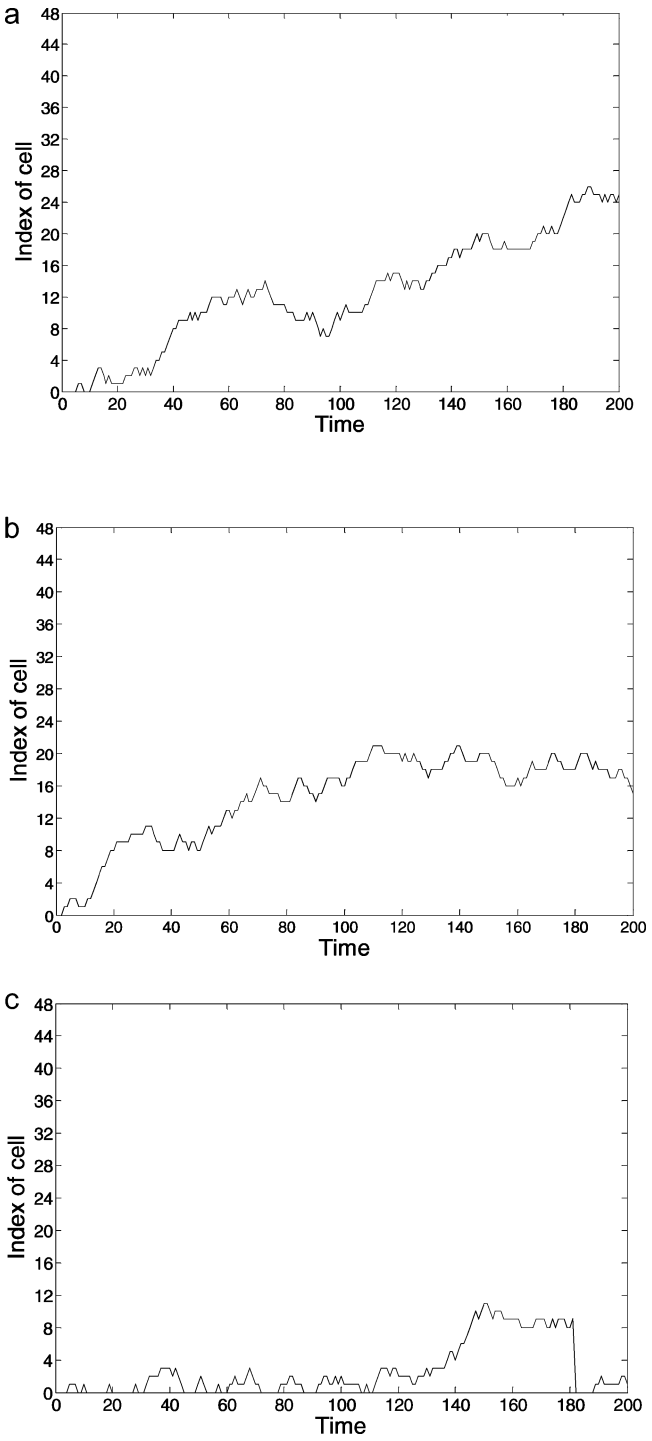

Figure 4. Simulation of a path in the discrete Markov model for three different parameter settings. In all cases, $L_1 = 16$, $L_2 = 32$, $\beta_i = 0.3$, $\delta_i = 0.2$, $\alpha_i = 0.5$, and return probability $\lambda_i = 0$ for all ranges (Figure 4a), $\lambda_i = 0.01$ (Figure 4b) and $\lambda_i = 0.05$ (Figure 4c) for the pumping zone. The horizontal axis denotes time, the vertical axis the index of the cell. Results display the average of 100 trials.

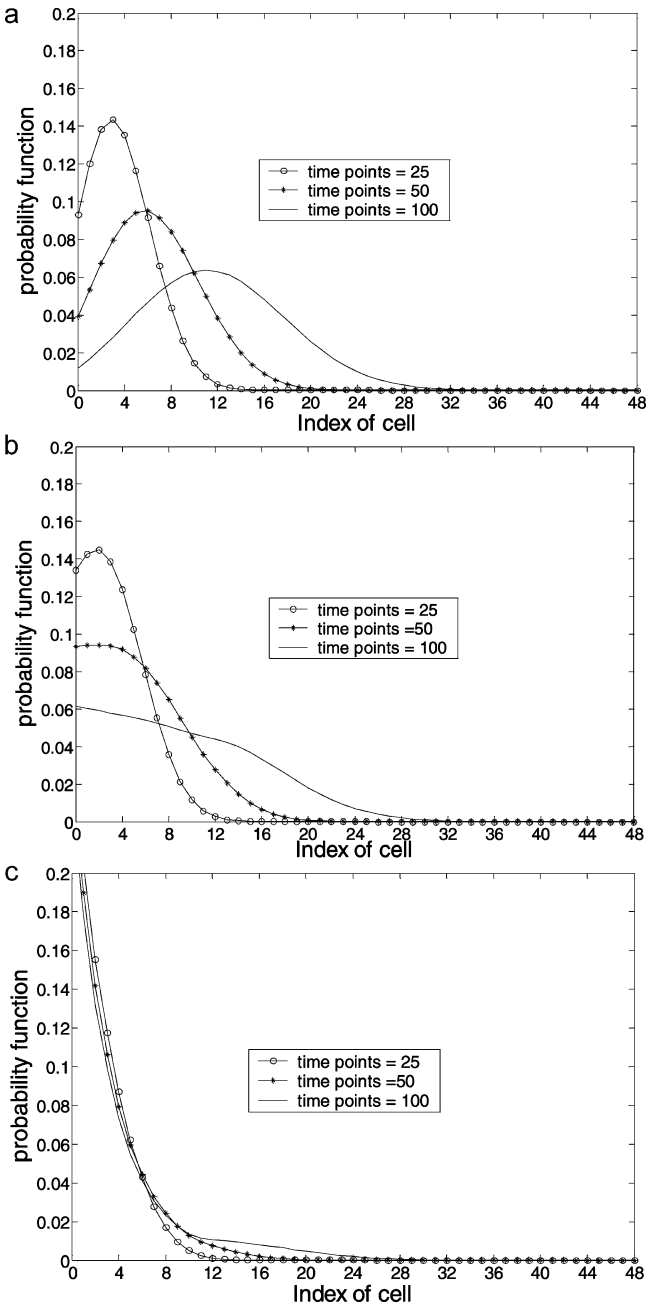

Figure 5. Evolution of particle density in the discrete Markov model for three different parameter settings. In all cases, $L_1 = 16$, $L_2 = 32$, $\beta_i = 0.3$, $\delta_i = 0.2$, $\alpha_i = 0.5$, and return probability $\lambda_i = 0$ for all cells (Figure 5a) and $\lambda_i = 0.01$ (Figure 4b), and $\lambda_i = 0.05$ (Figure 4c) for the pumping zone. The density is shown at time points $n = 25$, 50, and 100, with the horizontal axis showing the index of the cell and vertical axis showing the occupation probability.

available. There are several experimental techniques that can be used to track random paths of molecule across membrane, through channels, or coupled to transporters.[28,29] In the past decades with the arrival of molecular biology and modern imaging instruments, real-time spatial visualization analysis of the cellular or subcellular processes in living cells (or organisms) becomes more important than previously. Any

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such techniques require a high degree of spatial and temporal resolution. For example, Hopener et al.[28] used scanning near-field optical microscopy and directly visualized the transport of single particles through the single nuclear pore complex and other transporters. Another instrument, positron emission tomography (PET), can be used to visualize in vivo the expression and function of Pgp in living human brains.[29] It was found that the common approaches for studying presynaptic norepinephrine (NE) and dopamine (DA) transporters involve use of radiolabeled substrates or antagonists and this method possesses limited spatial resolution and offers limited opportunities for repeated monitoring of living preparations. To circumvent these issues, scientists at Vanderbilt University[24−27] explored two novel assay platforms that permit temporally resolved quantitation of transport activity and transporter protein localization. The binding and transport of ASP+, a fluorescent organic compound with micromolar potency for the human norepinephrine transporter, showed Gaussian-like distribution in the HEK-hNET cells (Figure 5A in ref 25), a phenomenon that can be explained by the Fokker−Planck equation (the model for binding and transport of transporter can also explained by the Fokker−Planck equation but is not presented here).

The Fokker−Planck equation has been used in modeling biomolecular transport processes.[30,31] The principle of transport of the efflux drug is same as that of the biomolecules, i.e., the particles have to cross the potential barrier height in a fluctuating environment,[11] and the movement involves random walk of a pulse of the particles.[16] Fitting the proposed stochastic model with the experimental data will be done once the experimental data is available.

Discussion

Under the framework of Brownian motion, we presented a stochastic model for diffusion of the efflux drug. The model is constructed on the basis of the recent experimental findings on the substrates and the efflux transporters, especially the cellular-based real-time studies.[8,11,24]

For the oral absorption, the intestinal membrane channels were designed by Nature to regulate the transport of biomolecules such as amino acids, proteins, and nucleic acid, and serve as pathways to metabolites.[32] Understanding the transport of drug under the influence of the membrane substances (enzyme, transporter, etc.) is essential to prodrug design. Levadny et al.[33] studied theoretically the binding of a polar macromolecule to a large ion channel and the effect of external conditions (applied voltage and ion strength of solution). They considered that molecule behavior in the bound state has random thermal fluctuations within a limited fraction of its phase space. With the framework of the first passage time approach and under the adiabatic approximation, they reduced the problem to one dimension and concluded that the angle between macromolecule dipole and channel axes is the key variable of the problem.

Pajeva and Wiese[34] developed a pharmacophore model of Pgp drugs, based on a highly diverse data set and related to the verapamil-binding site of the protein, to explain the broad structural variety of the Pgp substrates and inhibitors. The pharmacophore model consists of two hydrophobic points, three hydrogen bond (HB) acceptor points, and one HB donor point. The binding affinity of the drugs depended on the number of the pharmacophore points simultaneously involved in the interaction with Pgp. They proposed a hypothesis that (i) the verapamil binding site of Pgp has several points that can participate in hydrophobic and HB interactions and (ii) different drugs can interact with different receptor points in different binding modes.

The transport dynamics of the produg or its derivatives is a result of multiple factors including steric factors,[35] electrostatic interactions,[36] and hydrodynamics.[37,38] Gangwar et al.[39] determined the different conformations of the acyloxyalkoxy-linked cyclic prodrug of a model hexapeptide in solution and investigated the relationship between these solution conformations and the cellular permeability characteristics of the prodrugs. They found that the increased ability to permeate membranes could be due to reduction in the average hydrodynamic radius of the molecule facilitating paracellular flux and/or the reduction in the hydrogen bonding potential facilitating transcellular flux.

Elucidation of the transport dynamic of prodrug or its derivatives in the membranes is highly relevant to the growing field of microfluidics and in particular to recent

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efforts to miniaturize molecular biology reactions to the submicron scale.[40] The passage of the prodrug and its derivatives through the biological membrane to the destination can be seen as a one-dimensional version of particle "trapping" in random environments. Due to the nonequilibrium nature of living systems, a stochastic approach would be more appropriate. Besides, with the modern computation instruments, such as MATLAB, the stochastic model is easily made—at lease for a discrete approximation—and has a stronger intuitive appeal than a traditional deterministic model.

The simulation results showed that the pumping probability, although small, has a dramatic impact on its path to the destination, and its probability density. Theoretically, the model could be used to predict the targeting efficacy of the blocking strategy once we obtain the key parameters in eq 6 from the cellular-based temporal spatial image study. The model could also provide a mechanistic insight into the stochastic processes that drive drug interactions with the transporters.

Due to its lesser complexity, the deterministic kinetic approach, based on ordinary differential equations, can be considered as a first step in modeling drug transport. The blood−brain barrier (BBB), formed by brain capillary endothelial cells, is a functional barrier responsible for restricting the retry compounds from the circulating blood to the blood parenchyma cells.[41] In additional to this physical barrier, active efflux transporter on this barrier also played important roles in the BBB function. He and Liu[42] used rhodamine123 (Rh123, a florescent dye and a Pgp substrate) to examine the functional activity of Pgp in cultured bovine brain capillary endothelial cells (BCEC) and screen various principles extracted from Chinese herbal medicines including tretramethylpyrazine (TMP) on Pgp modulation in the BBB. They also compared the activity of the principles with well-known Pgp transporter inhibitors such as cyclosporin A and doxorubicin. They found that the accumulation of Rh123 was time-dependent and the steady state was attained at 90 min. Then they used the uptake at 90 min to characterize the transport of Rh123 and further investigated the effect of the eight principles on cellular accumulation of Rh123 in BCEC. They found that some isoquinoline alkaloids acted as Pgp reversal agents in the BBB. When the reversal agent was withdrawn, the physiological function of Pgp returned to the normal level. Tsai and Liang[43] used microdialysis techniques and measured the blood and brain levels of TMP in rats. Qi et al.[44] developed a simple kinetic model (one for partition transport across membrane, and one for mass transfer into brain, and one for reverse pumping) for TMP pharmacokinetics. Since TMP is a compound with an order of 10^{-6} cm/s permeability across human cadaver skin,[45] therefore, we can assume that the transport process reached an equilibrium state quickly (<30 min). However, for a nonequilibrium process and/or an inhomogeneous transport process, the probabilistic analysis is needed.[14−18,46]

While the mathematical structure introduced by Dehling et al.[22] is indeed transferable to the problem of drug efflux, one must admit that it is much more difficult to obtain the temporal and spatial particle motion data in the cellular world. Dehling et al.[22] conducted their study in fluidized beds; Turton and co-workers[47,48] developed a unique video imaging technique to capture the particle motion in the pan coater. In order to measure localized moieties (lateral distribution), positron emission tomography (PET) is applicable. To gather the lateral and longitudinal distribution, advanced image techniques, such as three-dimensional quantitative confocal microscopy[49] and DTI tractography,[50] are needed. Nano-biosensors with single-molecule sensitivity will enable scientists to explore the molecular mechanisms and cellular pathways of viral infections and nonviral gene delivery.[51] Image data analysis and estimation of the parameters in stochastic differential equations are also great tasks, and have to be done with statistical knowledge, i.e., the Markov chain Monte Carlo method[52] and Baynesian analysis.[53] It is necessary to note that particle motion in either fluidized bed or pan coater has no effect on the system

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functionality of the instruments, while chemical compounds could change the functionality of the biological membrane when they cross the membrane.[54] Nevertheless, we are optimistic about the progress in image technology and the potential application in drug discovery and drug delivery. The validation and modification of the proposed model will be done when data are available toward understanding the mechanism of drug efflux.

Application of the stochastic modeling in biopharmaceutics is still relatively limited. Acceptance of the stochastic approach has been slow to develop.[13,21,55,56] There are many problems that are too complex to be modeled by a deterministic model but could be tackled soundly by the stochastic approach. Stochastic mathematics deserves to be more widely studied and applied in biopharmaceutics.

Conclusions

A stochastic model was developed for diffusion of the efflux drug. The time-dependent spatial probability density function of the efflux drug was calculated with different pumping probabilities. The results showed that the pumping probability, although small, has a significant impact on the evolution of PDF. The study emphasized the importance for the real-time study that elucidated the functions and identified the locations of the efflux transporters.

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