Interdependent Effect of P-Glycoprotein-Mediated Drug Efflux and Intracellular Drug Binding on Intracellular Paclitaxel Pharmacokinetics: Application of Computational Modeling

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ABSTRACT

Intracellular concentration of paclitaxel is determined by the extracellular drug concentration, the level of the mdr1 P-glycoprotein (Pgp), and binding to intracellular proteins including tubulins/microtubules. The present study used a computational method to examine the effects of these factors, singly and in combination, on intracellular paclitaxel pharmacokinetics. The study was performed using our previously described intracellular pharmacokinetic model. The parameters representing Pgp-mediated drug efflux and intracellular drug binding (i.e., number of Pgp and binding sites and binding affinity) were altered systematically and used to generate computer simulations depicting the effects of biological parameters such as drug efflux transporters, drug binding sites, and binding affinity on intracellular paclitaxel pharmacokinetics at clinically relevant extracellular (e.g., plasma) drug concentrations. The simulation results indicate that all four factors played a role in determining the intracellular drug accumulation. The rank order of the importance of these parameters was extracellular drug concentration \( \gg \) intracellular binding capacity \( \gg \) intracellular binding affinity \( \gg \) Pgp expression. The results further showed that omission of one or more of these factors in the experimental design would lead to erroneous conclusions on the importance of other factors, as simultaneous changes in more than one parameter altered the relative importance and offset the effects of other parameters. In summary, results of the present study demonstrate the use of computational modeling to depict the effects of biological parameters such as drug efflux transporters, drug binding sites, and binding affinity on intracellular accumulation and retention of drugs that bind to cellular components.

Paclitaxel, one of the most important anticancer drugs developed in the past two decades, is active against multiple types of human solid tumors (Rowinsky et al., 1993). Paclitaxel enhances tubulin polymerization, promotes microtubule assembly, binds to microtubules, stabilizes microtubule dynamics, induces mitotic block at the metaphase/anaphase transition, and induces apoptosis (Parness and Horwitz, 1981; Manfredi et al., 1982; Jordan et al., 1993, 1996; Derry et al., 1995).

Paclitaxel resistance in several resistant sublines is correlated with reduced intracellular drug accumulation compared with the sensitive parent cell line (Lopes et al., 1993; Bhalla et al., 1994; Jekunen et al., 1994; Riou et al., 1994; Speicher et al., 1994). Changes in the levels of mdr1 P-glycoprotein (Pgp) and tubulin, as well as tubulin mutations, affect the intracellular paclitaxel accumulation and are considered the major mechanisms of paclitaxel resistance (Minnotti et al., 1991; Jachez et al., 1993; Bhalla et al., 1994; Haber et al., 1995; Dumontet et al., 1996; Giannakakou et al., 1997; Parekh et al., 1997; Ranganathan et al., 1998; Dumontet and Sikic, 1999). These earlier studies used cell lines in which changes in Pgp or tubulin were induced either by drug treatment or by gene transfection and, accordingly, studied each of these parameters as separate entities. However, multiple groups of investigators have shown that Pgp and tubulins/microtubules are often altered simultaneously in paclitaxel-resistant cells (Bhalla et al., 1994; Haber et al., 1995; Dumontet et al., 1996). The effect of simultaneous changes in Pgp and tubulin on the relative importance of the other parameters on intracellular accumulation is not known and is the subject of the present study.

As discussed in our previous publications (Kuh et al., 2000; Jang et al., 2001), the accumulation of paclitaxel in cells is determined by several processes, i.e., saturable and nonsaturable drug binding to intracellular and extracellular proteins, cell density, concentrations of tubulins/microtubules, and other cellular components.

ABBREVIATIONS: mdr1, multidrug resistance gene 1; Pgp, P-glycoprotein; \( J_{\text{max}} \), maximum efflux rate by Pgp per cell; \( B_{\text{max,c}} \), the number of saturable drug binding sites in cells; \( K_{\text{d,c}} \), dissociation constants for drug binding to saturable binding sites in cells.
which are the major intracellular drug binding sites, and drug transport by passive diffusion and by the energy-dependent Pgp-mediated efflux. These processes, in turn, are dependent on the extracellular and intracellular drug concentrations, and are interrelated. For example, the contribution of Pgp-mediated paclitaxel efflux to the overall efflux decreases with increasing extracellular drug concentrations (Jang et al., 2001). Paclitaxel binding to tubulins/microtubules is saturable, and the bound fraction diminishes with increasing extracellular drug concentrations (Manfredi et al., 1982; Jordan et al., 1993; Kuh et al., 2000). In addition, changes in the amount of tubulins/microtubules result in parallel changes in the intracellular paclitaxel binding capacity, and changes in the composition of β-tubulin isoforms alter the paclitaxel binding affinity, whereas mutation of β-tubulin reduces the binding affinity (Haber et al., 1995; Dumontet et al., 1996; Derry et al., 1997; Giannakakou et al., 1997; Ranganathan et al., 1998). These tubulin-related changes alter the fraction of free drug available for efflux and therefore may alter the importance of Pgp efflux. An examination of the effects of these various determinants of intracellular paclitaxel accumulation has not been possible because of the inherent difficulty of measuring cellular free drug concentrations with systematic changes in the levels of Pgp and/or tubulins/microtubules, and/or the drug binding affinity to tubulins/microtubules.

The present study used a computational approach to depict the effects of Pgp efflux and drug binding to tubulins/microtubules, singly and in combination, on intracellular paclitaxel accumulation. The studies were performed using our previously described intracellular pharmacokinetic model and model parameters obtained for human breast MCF7 cells and the corresponding mdr1-transfected subline (BC19), which showed a >9-fold higher Pgp level (Fairchild et al., 1990; Li and Au, 2001). The parameters representing Pgp-mediated drug efflux and intracellular binding (i.e., number of Pgp molecules, number of paclitaxel binding sites, and paclitaxel binding affinity) were altered, singly or in combination, and were used to generate computer simulations. The results demonstrate that the effects of these parameters on intracellular paclitaxel accumulation depended on extracellular drug concentrations and were interdependent. These data further provide quantitative measurements of the relationship between tubulins/microtubules, kinetics of Pgp efflux, extracellular (e.g., plasma) drug concentration, and intracellular drug accumulation and retention.

**Materials and Methods**

**Intracellular Pharmacokinetic Model.** We have constructed an intracellular pharmacokinetic model describing paclitaxel accumulation in cells, as a function of drug concentration and time. Detailed discussions of the theoretical basis for the model have been provided in previous publications (Kuh et al., 2000; Jang et al., 2001). Briefly, the model included 1) saturable binding of paclitaxel to proteins in the extracellular compartment (Song et al., 1996), 2) saturable and nonsaturable binding of paclitaxel to cellular components (Manfredi et al., 1982), 3) time- and concentration-dependent changes in microtubule mass (Jordan et al., 1993, Derry et al., 1995), 4) time- and concentration-dependent changes in cell number (Kuh et al., 2000), and 5) saturable Pgp-mediated drug efflux (Jang et al., 2001). We assumed that 1) drug uptake into cells is by passive diffusion, 2) drug efflux from cells is by passive diffusion and Pgp-mediated efflux, and 3) only unbound drug molecules participate in the uptake and efflux processes.

Equations 1 and 2 describe the time-dependent changes in intracellular and extracellular drug concentrations, respectively. Derivations of these equations have been described elsewhere (Kuh et al., 2000; Jang et al., 2001).

\[
\frac{dC_{\text{total},c}}{dt} = \left( CL_{d,c} \cdot C_{\text{free,c}} - CL_{d,m} \cdot C_{\text{free,m}} - \frac{J_{\text{max}} \cdot C_{\text{free,c}}}{K_{\text{d,Pgp}} + C_{\text{free,c}}} \right) \cdot \frac{1}{V_{\text{one cell}}} - k_{\text{cell number}} \cdot C_{\text{total,c}} \tag{1}
\]

\[
\frac{dC_{\text{total,m}}}{dt} = \left( -CL_{d,m} \cdot C_{\text{free,m}} + CL_{d,c} \cdot C_{\text{free,c}} + \frac{J_{\text{max}} \cdot C_{\text{free,c}}}{K_{\text{d,Pgp}} + C_{\text{free,c}}} \right) \cdot ICN \cdot e^{(\text{bolus intake})^2} \cdot \frac{1}{V_m} \tag{2}
\]

Where

\[
C_{\text{free,m}} = \frac{-(K_{d,m} + B_{\text{max,m}} - C_{\text{total,m}})}{2} + \sqrt{(K_{d,m} + B_{\text{max,m}} - C_{\text{total,m}})^2 + 4 \cdot K_{d,m} \cdot C_{\text{total,m}}} \frac{2}{2} \cdot (1 + \text{NSB}) \cdot K_{\text{d,c}} \cdot C_{\text{total,c}}
\]

\[
C_{\text{free,c}} = \frac{-A + \sqrt{A^2 + 4 \cdot (1 + \text{NSB}) \cdot K_{\text{d,c}} \cdot C_{\text{total,c}}}}{2 \cdot (1 + \text{NSB})}
\]

\[
A = (1 + \text{NSB}) \cdot K_{d,c} + B_{\text{max,c,initial}} \cdot (1 + h_{\text{max,c}} \cdot t) - C_{\text{total,c}}
\]

C_{\text{total,c}} and C_{\text{total,m}} are total (i.e., free plus bound) drug concentrations in cells and medium, respectively. A separate study found that >95% of the cell-associated paclitaxel was accounted for the drug located in cellular components and organelles (J. Kim and J. L.-S. Au, unpublished observations). Hence, intracellular drug concentration equals C_{\text{total,c}} - C_{\text{free,c}} and C_{\text{free,m}} represent free drug concentrations in cells and medium, respectively. V_c is the total cell volume. V_m is the volume of medium. CL_{d,c} is the clearance of free drug per cell by passive diffusion. J_{\text{max}} is maximum efflux rate by Pgp per cell. K_{\text{d,Pgp}} is the dissociation constant of Pgp-mediated efflux. B_{\text{max,c}} and B_{\text{max,m}} are the numbers of saturable drug binding sites in cells and medium, respectively. K_{d,c} and K_{d,m} are dissociation constants for drug binding to saturable binding sites in cells and medium, respectively. NSB is the proportionality constant for nonsaturable drug binding in cells. V_{\text{one cell}} is the average volume of a single cell. ICN is the initial cell number at time 0, k_{\text{cell number}} is the rate constant for changes in cell number. B_{\text{max,c,initial}} is B_{\text{max,c}} at time 0. k_{\text{max,c}} is the rate constant for changes in B_{\text{max,c}}.

**Simulation Studies.** Equations 1 and 2 were used to simulate the paclitaxel concentration-time profiles in cells as a function of initial extracellular drug concentration, intracellular drug binding capacity and affinity, and maximum Pgp efflux rate. These simulations provided a quantitative measure of the effects of maximum Pgp efflux and drug binding, individually and collectively, on intracellular paclitaxel pharmacokinetics. For the remainder of this report, changes in the maximum Pgp efflux rate are assumed to reflect changes in Pgp expression.

All simulations used an initial cell number of 1 million and a medium volume of 1 ml. Initial simulations were performed using the parameter values experimentally obtained in MCF7 and BC19 cells. At each condition, intracellular drug concentration-time profiles were simulated, and the intracellular drug concentrations at 4 h, which is the time interval when intracellular and extracellular drug concentrations reached an apparent steady state under most conditions, were compared (Jordan et al., 1996; Kuh et al., 2000; Jang et al., 2001). WIN-NONLIN (SCI Software, Lexington, KY) was used.
used for simulations. The parameter values were further altered according to literature reports, as described below and summarized in Table 1.

Evaluation of the effect of extracellular concentrations was conducted using four initial extracellular concentrations, i.e., 1, 2, 10, and 1000 nM, which are within the clinically relevant range and the range in which the role of Pgp-mediated efflux varies from major to minor relative to diffusion (Jang et al., 2001).

Changes in the amount of tubulins/microtubules were represented by altering the number of saturable intracellular binding site \( B_{\text{max,c}} \). The value of \( B_{\text{max,c}} \) in MCF7 and BC19 cells was 60 \( \mu \)M (Kuh et al., 2000; Jang et al., 2001). Resistant cells selected by continuous paclitaxel treatment showed a 2-fold increase in the tubulin amount \( \rho \text{,c} \). Hence, simulations used three \( B_{\text{max,c}} \) values: low binding capacity (30 \( \mu \)M), moderate binding capacity (60 \( \mu \)M), and high binding capacity (120 \( \mu \)M).

Changes in the drug binding affinity of tubulins/microtubules were represented by changes in the value of the dissociation constant of saturable intracellular drug binding \( K_{d,c} \). The \( K_{d,c} \) value in MCF7 and BC19 cells was 5 nM. To the best of our knowledge, there are no data on the binding affinity of paclitaxel to tubulins/microtubules in parent and resistant cancer cells. A study using different \( \beta \)-tubulin isotypes purified from bovine brain tubulin showed a 2- to 6-fold difference in the number of bound paclitaxel molecules required for tubulin polymerization (Derry et al., 1997). Hence, simulations used two \( K_{d,c} \) values: low binding affinity (20 nM) and high binding affinity (5 nM).

Changes in Pgp expression were represented by altering the maximum Pgp-mediated efflux rate \( J_{\text{max,c}} \) from 0 to 280 \( \times 10^{-6} \) pmol/h. These \( J_{\text{max,c}} \) values were observed in MCF7 cells with negligible Pgp expression and in \( mdr1 \) gene-transfected BC19 cells (Jang et al., 2001).

## Results

**General Comments.** The simulation results on the effects of extracellular drug concentrations, intracellular drug binding capacity and binding affinity, and Pgp expression on intracellular paclitaxel accumulation, as a function of time, are shown in Fig. 1. As we showed previously (Kuh et al., 2000), the time course of intracellular paclitaxel accumulation varies with extracellular drug concentration. At low extracellular concentration (<10 nM), the intracellular concentrations initially increased, reaching an apparent steady state at about 4 h, and, due to the formation of new cells (due to incomplete inhibition of cell proliferation) and redistribution of paclitaxel over the increased cellular mass, gradually declined after 12 h. At high extracellular concentrations of 1000 nM, the intracellular concentration rose rapidly to reach an apparent steady state at 4 h, and, due to the drug-induced enhancement of tubulin/microtubule levels and, consequently, the increase in intracellular drug binding, continued to increase slowly by ~35% from 4 to 24 h. A comparison of the intracellular concentrations, achieved at 4 h or before changes in cell number or tubulin/microtubule content occurred, showed that clinically relevant changes in each of the four above-mentioned parameters resulted in 1.04- to 1243-fold changes in intracellular drug concentration (Table 1).

As shown in Table 1, the changes in intracellular concentration due to increases in extracellular concentration are different in cells that express different Pgp levels, different binding capacity, or different binding affinity. Hence, the data in Table 1 were further analyzed to depict the changes of intracellular concentration for various combinations of the four parameters. The results are summarized in Tables 2 to 5. For example, Table 2 shows the effect of changing the extracellular concentration in the presence of different Pgp expression (low or high), different binding affinity (low or high), or different binding capacity (low, moderate, or high). Similarly, Tables 3 to 5 show the effects of intracellular binding capacity, binding affinity, and Pgp expression in the presence of different values of the other parameters, respectively.

In the following discussions, the numerical values of the four parameters are as follows: initial extracellular concentrations, 1 (low), 2 (low), 10 (moderate), and 1000 nM (high); binding capacity or \( B_{\text{max,c}} \) 30 (low) 60 (moderate), and 120 \( \mu \)M (high); binding affinity or \( K_{d,c} \) 20 (low) and 5 nM (high); and Pgp expression or \( J_{\text{max,c}} \) values, 0 (low) and 280 \( \times 10^{-6} \) pmol/h (high).

### Effect of Extracellular Drug Concentrations on Paclitaxel Accumulation in Cells.

As expected, higher extracellular paclitaxel concentrations resulted in higher intracellular concentrations; 2-, 10-, and 1000-fold increases in extracellular drug concentration yielded, on average, 2-, 9.9-, 5.4-, and 13.2-fold increases in intracellular drug concentration, respectively.
and 573-fold increases in intracellular accumulation, respectively (Table 2).

Two differences were observed at low (1–10 nM) and high (1000 nM) extracellular drug concentrations. First, when extracellular paclitaxel concentration increased from 1 to 2 or 10 nM, intracellular concentration increased linearly with extracellular concentration. However, at a much higher extracellular concentration of 1000 nM, intracellular concentration increased linearly with extracellular concentration only under the condition of low binding affinity and high Pgp expression (irrespective of binding capacity), whereas all other conditions resulted in less than a 1:1 linear increase (Tables 1 and 2). Second, at low extracellular concentration range (1–10 nM), the effect of extracellular concentrations on intracellular accumulation was constant regardless of the values of the other three parameters. In comparison, when extracellular paclitaxel concentration increased from 1 to 1000 nM, the effect of extracellular concentrations on intracellular accumulation was dependent on the values of the other three parameters, was greater at low intracellular drug binding affinity compared with high binding affinity (2-fold difference), and was greater at high Pgp expression compared with lower Pgp expression (2.5-fold difference), but was only slightly altered by a 4-fold change in binding capacity (<20% difference) (Table 2).

**Effect of Intracellular Drug Binding Capacity on Paclitaxel Accumulation in Cells.** In general, increases in binding capacity resulted in increased intracellular drug accumulation, whereas decreases in binding capacity resulted in decreased intracellular accumulation; a 2-fold increase in binding capacity yielded ~50% increase in intracellular accumulation and a 2-fold decrease produced a ~30% decrease (Table 3). The effect of binding capacity on intracellular drug accumulation was dependent on the values of the other three parameters. In comparison, when extracellular paclitaxel concentration increased from 1 to 1000 nM, the effect of extracellular concentrations on intracellular accumulation was dependent on the values of the other three parameters, was greater at low intracellular drug binding affinity compared with high binding affinity (2-fold difference), and was greater at high Pgp expression compared with lower Pgp expression (2.5-fold difference), but was only slightly altered by a 4-fold change in binding capacity (<20% difference) (Table 2).

**Fig. 1.** Application of computational model: effect of simultaneously changing Pgp expression, intracellular binding capacity, and binding affinity on intracellular drug accumulation. Simulations were performed at two initial extracellular concentrations, 1 and 1000 nM. The number of intracellular drug binding sites ($B_{\text{max},c}$) was altered from 60 to 30 and 120 μM. The dissociation constant, $K_{d,c}$, was altered from 5 to 20 nM. The maximum Pgp efflux rate, $J_{\text{max}}$, was altered from 0 to $280 \times 10^{-6}$ pmol/h.
<table>
<thead>
<tr>
<th>Initial Extracellular Drug Concentration</th>
<th>Overall</th>
<th>Pgp Expression</th>
<th>Binding Affinity</th>
<th>Binding Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-fold increase</td>
<td>Mean ± S.D. (fold)</td>
<td>+2.00 ± 0.005</td>
<td>+1.99 ± 0.004</td>
<td>+2.00 ± 0.004</td>
</tr>
<tr>
<td>Median (fold)</td>
<td>+2.00</td>
<td>+1.99</td>
<td>+2.00</td>
<td>+1.99</td>
</tr>
<tr>
<td>Range (fold)</td>
<td>+1.00</td>
<td>+0.00</td>
<td>+1.00</td>
<td>+0.00</td>
</tr>
<tr>
<td>10-fold increase</td>
<td>Mean ± S.D. (fold)</td>
<td>+9.21 ± 0.11</td>
<td>+9.85 ± 0.11</td>
<td>+9.60 ± 0.09</td>
</tr>
<tr>
<td>Range (fold)</td>
<td>+0.0</td>
<td>+0.0</td>
<td>+0.0</td>
<td>+0.0</td>
</tr>
<tr>
<td>1000-fold increase</td>
<td>Mean ± S.D. (fold)</td>
<td>+573 ± 383</td>
<td>+329 ± 91</td>
<td>+826 ± 400</td>
</tr>
<tr>
<td>Median (fold)</td>
<td>+432</td>
<td>+333</td>
<td>+813</td>
<td>+788</td>
</tr>
<tr>
<td>Range (fold)</td>
<td>+187 to +1243</td>
<td>+187 to +439</td>
<td>+428 to +1243</td>
<td>+324 to +1243</td>
</tr>
</tbody>
</table>

The table shows the effect of extracellular drug concentration on paclitaxel accumulation in cells. Simulations were performed as described in Table 1. The intracellular drug concentrations listed in Table 1 were used to calculate the changes resulting from altering the different parameters. For each parameter, the mean ± SD, median, and range of values (fold) for the multiple conditions as listed in Table 1 are shown. For example, for low or high binding affinity, the range encompasses the values for the following conditions: low and high Pgp expression and low, moderate, and high binding capacity.
TABLE 3
Effect of intracellular binding capacity on paclitaxel accumulation in cells
Simulations were performed as described in Table 1. The intracellular drug concentrations listed in Table 1 were used to calculate the changes resulting from altering the different parameters as described in Table 2. Values are mean ± SD, median, and range (%). Results of two extracellular drug concentrations (i.e., 1 and 1000 nM) are presented.

<table>
<thead>
<tr>
<th>Intracellular Binding Capacity</th>
<th>Difference Compared with Moderate Intracellular Binding Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2-fold increase</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D. (%)</td>
<td>+54 ± 24</td>
</tr>
<tr>
<td>Median (%)</td>
<td>+67</td>
</tr>
<tr>
<td>Range (%)</td>
<td>+9 to +75</td>
</tr>
<tr>
<td>2-fold decrease</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D. (%)</td>
<td>−34 ± 9</td>
</tr>
<tr>
<td>Median (%)</td>
<td>−36</td>
</tr>
<tr>
<td>Range (%)</td>
<td>−14 to −44</td>
</tr>
</tbody>
</table>

TABLE 4
Effect of intracellular binding affinity on paclitaxel accumulation in cells
Simulations were performed as described in Table 1. The intracellular drug concentrations listed in Table 1 were used to calculate the changes resulting from altering the different parameters as described in Table 2. Values are mean ± SD, median, and range (%). Results of two extracellular drug concentrations (i.e., 1 and 1000 nM) are presented.

<table>
<thead>
<tr>
<th>Intracellular Binding Affinity</th>
<th>Difference Compared with High Intracellular Binding Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4-fold decrease</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D. (%)</td>
<td>−28 ± 24</td>
</tr>
<tr>
<td>Median (%)</td>
<td>−17</td>
</tr>
<tr>
<td>Range (%)</td>
<td>−4.1 to −68</td>
</tr>
</tbody>
</table>

TABLE 5
Effect of maximum efflux rate by Pgp on paclitaxel accumulation in cells
Simulations were performed as described in Table 1. The intracellular drug concentrations listed in Table 1 were used to calculate the changes resulting from altering the different parameters as described in Table 2. Values are mean ± SD, median, and range (%). Results of two extracellular drug concentrations (i.e., 1 and 1000 nM) are presented.

<table>
<thead>
<tr>
<th>Maximum Efflux Rate by Pgp</th>
<th>Difference Compared with Low Pgp Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td>Increase from 0 to 280 × 10⁻⁶ pmol/h</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D. (%)</td>
<td>−39 ± 26</td>
</tr>
<tr>
<td>Median (%)</td>
<td>−29</td>
</tr>
<tr>
<td>Range (%)</td>
<td>−9.3 to −79</td>
</tr>
</tbody>
</table>

in cells with high Pgp expression \( (J_{\text{max}} \text{ of } 280 \times 10^{-6} \text{ pmol/h}) \), high binding capacity \( (B_{\text{max,e}} \text{ of } 120 \mu M) \), and high binding affinity \( (K_{d,e} \text{ of } 5 \text{ nM}) \) was higher than in cells with low Pgp \( (J_{\text{max}} \text{ of } 0 \text{ pmol/h}) \), low binding capacity \( (B_{\text{max,e}} \text{ of } 30 \mu M) \), and low binding affinity \( (K_{d,e} \text{ of } 20 \text{ nM}) \).

**Discussion**

Results of the present study demonstrate the use of computation, together with an intracellular pharmacokinetic model, to predict the intracellular drug concentration as a function of extracellular drug concentration and changes in tumor biological factors, including Pgp expression, and amount and binding affinity of tubulins/microtubules. The simulation results show that all of these four biological factors played a role in determining the intracellular drug accumulation. The rank order of the importance of these parameters on intracellular drug accumulation was extracellular drug concentration (average of 200, 9,900, and 57,300% increases in intracellular accumulation with 2-, 10-, and 1,000-fold increases in extracellular concentration, respectively) \( \gg \) intracellular binding capacity (average of 50% increase in intracellular accumulation with a 2-fold increase in binding capacity and 30% decrease with a 2-fold decrease) \( \gg \) intracellular binding affinity (average of 30% increase in intracellular accumulation with a 4-fold tighter binding affinity). In comparison, the effect of Pgp expression is relatively minor, because a >280-fold increase in the Pgp-mediated efflux rate produced only \( \sim 40\% \) decrease in intracellular accumulation. These results, in turn, suggest that although Pgp efflux, paclitaxel binding capacity, and affinity play a role in intracellular drug accumulation, their effects are far less important compared with extracellular drug concentration. Our results indicate that within the clinically relevant drug concentration range of 1 to 1,000 nM, the
presentation or delivery of paclitaxel to tumor cells, rather than tumor biological factors including Pgp expression, and amount and binding affinity of tubulins/microtubules, is the major determinant of intracellular drug accumulation.

Our results further indicate that the relative contributions of the four parameters to the intracellular paclitaxel accumulation were interdependent, as follows. The relationship between extracellular and intracellular drug concentrations was highly dependent on intracellular drug binding affinity and Pgp expression but was only slightly affected by intracellular binding capacity. The relationship between intracellular drug binding capacity and intracellular drug accumulation was highly dependent on the extracellular drug concentration and Pgp expression but was only slightly affected by intracellular binding affinity. The relationship between intracellular binding affinity and intracellular drug accumulation was highly dependent on extracellular drug concentration and Pgp expression but was only slightly affected by intracellular binding capacity. Finally, the relationship between Pgp expression and intracellular drug accumulation was highly dependent on extracellular drug concentration but was only slightly affected by intracellular drug binding capacity or intracellular binding affinity.

We have also shown that omission of one or more of these factors in the experimental design would lead to erroneous conclusions on the importance of other factors. For example, an experiment conducted using 1000 nM extracellular drug concentration would suggest binding affinity of tubulins/microtubules as an unimportant determinant of drug accumulation since a 4-fold change in this parameter resulted in only minor changes in drug accumulation, whereas a similar experiment conducted at 1 nM concentration would indicate the opposite. Likewise, simultaneous changes in the Pgp efflux rate, drug binding sites, and binding affinity may alter the relative importance and offset the effects of each other; e.g., the effect of a 3-fold increase in the Pgp efflux rate was offset by a 2-fold increase in intracellular saturable drug binding.

Hence, depending on the status of the intracellular drug binding, changes in intracellular drug accumulation may occur with or without changes in Pgp expression. Conversely, our results indicate that the importance of Pgp is dependent on the amount and drug binding affinity of tubulins/microtubules in cells as well as the extracellular drug concentration, and that changes in Pgp expression may or may not translate to altered drug accumulation. This may, in part, explain the controversy regarding the importance of Pgp in tumor sensitivity/resistance to paclitaxel; some studies indicate Pgp as an important prognostic indicator of tumor response, whereas other studies indicate the opposite conclusion (Arbuck et al., 1994; Fisher and Sikic, 1995). This issue deserves consideration since it is well known that paclitaxel-resistant cells show simultaneous changes in Pgp expression, total tubulin amount, and composition of tubulin isotypes (Haber et al., 1995; Dumontet et al., 1996).

In conclusion, we have demonstrated the complex interdependent effects of extracellular drug concentration, Pgp expression, intracellular drug binding capacity, and binding affinity on intracellular paclitaxel accumulation. Our results further demonstrate that omission of one or more of these factors may result in erroneous conclusions. We propose that studies on the effects of Pgp expression and changes in tubulins/microtubules on intracellular paclitaxel accumulation should include multiple extracellular drug concentrations within the clinically relevant range, and include evaluation of simultaneous changes of these factors. Furthermore, our simulation results suggest that within the clinically relevant drug concentration range, extracellular drug concentration is the most important determinant of intracellular drug accumulation. These results justify further studies to verify that changes in dosing or treatment schedule to provide greater drug delivery to tumor cells may be more effective than modifying tumor biological factors for increasing drug accumulation in tumor cells, and to establish pharmacokinetic-pharmacodynamic models to examine the schedule-depen-
dent antitumor activity of paclitaxel. Finally, the present study represents an attempt to use computational modeling to address the complex interplay between Pgp and drug binding to intracellular macromolecules. Such an approach can be expanded to evaluate other agents that are Pgp substrates and are highly bound to intracellular macromolecules.

References


