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Multidrug Resistance and its Reversal: Mathematical Models

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Classic multidrug resistance (MDR) is a phenomenon by which cells nonspecifically extrude noxious agents from the cytoplasm before lethal concentrations build up. Some chemotherapeutically treated tumors exhibit these same dynamics. In tumor systems, the most common mechanism of facilitating MDR is the upregulation of the P-glycoprotein pump. This protein forms a transmembrane channel, and after binding the chemotherapeutic agent and 2ATP molecules, forces the noxious agent through the channel. Hydrolysis of ATP to ADP provides the energy component of this reaction. General mathematical models describing drug resistance are reviewed in this article. One model describing the molecular function of the P-glycoprotein pump in MDR cell lines is developed and presented in detail. The pump is modeled as an energy-dependent facilitated diffusion process. A partial differential equation is linked to a pair of ordinary differential equations to form the core of the model. To describe MDR reversal, the model is extended by adding an inhibitor to the equation system. Equations for competitive, one-site non-competitive, and allosteric non-competitive inhibition are then derived. Numerical simulations have been run to describe P-glycoprotein dynamics both in the presence and absence of inhibition, and these results are briefly reviewed. The character of the pump and its response to inhibition are discussed within the context of the models.

Keywords: Multidrug Resistance, Mathematical Model, Numerical Simulation

INTRODUCTION

In vitro experiments with tumor cell lines have revealed a series of very complicated mechanisms, both at the genetic and biochemical levels, that can account for drug resistance (Curt, *et al.*, 1984; Schimke, 1984; Harris, 1984; Marx, 1986; Warr and Atkinson, 1988). The most common include: (1) decreased drug uptake into the cytoplasm, (2) increased drug efflux from the cytoplasm, (3) increased degradation/metabolism of drug, (4) upregulation of the drug target, and (5) alteration in the drug target's biochemical properties. While some cells may become resistant to only single agent, in some cases a cell line may become resistant to many agents which are structurally and mechanistically diverse. This latter form of drug resistance is called multiple or

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pleiotropic drug resistance (Pastan and Gottesman, 1987; Moscow and Cowan, 1988; Gottesman and Pastan, 1988; Croop *et al.*, 1988).

Classical multidrug resistance (MDR) falls into this category, and the mechanism underlying it involves the overexpression of an energy-dependent efflux pump that spans the plasma membrane (Ling and Thompson, 1974; Juliano and Ling, 1976; Inaba, *et al.*, 1979; Tsuruo, *et al.*, 1982; Kartner *et al.*, 1983). The pump is a transmembrane glycoprotein referred to as p170 or P-glycoprotein. In the laboratory, one often finds that stepwise selection of cells with one agent, say doxorubicin, leads to the generation of a cell line that is also resistant to other natural product agents including anthracyclines, vinca alkaloids, podophyllotoxins, and colchicine (reviewed by Pastan and Gottesman, 1987; Gottesman and Pastan, 1988). The resultant cell line often expresses an active P-glycoprotein pump.

Coincident with the development of the experimental data, a considerable amount of mathematical theory has evolved to describe it. Therefore, to describe a particular model of the P-glycoprotein pump in detail (see below), I have decided to 'zoom in' on the appropriate models of MDR by moving from the general case, the emergence of resistance, to models of tumor-wide resistance, to models of cellular resistance, to models of MDR and its reversal.

GENERAL MODELS OF DRUG RESISTANCE

Stochastic Models of Emergence

Given the complexities in both definition and characterization of drug resistance, it has been difficult to develop manageable mathematical models to describe its emergence. Goldie and Coldman have proposed some of the most notable examples (Goldie and Coldman, 1979, 1984; Goldie *et al.*, 1982; Coldman and Goldie, 1983; Coldman *et al.*, 1985). Their models are phenomenological, in that they predict that single-agent resistance is a population-wide phenomenon, and depends upon an unspecified underlying genetic mutation. They predict that the frequency of cross-resistance to several agents is the product of the individual underlying single mutation frequencies, and account for multiple resistance on the *tumor level* in this manner. Additionally, in their earliest work, they assumed that 'resistant' meant completely resistant, i.e. that no clinically acceptable level of drug could kill the resistant subclone. This assumption has been relaxed in subsequent models.

Simple analyses highlight the limitations of this model. Assume a small viable mutation rate (i.e. 1 mutation in 10^6 mitoses). Tumors presenting at an early clinical stage (approximately 1 cm^3) contain between 10^8 and 10^9 cells. Therefore, these tumors have negligible chance of being homogeneously chemosensitive. However, the likelihood is that multiply resistant subpopulations have not yet had a chance to emerge and grow. If one assumes, as Goldie and Coldman once did, that resistant cells are *totally* resistant, then one must conclude that upon presentation, the chances of curing any moderately sized tumor depend strictly on finding the correct combination of chemotherapeutic agents to overcome the singly resistant subclones that are present in that particular tumor.

Goldie and Coldman address multiple drug resistance on the cellular level incidentally. They assume that individual subpopulations emerge independently, and that these subclones are resistant to structurally different drugs. MDR is then quite rare, the product of two independent mutations, yielding a cell that is doubly mutated. It is not a dynamic process and cannot evolve within one particular resistant subpopulation under selection pressure. Therefore, on a cellular level, the odds of achieving resistance to three or more drugs are astronomical. Thus, in their model, resistance to an array of drugs is a tumor-wide, not cellular, phenomenon.

Day (1986) extended Goldie and Coldman's work to include asymmetry in growth, mutation, and death rates to show that in multiply resistant tumors, optimal treatment is always achieved with multiple drug regimens, and that the sequencing strategy employed depends upon the underlying transition

rates established independently for each subpopulation. However, Day states quite clearly that this type of model may be insufficient when considering MDR as expressed by the P-glycoprotein pump.

Deterministic Models of Therapy

Like the stochastic models outlined above, the deterministic models that portray drug resistance mathematically define 'resistance' as a classifier, i.e. cells are either 'sensitive' or 'resistant'. There is no attempt to represent drug resistance as a continuous variable over a spectrum of response. The models usually define a resistant subpopulation in generic terms, e.g. by a decreased death rate in the presence of drug. Overall tumor growth is modeled as a process in which the two populations vie for survival in a hostile (drug-treated) environment. None of these models directly describes resistance mechanistically on the molecular level.

In three related papers (Hokanson, *et al.*, 1986; Birkhead *et al.*, 1986; Gregory, *et al.*, 1988) the rate of tumor growth and overall tumor volume at the time of presentation are used as predictors of response in the presence of single-drug therapy. However, the authors state explicitly that their original intention was to describe resistance phenomenologically, and that no attempt was made to model it mechanistically. As a case in point, they assumed that sensitivity and resistance are non-acquirable traits, fixed in an altering environment. This kind of assumption totally ignores the effects of gene amplification as seen in dihydrofolate reductase (DHFR) upregulation in methotrexate treated tumors (Schimke, 1984), and no explicit definition or attempt to model MDR as a molecular mechanism is presented.

In a more mechanistic model, Duc and Nickolls (1987) link the pharmacokinetic profile of a single course of drug therapy to a standard tumor growth model. The distribution of drug is modeled deterministically using three physiological compartments (the plasma, the sensitive tumor, and the resistant tumor). They assumed that each tissue compartment exhibits first order kinetics, and that the sensitive and resistant tumor cell compartments are completely segregated, unable to communicate biophysically. A standard set of ordinary differential equations were then derived and solved.

Cell growth within each compartment was modeled independently. The presence of one population does not affect growth or loss in the other. The level of drug at each site was determined separately. The general growth-death model for cycle specific drugs took the following form:

$$\frac{dN}{dt} = F(N)N(t) - kf(t)m_x F(N)N(t) \qquad (1)$$

For cycle non-specific drugs the model looked like:

$$\frac{dN}{dt} = F(N)N(t) - kf(t)m_x N(t) \qquad (2)$$

where, $F(N)$ represents a logistic growth modifier. The term $kf(t)$ represents the time-dependent distribution of drug in a particular tissue compartment. The loss terms in these equations represents cell death due to drug treatment. Resistance is modeled as a tumor-wide effect and is expressed entirely as a difference in the cell death rates. In these equations, that difference is accounted for by the term m_x, which is large if the population is sensitive and small if it is resistant.

One could modify this model to implicitly represent classical MDR by asymmetrically representing the pharmacokinetics of two segregated tissue compartments, e.g. by allowing for increased efflux of drug from the resistant tissue compartment. Then $kf(t)$, which represents whole tissue and not intracellular concentrations, would be modified to represent tissue specific dynamics in the two populations. The authors do not address this particular aspect of resistance explicitly.

The Hybrid

Michelson and Slate (1989, 1991) developed a mathematical model which describes drug resistance in a more mechanistic manner, by defining it as any of the five physiologic pathways listed in the Introduction above. The model is a stochastic birth–death–migration process. As such, any

change in cellular status, e.g. growth, death, or acquisition/loss of resistance, is expressed stochastically for individual cells. However, as opposed to the classic stochastic process, the transition probabilities are dynamic, determined by the level of drug at the target site of the average tumor cell (usually the nucleus).

The model assumes that: (1) Each transition occurs with a given probability distribution during a given time step, and that these probability distributions are independent. (2) Each cell acts independently and does not depend on its neighbors for signals or controls. (3) The lifelength of each cell is a random variable and that it is identically and independently distributed throughout the cell population. (4) Drug is uniformly distributed throughout the tumor cell population, i.e. no spatial hindrance in access of drug to any cell is encountered. (5) Cell death is due strictly to the cytotoxic effects of the drug, and that the risk of cell death is proportional to the concentration of drug at the target site in the cell.

Thus, to determine the transition probabilities as functions of drug concentration at the target site, the distribution of drug moieties throughout the average cell is modeled using a standard concentration-dependent first-order compartment model. The compartments of interest are the interstitial spaces, the cytoplasm, and the target site (usually the nucleus).

While the variable expression of any of the resistance mediators listed in the Introduction may be due to any number of underlying processes (e.g. gene amplification, alterations in transcriptional or translational efficiency, etc.), this model deals only with their functional consequences (e.g. decreased uptake, increased efflux, differential target sensitivity, etc.). The very simple assumption is that cell death is strictly proportional to the concentration of drug at the target site: If the concentration of an active agent at a cell's target site is high enough, the cell will very likely die, and the more drug that is present, the more likely death is to occur.

Cell death thus becomes a probabilistic event, based entirely upon the ability of a drug to penetrate, distribute, and accumulate at the target site in the average tumor cell. Using these models, we have shown that any cell which can pump out enough drug from the cytoplasm so that its concentration at the target site remains 'low enough', significantly enhances its chances for survival (Michelson and Slate, 1989; Slate and Michelson, 1991). And though the molecular structure of the P-glycoprotein pump is not explicitly addressed in this model, a simple first-order pump is included in the model as a first approximation of its dynamics. This type of hybrid model shifts our attention of the definition of drug resistance from a tumor-wide phenomenon to a more cellular-based mechanism.

There are, however, significant limitations inherent in this model. Clearly, in large, poorly vascularized tumors, cell–cell interactions (including 'competition' for the drug) can not be ignored. Any claim that drug is equally distributed across a large tumor mass is self-evidently erroneous. However, our goal was to determine whether, on the micropharmacological level — the level at which the P-glycoprotein pump works — the distribution of drug within the tumor cell yielded a potential process by which MDR was conferred, and whether the P-glycoprotein pump was a potential target for its reversal. It was this simple hypothesis that motivated the development of the more mechanistic models described below.

SPECIFIC MODELS FOR MULTIDRUG RESISTANCE

Intracellular Micropharmacology

Demant *et al.* (1990) developed a model which asked whether endosomal transport of drug, as an alternative to the P-glycoprotein pump, could, under varying pH conditions, account for a major portion of drug efflux in MDR cell lines. Their model described three basic compartments: the extracellular medium, the cytoplasm, and the endosomal vesicles. Within the cytoplasm, the drug could exist in three distinct states: (1) free, (2) bound to low-affinity membrane binding sites, or (3) bound to high-affinity nuclear binding sites. Assuming that the amount of drug bound to

membrane sites is significantly smaller than the number of possible sites involved, i.e. ignoring saturation, and by assuming that equilibrium is rapidly achieved in the cytoplasm, Demant *et al.* derived a mass action-type equation for calculating the dissociation constant for membrane binding. Active transport across the membrane was then defined by Michaelis–Menten kinetics. From their model they concluded that active transport is, in fact, the primary efflux mechanism in MDR cell lines, and that diffusion and exocytosis are simply not fast enough to account for the rapid drug efflux observed experimentally.

Michelson and Slate (1992, 1994), on the other hand, modeled the P-glycoprotein pump directly. Our model let drug leave the cytoplasm via normal diffusion processes and via the energy-dependent pump. The mathematical representation of the active transport mechanism is as a facilitated diffusion process and is discussed in detail below.

Calculation of Pump K_M

Most mathematical models used to describe MDR transport on a molecular level assume that the pump binds to a cytotoxic target drug before actively transporting it out of the cytoplasm. The binding and facilitation of the transport are biologically identical to those observed in enzyme kinetics. Therefore, most (all?) theoreticians have based their descriptions of the pump and its dynamics on the Michaelis–Menten rate equation. Horio *et al.* (1990) developed a model to mimic an experiment in which apical-to-basal and basal-to-apical flux across MDCK epithelial cells was measured. Based upon differential flux characteristics and relative diffusion rates, they derived a final equation for the apparent K_M of the system.

In a similar system, Spolestra *et al.* (1992) modeled the pump as an experimental flow through process. Flux, defined as the time derivative, d/dt, represents net diffusion and Michaelis–Menten transport. By assuming equal membrane diffusibility, i.e. equal diffusion both into and out of the cell, they derived net flux estimates based upon the intracellular and extracellular concentration–time profiles observed in their experiments. Using standard Scatchard plots, they derived estimates for the number of binding sites available to the target drug and their average affinity. However, they also observed Hill slopes in their Scatchard lines markedly greater than one, making strict interpretation of their data difficult.

Energy-Dependent Facilitated Diffusion

Michelson and Slate (1992) developed a model of the MDR pump based upon the following washout experiment: tumor cells are pre-loaded with radioactively tagged drug, removed from the drug-loaded medium, washed, and restored to a new, drug-free medium. The intracellular drug concentrations are then monitored over time using flow cytometry, scintillation counters, etc.

Since the P-glycoprotein pump associated with MDR is an energy dependent process, we described total pump efflux using the following enzyme kinetics scheme:

$$\begin{aligned} E + 2\text{ATP} &\rightleftarrows E{\cdot}2\text{ATP} \\ E{\cdot}2\text{ATP} + S_{(\text{inside})} &\rightleftarrows E{\cdot}2\text{ATP}\cdot S \\ E{\cdot}2\text{ATP}\cdot S &\rightleftarrows E + 2\text{ADP} + 2\text{P} + S_{(\text{outside})} \end{aligned} \tag{3}$$

E is the concentration of the P-glycoprotein pump and S is the concentration of the substrate (chemotherapeutic drug). Both concentrations are measured at the cytoplasm–membrane interface. The rate equation for the first reaction is given by the typical Michaelis–Menten formula

$$V_1 = \frac{V_{\text{ATP}\cdot\text{ATP}}}{K_A + \text{ATP}} \tag{4}$$

The rate equation for the second reaction is given by

$$V_2 = \frac{V_S \cdot S}{K_M + S} \tag{5}$$

Because the total flux of substrate out of the cell is limited by the slower of the two rates, V_1 and V_2

we define V_{MAX}, the maximum efflux rate, as the minimum of the V_1 and V_2. Thus, the overall flux, F, is given by

$$F = \frac{V_{MAX} \cdot \text{ATP} \cdot S}{(K_A + \text{ATP})(K_M + S)} \tag{6}$$

The level of ATP in a resting cell is maintained dynamically by conversion from ADP and free phosphate. For the purposes of the model, we assumed that the cell is a homogeneously mixed compartment packed with enzyme processes which maintain this homeostatic condition. We also assumed (for numerical purposes) that this array of enzyme reactions could be conglomerated into a single energy maintenance process which follows Michaelis–Menten kinetics. Mathematically, then, the energy pools of a resting cell can be described by:

$$\begin{aligned} \frac{d\text{ATP}}{dt} &= -\frac{V'\text{ATP}}{K' + \text{ATP}} + \frac{V^*\text{ADP}}{K^* + \text{ADP}} \\ \frac{d\text{ADP}}{dt} &= \frac{V'\text{ATP}}{K' + \text{ATP}} - \frac{V^*\text{ADP}}{K^* + \text{ADP}} \end{aligned} \tag{7}$$

where (V^*, K^*) and (V', K') are the Michaelis-Menten constants for the ADP–ATP conversion process.

When a cell is challenged with a cytotoxic agent, the P-glycoprotein begins pumping the drug from the cytoplasm into the interstitial space. The ATP pool is decreased by two molecules for each molecule of drug pumped from the cytoplasm. Consequently, for each molecule of drug pumped from the cell two molecules of ADP are produced. Therefore, in a challenged cell:

$$\begin{aligned} \frac{d\text{ATP}}{dt} &= -2F - \frac{V'\text{ATP}}{K' + \text{ATP}} + \frac{V^*\text{ADP}}{K^*\text{ADP}} \\ \frac{d\text{ADP}}{dt} &= 2F + \frac{V'\text{ATP}}{K' + \text{ATP}} - \frac{V^*\text{ADP}}{K^* + \text{ADP}} \end{aligned} \tag{8}$$

Here F represents the total flux (in molecules/second) of the active drug.

Thus, the entire pump and its energy dependent machinery, are given as follows:

$$\frac{\partial S}{\partial t} - \alpha \frac{\partial^2 S}{\partial x^2} - F = 0$$

$$F = \frac{V_{MAX} \cdot \text{ATP} \cdot S}{(K_A + \text{ATP})(K_M + S)}$$

$$\begin{aligned} \frac{d\text{ATP}}{dt} &= -2F - \frac{V'\text{ATP}}{K' + \text{ATP}} + \frac{V^*\text{ADP}}{K^* + \text{ADP}} \\ \frac{d\text{ADP}}{dt} &= 2F + \frac{V'\text{ATP}}{K' + \text{ATP}} - \frac{V^*\text{ADP}}{K^* + \text{ADP}} \end{aligned} \tag{9}$$

where α is the normal diffusion rate constant for drug through the membrane (from the cytoplasm to the interstitial space) and x represents the perpendicular radial distance through the membrane from inside to out.

The spatial characteristics of the model assume that the concentration of substrate in the cytoplasm is derived from a well-mixed compartment, and that the concentration of substrate at the internal membrane surface is representative of the entire cytoplasmic concentration. A similar assumption is made about the external cell surface. We also assumed a normalized cell volume so that we could deal with concentrations (data from the literature were available in those forms) in a straightforward manner.

What this model says then, is that a drug would normally leave a preloaded cell via diffusion proportional to rate α. This assumption is similar to that made by Spolestra *et al.* (1992). Mathematically, simple diffusion over a spatial distance, x (in our case, radially through a membrane) is described by the partial differential equation given in System (9) when $F = 0$. To facilitate this diffusion, i.e. to model the active P-glycoprotein pump, we added the flux term, F. The derivation of the flux term is the crux of the model. It is this term which explicitly accounts for the energy dependence of the pump and its binding characteristics for the drug. Suspected inhibitors designed to act on the pump should, therefore, act on this flux term. Therefore, characterization of flux is necessary if one is to describe MDR reversal in a mechanistic way (see below).

Numerical simulations were run to confirm the reasonableness of the model. A drug washout experiment was mimicked. The pre-loaded cells were allowed to equilibrate in fresh medium, and the cytoplasmic concentration profiles for the target

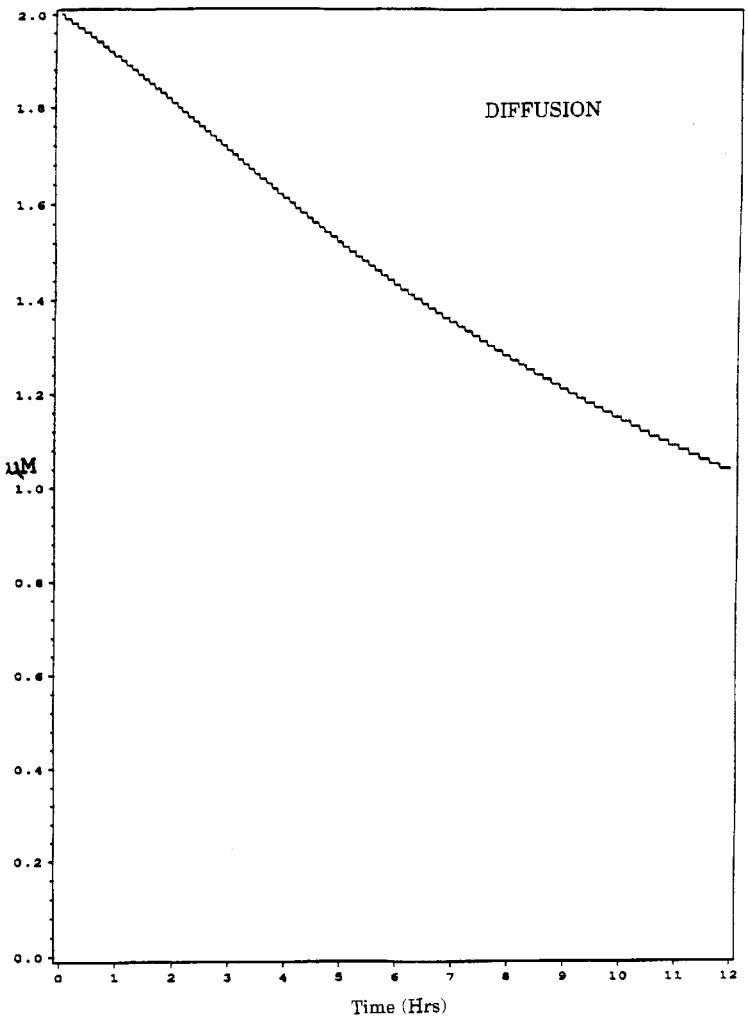

FIGURE 1 Concentration-time curves for simple diffusion kinetics from the cytoplasm. Time axis is measured in hours, and the half-life of the active agent in the cytoplasm is 12 hours.

drug were plotted over time (see Figures 1-3). Without the pump, the half-life of drug, as defined by pure diffusion from the cytoplasm, was 12 hours (Figure 1). With the pump, the half-life was about 30 minutes (similar to the dynamics we have observed in laboratory experiments; see Figure 2 and note the change of time scale). Rate constants for these simulation studies were derived from both laboratory measurements and from standard literature estimates (cited in the references of Michelson and Slate, 1992).

These estimates include: (1) The diffusion rate out of the cell (assumed to have exponential shape and a half-life of about 12 hours); (2) the rate limiting velocity of the entire pumping system (i.e. the minimum of V_1 and V_2), given as 64 micromolar per minute — V_A is approximately 375 micromolar per minute for ATP and V_S is 64 micromolar per minute for vincristine; (3) K_M, the Michaelis-Menten binding constant for drug — $K_M = 0.6$-0.7 micromolar for vincristine and approximately 1.1 micromolar for vinblastine; (4) K_{ATP}, the Michaelis-Menten binding constant for ATPase activity of the pump, is approximately 150 micromolar; (5) Initial equilibrium levels of ATP in the cell are between 500 and 600 micromolar.

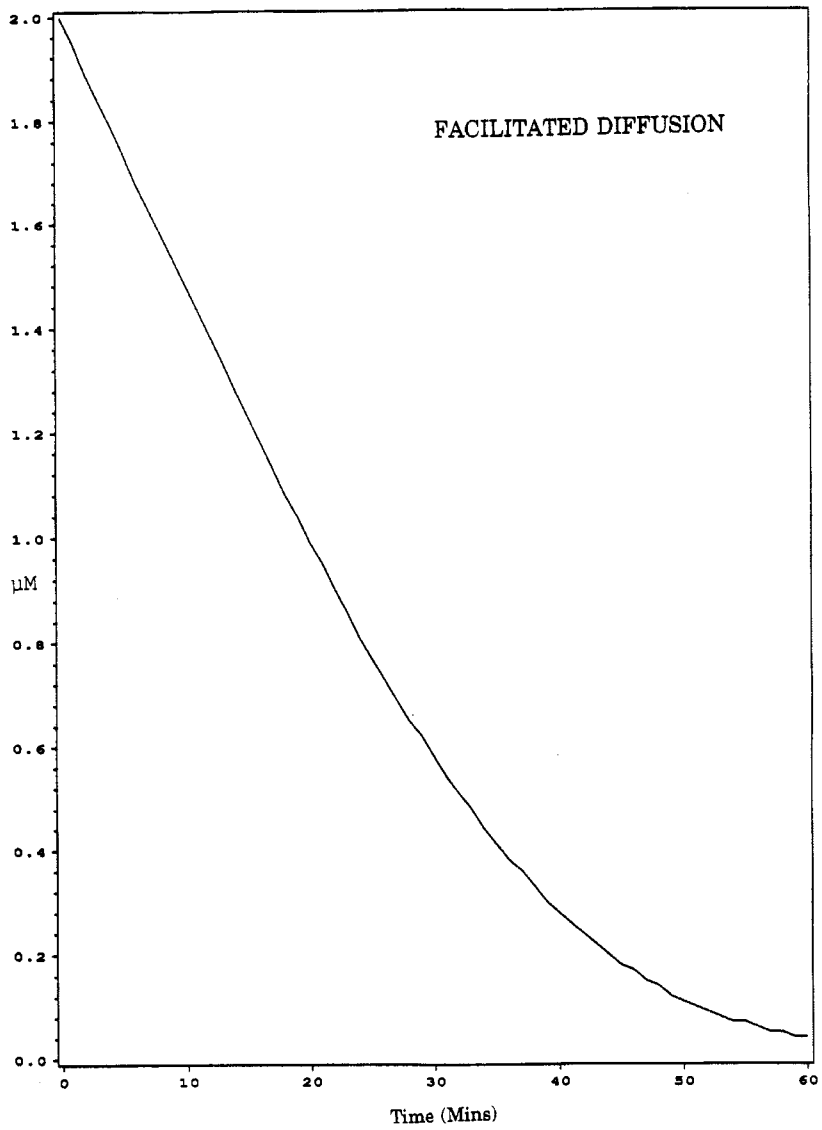

FIGURE 2 Concentration-time curves for facilitated diffusion kinetics from the cytoplasm. Time axis is measured in minutes, and the half-life of the active agent in the cytoplasm is approximately 30 minutes (corresponding to laboratory measurements with Adriamycin).

The most difficult parameters to estimate were the Michaelis-Menten parameters for the conglomerated enzyme system which maintains the ATP-ADP balance in the cell. To maintain the cell, hundreds of energy consuming processes occur simultaneously. The restoration of ATP levels is carried out via a number of pathways, some of which are specific for the energy-expending activity involved, some not. The simulations assumed that in the unchallenged cell, ATP and ADP are in a fairly steady state. The Michaelis-Menten constants are 300 micromolar for the ATP to ADP reaction and 270 micromolar for the ADP to ATP reaction. These values were based on conglomerate estimates for pyruvate transferase, polyphosphate kinase, and adenylate kinase as derived from standard enzyme tables.

One of the questions raised during our analysis was whether the energy dependence of the pump provided

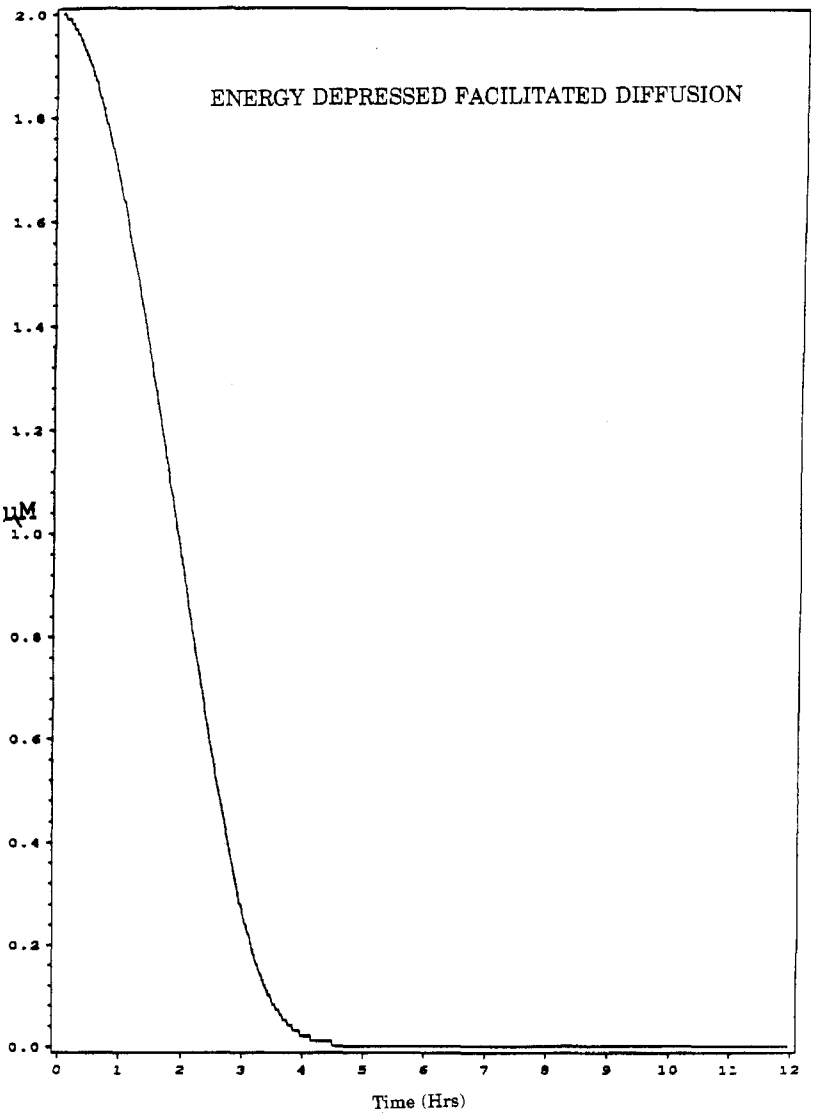

FIGURE 3 Concentration-time curves for facilitated diffusion kinetics from the cytoplasm in energy-deprived cells. Time axis is measured in hours, and the half-life of the active agent in the cytoplasm is approximately 2 hours. Note, the non-exponential shape of the curve.

a reasonable target for inhibition and reversal of MDR. To that end we simulated a cell depleted of all its ATP while keeping the ADP levels constant. This is a worst case scenario for this type of therapy. The results of this study are shown in Figure 3, which shows the time-dependent concentration curve for the substrate drug in an energy deprived cell. Further analyses suggest that as long as the rate at which ADP and inorganic phosphate can be joined to re-establish ATP exceeds the rate at which the pump can bind and extrude the substrate drug (64 micromolar per minute in these particular studies) then the ATP levels of the cell can be maintained at levels high enough to assure adequate pumping. And even though the pump is not as efficient as that observed in the unperturbed state (compare Figures 2 and 3), it is probably efficient enough to insure decreased levels of drug at the cellular target sites.

MODEL FOR REVERSAL OF MDR

Though the energy specific pathway may represent a potential target for resistance reversal, this strategy completely ignores the fact that manipulating cellular energy systems needs to be exquisitely precise for one to minimize toxicity *in vivo*. Our objection to this means of MDR reversal, however, is based more upon the theoretical fact that, with respect to ATP levels, the pump is, in fact, a self-regulating mechanism.

Once a cytotoxic challenge begins, and if sufficient levels of ATP and target drug are around, the pump will become saturated, forcing flux to some positive constant, F^*, which is less than or equal to V_{MAX}. During the transition phase from a completely inactive pump to one which is totally saturated, the steady-state concentrations of ATP and ADP shift in their phase space towards the ADP axis. If there is enough ATP present, when the pump is fully saturated flux equals F^*, and a steady state is established. If the level of drug in the cytoplasm is such that the energy control mechanisms can maintain ATP levels sufficient for the pump to function, all the drug will be pumped out, flux will tend to zero, and the original ATP–ADP steady state will be re-established. If, on the other hand, ATP levels are depressed or the amount of drug in the cytoplasm is such that pumping it out begins to exhaust the ATP pool, then flux will decrease appropriately (via the V_{MAX} term), and the pump slows. When that occurs, overall flux is re-established at a new lower level, and when all the drug is pumped out of the cell, the flux again tends to zero, and the original ATP–ADP steady state is re-established.

Given that the energy pool is probably a suboptimal target for MDR reversal, the question arose as to how one would inhibit the P-glycoprotein pump. A possible inhibitor could:

1. Attack the outward diffusion kinetics through the membrane,
2. Attack the transport activity of the pump at the inner surface of the membrane (e.g. its ability to bind drug), And/or
3. Attack the efflux efficiency of the pump by 'clogging' the transmembrane pore complex extracellularly.

Strategy 2 is the one being pursued most actively in the clinic. Essentially, this is the motivation for trying the calcium channel blocker, verapamil, and other channel-type inhibitors as MDR reversal agents. To accommodate these newer therapeutic strategies, we extended our original model to include theoretical reversal agents (Michelson and Slate, 1994).

Suppose one introduces a competitive inhibitor, I, into the system. Then, to accommodate this inhibition, the V_2 equation above (equation 5), must be altered as follows:

$$V_2 = \frac{V_1 \cdot S}{K_M[1 + (I/K_I)] + S} \tag{10}$$

where K_I is the dissociation constant of the P-glycoprotein-inhibitor complex. Efflux thus becomes

$$F = \frac{V_{MAX} \cdot \mathrm{ATP} \cdot S}{(K_A + \mathrm{ATP})[K_M(1 + (I/K_I)) + S]} \tag{11}$$

Adding inhibitor diffusion and efflux to the original system yields:

$$\begin{aligned}
&\frac{\partial S}{\partial t} - \alpha \frac{\partial^2 S}{\partial x^2} - F_1 = 0 \\
&\frac{\partial I}{\partial t} - \beta \frac{\partial^2 I}{\partial x^2} - F_2 = 0 \\
&F_1 = \frac{V_{MAX} \cdot \mathrm{ATP} \cdot S}{(K_A + \mathrm{ATP})[K_M(1 + (I/K_I)) + S]} \\
&F_2 = \frac{V_{MAX} \cdot \mathrm{ATP} \cdot I}{(K_A + \mathrm{ATP})[K_I(1 + (S/K_S)) + I]} \\
&\frac{\mathrm{dATP}}{\mathrm{d}t} = -2(F_1 + F_2) + \frac{V^* \mathrm{ADP}}{K^* + \mathrm{ADP}} \\
&\frac{\mathrm{dADP}}{\mathrm{d}t} = 2(F_1 + F_2) - \frac{V^* \mathrm{ADP}}{K^* + \mathrm{ADP}}
\end{aligned} \tag{12}$$

where β is the diffusion constant for the inhibitor, F_1 is the efflux of the active drug, and F_2 is the efflux of the inhibitor. Since the same fixed number of ATP molecules is required to transport each molecule out of the cytoplasm (whether S or I), and since the two substrates are competitive inhibitors, a total

efflux term, $2(F_1+F_2)$, must be added to the energy conversion equations.

A similar analysis was performed for a non-competitive inhibitor. By replacing V_2 in equation (5) with

$$V_2 = \frac{V_1 \cdot S}{(K_M + S)(1 + (I/K_I))} \tag{13}$$

one re-derives the diffusion equation with new flux terms

$$F_1^* = \frac{V_{MAX} \cdot ATP \cdot S}{(K_A + ATP)[(K_M + S)(1 + (I/K_I))]}$$

$$F_2^* = \frac{V_{MAX} \cdot ATP \cdot I}{(K_A + ATP)[(K_I + I)(1 + (S/K_S))]} \tag{14}$$

Simple inspection of equations (10) and (13) shows that the difference between a competitive inhibitor and non-competitive one is that a competitive inhibitor increases K_M by a factor of $(1+I/K_I)$, shifting the half-maximum concentration to the right in concentration space. A non-competitive inhibitor, on the other hand, decreases the transport (reaction) rate, V_{MAX}, by a factor of $1/(1 + I/K_I)$. In each case, the concentration of I is normalized to its own binding affinity, I/K_I. For pure competition, the substrate competes with the competitive inhibitor, and will eventually overcome the inhibition given a high enough concentration. For the non-competitive inhibitor, no amount of substrate can overcome the blockade; the actual reaction rate is depressed as long as inhibitor is present.

The questions we then asked were, What should the initial loading of an inhibitor be given its (1) inhibitive character and (2) its affinity for the binding site? Is one type of inhibitor better than any other? Does one need to worry about the timing and strategy for administration?

We found that over a fixed range of initial loading and inhibitor affinities, non-competitive inhibitors are more efficacious than competitive inhibitors, but that these advantages are only evident at the lower loading concentrations. If enough competitive inhibitor can get into the tumor cells prior to drug therapy, and if it can be maintained there at high enough levels despite the pump's activity, then competitive inhibitors with proper pharmacokinetic profiles may be acceptable as clinically relevant MDR reversal agents.

We also looked at the question of whether a one site non-competitive inhibitor — one which is pumped out of cell — is significantly different from an inhibitor which is not pumped from the system, an allosteric non-competitive inhibitor. Our simulation studies showed that if an inhibitor is not extruded by the pump, then its advantage over a compound with similar inhibitory properties is exerted only at high affinity-low concentration combinations. If both inhibitors are of low affinity or if the concentration of each is high enough, no real advantage was observed.

IMPLICATIONS OF THE THEORY

A cautionary word is in order before one ventures to interpret the theoretical results described above. First, the models of Spolestra, Horio, and Michelson and Slate are all variations upon the Michaelis–Menten transport theme. Each, in its own way, describes transport as a saturable, rate-limited, phenomenon depending upon the mass-action chemistry of the pump molecule. The differences between the three models reside in the detailed descriptions of diffusion, energy dependence, etc. Each is based upon an experimental design of choice.

Second, these models are evolving constantly. For example, Michelson and Slate's earlier results (1989, 1991) did not adequately mimic the activity or inhibition of P-glycoprotein pump. We did not account for the stereochemistry of the ATP binding sites, the nature of the inhibitor (competitive, non-competitive, or allosteric), the stoichiometry of the drug binding site(s), etc. When we added these extra layers of complexity to our theory, a more sophisticated model emerged (Michelson and Slate, 1992, 1994). More complicating factors have yet to be included.

For example, in the Michelson and Slate model (1994) outlined above, we modeled diffusion as the only other mechanism by which inhibitor is lost. This is especially important when considering

the case of a two-site allosteric non-competitive compound. But suppose the inhibitor is the target of biochemical degradation, metabolism, etc. Then one should expect that target drug efflux will accelerate with inhibitor degradation, and might even approach that observed when a one-site non-competitive inhibitor is used. In a worst case scenario, if the degradation rate of the two-site inhibitor is faster than the efflux rate of the one-site inhibitor, the effectivity of the stable one-site inhibitor might surpass that of the two-site one.

The models that have been developed thus far can only be used to make simple predictions about how MDR reversal agents could be optimally employed to block pumping activity. In order to create a more realistic model, one must consider other complexities of P-glycoprotein function. For example, How many binding sites are there and what are their structures? How does binding affect ATPase activity? Are all P-glycoprotein molecules identical? How does post-translational modification of the protein affect its transport and binding characteristics? It is the evolution of these questions, derived from the experimentalist, that drives the theorist to develop newer mathematical models of MDR and its reversal.

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