Effects of Orally Active Taxanes on P-Glycoprotein Modulation and Colon and Breast Carcinoma Drug Resistance

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Background: The taxane paclitaxel (Taxol) is often of limited efficacy in chemotherapeutic regimens because some cancer cells express high levels of the efflux pump, P-glycoprotein (Pgp), which removes the drug from the cells. The orally active paclitaxel analog IDN-5109 has been reported to overcome Pgp-mediated drug resistance. We tested whether IDN-5109 acts by modulating Pgp activity. Methods: Human MDA435/LCC6mdr1 and MDA435/LCC6 breast carcinoma cells, which express and do not express Pgp, respectively, were incubated with [3H]IDN-5109 and paclitaxel to determine intracellular drug accumulation. Flow cytometry was used to analyze intracellular retention of two Pgp substrates, rhodamine 123 (Rh-123) and doxorubicin, in both breast carcinoma cell lines and in human colon carcinoma cells (SW-620, DLD1, and HCT-15, whose Pgp levels vary) treated with different taxanes. The effects of IDN-5109 and paclitaxel on tumor growth in vivo were studied with the use of tumors established through xenografts of Pgp-expressing SW-620 and DLD1 cells in severe combined immunodeficiency mice. All statistical tests were two-sided. Results: Pgp-expressing cells treated with IDN-5109 or with the taxane-based drug resistance reversal agent tRA96023, which blocks Pgp activity, retained 8.1- and 9.4-fold more Rh-123 (P = .0001), respectively, and 1.7- and 1.9-fold more doxorubicin (P = .001), respectively, than cells treated with paclitaxel. Non-Pgp-expressing cells treated similarly demonstrated no increased retention of either substrate. MDA435/LCC6mdr1 cells retained 5.3-fold more [3H]IDN-5109 than [3H]paclitaxel after 2 hours (P = .01). IDN-5109 showed statistically significantly higher tumor growth inhibition than paclitaxel against the SW-620 xenograft (P = .003). Conclusions: IDN-5109 modulates Pgp activity, resulting in superior tumor growth inhibition against Pgp-expressing tumors as compared with paclitaxel. IDN-5109 may broaden the spectrum of taxane use to include colon tumors. [J Natl Cancer Inst 2001;93:1234-45]

The taxane paclitaxel (Taxol) is one of the most active cancer chemotherapeutic agents known. It is effective against a variety of human tumors, including ovarian, breast, and non-small-cell lung tumors, as well as head and neck carcinomas (1–5). Paclitaxel causes cells to arrest at G2/M phase of the cell cycle (6,7) through drug-induced tubulin polymerization and stabilization (8–12), and it induces cell death by apoptosis (13–17).

Although paclitaxel is the drug of choice for many chemotherapeutic regimens, its effectiveness is often limited because many tumors display drug resistance. Cells can acquire resistance to paclitaxel by at least two different mechanisms (18). First, alterations in either the assembly or stability of microtu-
carcinoma cells (34), A2780 ovarian carcinoma cells (35), and HT-1080 sarcoma cells (36). We also used multidrug-resistant human tumor cell lines that had been derived from those drug-sensitive, Pgp-negative cell lines by exposing them to increasing levels of doxorubicin in culture. These drug-resistant cells included MCF-7Adr® (37) and A2780-DX5 (38) cells, both of which express Pgp, and HT-1080/DRA4 cells, which express the multidrug-resistance-related protein (MRP) and lung resistance protein (LPR) but not Pgp (39). MDA435/LCC6® cells, which express high levels of Pgp, were derived from MDA435/LCC6 cells that were transfected with a retrovirus engineered to constitutively express the mdr1 gene (33) and were provided by Dr. R. Clarke, Georgetown University Medical School, Washington, DC. The human colon carcinoma cell lines SW-620, DLD1, and HCT-15, which express low, intermediate, and high levels of Pgp, respectively, were also used (40–42). All of the other human tumor cell lines were obtained from the Division of Cancer Treatment Tumor Repository, National Cancer Institute (Frederick, MD) or the American Type Culture Collection (Manassas, VA). Two animal cell lines—Chinese hamster ovary (CHO) cells and Tax-18 CHO cells, a paclitaxel-dependent mutant CHO cell line with impaired mitotic spindle assembly—were described previously (43). All of the cells were maintained at 37 °C in 5% CO2 in RPMI-1640 medium supplemented with 5% Nu-serum, 5% fetal bovine serum (Atlanta Biological, GA), 10 mM L-glutamine (Life Technologies Inc. [GIBCO BRL], Rockville, MD).

### Drugs and Reagents

Rh-123, supplied by Dr. Hans Minderman (Roswell Park Cancer Institute, Buffalo, NY) or purchased from Sigma Chemical Co. (St. Louis, MO), was diluted in water to give a 1 mg/mL stock solution. Doxorubicin and verapamil (Sigma Chemical Co.) were dissolved in 10% dimethyl sulfoxide (DMSO) and water, respectively, to give stock solutions of 4 mM each. For in vitro experiments, the Pgp drug resistance reversal agent, tRA96023 (SB-RA-30112), and taxane analogues 94004 (SB-T-10113), 94045 (SB-T-10114), 94042 (SB-T-10114), 95049 (SB-T-10117), and 95048 (SB-T-10117) were synthesized as described previously (23–28). Each was then dissolved in DMSO at a concentration of 4 mM. Paclitaxel, docetaxel, IDN-5109, IDN-5102, IDN-5106, and IDN-5111 (supplied by Indena S.p.A., Milan, Italy) were also dissolved in DMSO at a concentration of 4 mM. [3H]Paclitaxel (specific activity, 2.6 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). [3H]IDN-5109 (specific activity, 60 Ci/mmol) was synthesized by American Radiolabeled Chemicals, Inc. (St. Louis, MO).

### In Vitro Growth-Inhibition Assay

The inhibitory effects of various drugs on cell growth were assessed by use of sulforhodamine B (SRB) (Sigma Chemical Co.), a dye-based assay, which indirectly determines cell number by measuring membrane-associated proteins, as described previously (44). Briefly, 1 x 10^6 exponentially proliferating cells were seeded on 96-well microtiter plates in complete growth medium and incubated at 37 °C for 15–18 hours to allow the cells to attach to the substrate before taxes were added. For each taxane tested, five 96-well microtiter plates were screened in parallel. All cell lines were exposed to 10^−4 M concentrations of drugs, covering a 5- to 6-log range of concentrations, at 37 °C. MCF-7, MCF-7R, MDA435/LCC6®, MDA435/LCC6®mdr1, A2780, A2780/DX5, HT-1080, SW-620, DLD1, and HCT-15 cells were exposed to drugs for 72 hours (approximately 3.3 cell doublings), whereas HT1080/DR4 cells were exposed to drugs for 100 hours (approximately 3.5 cell doublings). Cells were fixed in situ for 1 hour at 4 °C with ice-cold 50% trichloroacetic acid. The plates were then washed six times with water, and 150 μL of 0.4% SRB was added to each well. After a 5-minute incubation at room temperature, the plates were rinsed with 0.1% acetic acid and air-dried. Bound SRB was solubilized by adding a 100 μL 10 mM Tris base (pH 10.5) per well and was allowed to stand at room temperature for 5 minutes. The optical density (OD) of each well was measured at 570 nm. Under these conditions, cell number is proportional to OD. We determined the concentration of each drug that inhibited 50% of cell growth (IC₅₀) using empirically determined concentrations of drugs between 10 μM to 30 mM. The IC₅₀ was obtained by plotting a concentration–effect curve as described in the “Statistical Methods” section. A minimum of three separate experiments was used to derive the IC₅₀ values presented. The R/S ratio, which is a measure of cellular resistance to a particular chemotherapeutic agent, was calculated by dividing the IC₅₀ obtained for a drug-resistant cell line by the IC₅₀ of the corresponding drug-sensitive cell line (e.g., MCF-7R IC₅₀/MCF-7 IC₅₀).

### Colon-Forming Assay

Equal numbers (1 x 10^6) of CHO and TAX-18 CHO cells were seeded in 24-well plates in complete growth medium containing various concentrations of paclitaxel or IDN-5109 and incubated at 37 °C in 5% CO₂ for 6 days until visible colonies formed. Cells were then stained with 0.05% methylene blue, as described previously (43), to visualize the number of viable colonies and were photographed.

### Tubulin Polymerization Assay

Temperature-induced microtubule assembly or disassembly in vitro was assayed as described previously (45, 46). Briefly, bovine brain tubulin (Cytoskeleton, Inc., Denver, CO) at 10 mg/mL in G-PEM buffer (i.e., 80 mM piperezine 1,4-diethane sulfonic acid, 0.5 mM MgCl₂, 1 mM EGTA, and 1 mM guanosine 5’-triphosphate) was thawed on ice and diluted to 0.7 μg/mL in G-PEM buffer. To the tubulin solution, either paclitaxel or IDN-5109 in DMSO was added, bringing the final concentration of taxanes to 10 μM in 2% DMSO. Samples were mixed by vortexing and were placed on ice. Duplicate samples were incubated in a 32 °C water bath, and the absorbance of each sample at 340 nm, indicating tubulin polymerization, was measured every 30 seconds for 20 minutes by use of a Beckman DU640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). The absorbance readings for duplicate samples were averaged, and the background was subtracted.

### Measuring Cellular Accumulation of [3H]Paclitaxel and [3H]IDN-5109

Exponentially growing MDA435/LCC6 or MDA435/LCC6®mdr1 (1 x 10^5) were seeded into 24-well tissue culture plates and incubated at 37 °C in 5% CO₂ for 12 hours to allow for adherence. [3H]Paclitaxel and [3H]IDN-5109 were diluted in RPMI-1640 growth medium that contained or lacked tRA96023. Cells were washed once with phosphate-buffered saline (PBS), and the diluted radio-labeled drugs were added to the cells at a concentration of 0.2–0.6 μCi/mL per well. After a 2-hour incubation at 37 °C, adherent cells were carefully washed twice with PBS, fresh medium containing or lacking tRA96023 was added, and the cells were returned to the incubator to allow the efflux of radiolabeled drugs. At 2-, 4-, 8-, and 10-hour efflux time points (i.e., after the addition of fresh medium), the medium was removed from the well corresponding to that time point and the cells were washed twice with PBS and then solubilized in 200 μL 1 N NaOH at 37 °C for 30 minutes. Aliquots (35 μL) of solubilized cells were removed for protein quantification by use of the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Then 1 N HCl (150 μL) was added to a separate 150 μL aliquot of solubilized cells, and total radioactivity (in counts per minute [cpm]) was measured by use of a Model LS-6500 scintillation counter (Beckman Instruments, Wakefield, MA). Drug accumulation is expressed as counts per minute per milligram protein.

### Measurement of Rh-123 and Doxorubicin Uptake and Efflux by Flow Cytometry

Aliquots of approximately 1 x 10⁶ tumor cells in complete growth medium were placed into 5-mL polystyrene tubes. A solution of 5 μg/mL Rh-123 or 10 μM doxorubicin containing IDN-5109, tRA96023, paclitaxel, docetaxel, verapamil, or 94004 at 1 μM was added to the cells, which were then incubated at 37 °C for 1 hour (for Rh-123-containing additions) or 2 hours (for doxorubicin-containing additions). After this incubation, the cells were washed with PBS, and fresh medium containing only the original modulator (i.e., IDN-5109, tRA96023, paclitaxel, docetaxel, verapamil, or 94004) at 1 μM was added to the cells. The cells were incubated at 37 °C for 4 hours to allow the efflux of Rh-123 or doxorubicin. The cells were then washed and resuspended in ice-cold PBS and subjected to flow cytometry to analyze the intracellular levels of Rh-123 and doxorubicin.

Flow cytometry was carried out as described previously (47–53) by use of a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) interfaced with Macintosh Cell Quest software (Becton Dickinson Immunocytometry Systems). Briefly, cells incubated with Rh-123 and doxorubicin were excited with a single-beam argon laser running at 15-mW output. Rh-123 fluorescent emission was collected through a 530/30-nm band-pass filter, and doxorubicin fluorescent emission was collected though a 680/20-nm long-pass filter, with photomultiplier pulses logarithmically amplified in both cases. Cells were selected for analyses based on their forward-angle scatter and
right-angle scatter. The resulting data were analyzed with Winlist software (Version Software House, Torsham, ME). Fluorescence intensities of drug-treated cells were quantified by use of arbitrary units and were compared with those of control cells treated with the specific vehicle for each drug evaluated (i.e., DMSO, for taxanes; water, for verapamil; and 10% DMSO, for doxorubicin).

Animals and Tumor Xenografts

Female athymic nude (nu/nu) mice, 6–10 weeks of age and weighing 20–25 g, were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and housed at the Medical Research Complex at Roswell Park Cancer Institute. Animal care complied with the Institutional Animal Care and Use Committee guidelines. SW-620 or DLD1 human tumor cells were harvested by trypsinization, washed twice in ice-cold PBS, and adjusted to 1 × 10^7 viable cells/mL by use of trypan blue dye exclusion as the criterion for viability. Then 0.2 mL of each cell suspension was injected subcutaneously into the right flank of each mouse (54, 55). After the tumors reached a palpable size of 50–100 mm^3, the mice (in groups of five each) were given four separate doses (either by the oral route or by intravenous injection at 4-day intervals) of each drug. Oral paclitaxel treatment began on day 7, intravenous paclitaxel treatment began on day 10, oral IDN-5109 treatment began on day 11, and intravenous IDN-5109 treatment began on day 13 after tumor cell injection. The experiment concluded on day 150 after tumor cell injection.

Drug Preparation for In Vivo Experiments

Paclitaxel was prepared as a 6-mg/mL stock solution in equal parts of Cremorphor ELP (BASF, Ludwigshafen, Germany) and absolute ethanol. IDN-5109 was prepared as a 30-mg/mL stock solution in equal parts of Tween 80 (polyoxyethylene–sorbitan monooctate; from Sigma Chemical Co.) and absolute ethanol. Each stock solution was diluted further immediately before use in 0.9% NaCl (saline) so that the appropriate concentration of each drug could be injected in a volume of approximately 0.4 mL for a 20-g mouse. TPA96023 was prepared as a 10-mg/mL stock solution in equal parts of Cremorphor ELP and absolute ethanol and diluted to 1 mg/mL in saline before use.

Statistical Methods

In vitro cell growth-inhibition assay. The four-parameter Hill model (shown in equation 1) was used as the structural model for the concentration–effect curve of each single agent (56, 57). Equation 1 was fitted to the experimental data with iteratively reweighted nonlinear regression, with the estimation of the four parameters and accompanying error. In equation 1, E is the measured effect, C is the concentration of drug, E0 is the control response at zero drug concentration, B is the background response at infinite drug concentration, IC50 is the concentration of drug inducing a 50% inhibition of the maximal possible cell growth (E0 − B), and m is the slope parameter of the concentration–effect curve. When m is positive, the concentration–effect curve rises with increasing agent concentration; when m is negative, the curve falls with increasing concentration. As the absolute value of m increases, the curve becomes steeper (on linear–log coordinates). The weighting factor used was the reciprocal of the square of the predicted response. This assumes a constant coefficient of variation for the data error structure, which is a common and reasonable assumption (56, 57).

\[ E = B + \left( \frac{E_0 - B}{IC_{50}} \right)^m \left( \frac{C}{IC_{50}} \right)^m + 1 \]

MicroSoft FORTRAN, Microsoft, Bothell, WA, was used to develop the nonlinear curve-fitting software. The nonlinear regression procedure was coded with the Nash (58) version of the Marquardt algorithm.

In vivo tumor growth assay. For each animal, the tumor length (l) and width (w), each in mm, were measured by use of electronic calipers and recorded every 3–4 days. Tumor volume (V), in mm^3, was calculated by use of the following formula: \( V = 0.4(l \times w)^2 \). The time in days to the predetermined target tumor volume of 600 mm^3 was linearly interpolated from a plot of log(volume) versus time. Statistically significant differences in tumor volumes between control and drug-treated mice were determined by the Cox–Mantel test (59). For the Cox–Mantel test, the time-to-event data for animals that did not reach the target tumor volume, either because of long-term cure (defined as those animals that were still alive at the conclusion of the experiment whose tumors either completely regressed or did not reach the preset target volume) or early death because of drug toxicity, were treated as censored data. All statistical tests were two-sided.

RESULTS

In Vitro Activity of Taxanes

To begin to investigate the mechanism of action of IDN-5109, we first compared the growth-inhibitory properties of various taxanes in drug-sensitive cell lines and in drug-resistant cell lines that were derived from them. For three of these pairs of cell lines, the drug-resistant line (i.e., MCF-7Adr, MAD435/LCC6mdr1, or A2780-DX5) expresses Pgp. For the remaining pair of cell lines, of which neither cell line expresses Pgp, the drug-resistant line, HT-1080/DR4, expresses MRP and LRP, two other membrane transporters believed to confer cellular multidrug resistance. Fig. 1 shows the structures of paclitaxel, docetaxel, and the various taxane analogues used in this study. Minor alterations to the chemical structure of paclitaxel were associated with a decrease in the concentration of drug necessary to cause a 50% inhibition of growth (IC50) for a variety of Pgp-expressing, multidrug-resistant cell lines (Table 1). However, such alterations had minimal effects on growth inhibition for the corresponding drug-sensitive cell lines that did not express Pgp. For example, lower concentrations of taxanes that have an acetyl group at the C-10 position of the moiety designated R5 in Fig. 1 (such as IDN-5109, IDN-94045, and IDN-95049) were required to inhibit the growth of drug-resistant cell lines that express Pgp than were required of taxanes that have a hydroxyl group at this position (such as 94004, 94042, and 95048).

The growth of Pgp-expressing tumor cell lines was inhibited by lower concentrations of IDN-5109 compared with the other taxanes studied. IDN-5109 was approximately 177 and 48 times more growth inhibitory than docetaxel and paclitaxel, respectively, against the Pgp-expressing MCF-7Adr multidrug-resistant cell line (Table 1). Likewise, the R/S ratio (the growth inhibition achieved by a drug in a drug-sensitive cell line that does not express Pgp divided by that achieved in the Pgp-expressing drug-resistant derivative of that cell line), another measure of taxane activity, was substantially lower for IDN-5109 than for either paclitaxel or docetaxel treatment. These results suggest that IDN-5109 may be more effective than paclitaxel or docetaxel in overcoming Pgp-mediated drug resistance. The MRP- and LRP-positive tumor line HT1080/DR4 was sensitive to taxanes (R/S values, 0.8–2.8) but highly resistant to doxorubicin (R/S value, 62), indicating that these membrane-efflux pumps do not appear to confer cellular resistance to taxanes. Thus, the enhanced growth-inhibitory activity of IDN-5109 is specific for human tumor cell lines that express Pgp and are highly resistant to paclitaxel and docetaxel.

Effect of IDN-5109 on Tubulin PolymORIZATION and Cell Growth

Because paclitaxel promotes the polymerization of tubulin both in vivo and in cell-free systems, we tested whether IDN-5109 was more effective than paclitaxel in stabilizing tubulin and promoting its polymerization. Both IDN-5109 and paclitaxel gave similar absorbance profiles in a temperature-dependent tubulin polymerization assay, suggesting that enhanced tubulin stabilization and/or polymerization could not account for greater
growth-inhibitory activity of IDN-5109 compared with paclitaxel (Fig. 2, A). Further support for this conclusion came from the observation that CHO-TAX-18 cells, which express a mutant form of tubulin that causes impaired mitotic spindle assembly and normally require low levels of paclitaxel for growth, required 20- to 40-fold lower concentrations of IDN-5109 than paclitaxel for growth (Fig. 2, B). This finding suggests that, in these cells, IDN-5109 is more potent in its ability to sustain cell growth and/or that its uptake and/or retention by these cells is increased. Moreover, the growth of both wild-type CHO cells and mutant CHO-TAX-18 cells was inhibited by IDN-5109 concentrations 10- to 20-fold lower than the concentrations of paclitaxel required to inhibit the growth of these cells. In contrast, the taxane-based drug resistance reversal agent tRA96023, which lacks the C-13 side chain necessary for taxane–tubulin interaction, had no effect on tubulin polymerization (Fig. 2, A) or on cell proliferation (data not shown) when present at 30 mM, its maximal soluble concentration in tissue culture.

Effects of IDN-5109 on Retention of Pgp Substrates

The superior growth-inhibitory effect of IDN-5109 in Pgp-expressing tumor cell lines, as compared with paclitaxel, could be because it is a poor substrate for Pgp, as was suggested previously (29–32) or because it binds to Pgp, blocking the action of the pump and, consequently, its own efflux from the cell. We tested the latter possibility by measuring the ability of IDN-5109 to modulate Pgp activity. Specifically, we used flow cytometry to measure the accumulation of Rh-123, a fluorescent Pgp substrate, in three human colon carcinoma cell lines, SW620, DLD1, and HCT-15, that express increasing amounts of the messenger RNA (mRNA) that encodes Pgp, respectively (60). The cells were incubated with Rh-123 for a brief uptake period, then washed and incubated in the absence of Rh-123 for a 4-hour efflux period (Fig. 3, A). Flow cytometry was used to generate histograms representing Rh-123 fluorescence within a population of cells. Separate histograms were generated for each cell line/treatment group before incubation with Rh-123 (auto-fluorescence), immediately after incubation with Rh-123 (uptake), and after the 4-hour efflux period (uptake and efflux). Those histograms were then superimposed to generate one histogram per drug treatment group for each cell line. For each of these vehicle-treated, Pgp-expressing colon tumor cell lines, the intracellular levels of Rh-123 decreased during the efflux period, as demonstrated by the substantial numbers of cells having

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**Fig. 1.** Chemical structures of the semisynthetic taxanes—structures of paclitaxel, docetaxel, and the various taxane analogues used in this study. Key substitutions on the taxane backbone at positions marked R1, R2, R3, R4, and R5 and the tubulin-binding side chain at C-13 are illustrated. The structure of the nontoxic, taxane-based, drug-resistance reversal agent tRA96023 is also shown. Bzl = benzyl group; Ac = acetyl group.
Table 1. IC_{50} of various chemotherapeutic agents against a panel of tumor cell lines

<table>
<thead>
<tr>
<th>Agent</th>
<th>MCF-7</th>
<th>MDA435/LCC6</th>
<th>A2780</th>
<th>HT-1080</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S†</td>
<td>AdrR†</td>
<td>R/S</td>
<td>S†</td>
</tr>
<tr>
<td>Dox</td>
<td>40 ± 20</td>
<td>3600 ± 1700</td>
<td>90</td>
<td>180 ± 30</td>
</tr>
<tr>
<td>PACTx</td>
<td>1.8 ± 0.9</td>
<td>863 ± 26</td>
<td>479</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>0.5 ± 0.1</td>
<td>3200 ± 45</td>
<td>6400</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>IDN-5102</td>
<td>1.0 ± 0.46</td>
<td>194 ± 23</td>
<td>194</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>IDN-5106</td>
<td>0.51 ± 0.1</td>
<td>102 ± 19</td>
<td>200</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>IDN-5111</td>
<td>0.45 ± 0.03</td>
<td>32 ± 3.0</td>
<td>71</td>
<td>1.4 ± 0.27</td>
</tr>
<tr>
<td>IDN-5109</td>
<td>0.4 ± 0.04</td>
<td>18 ± 6.5</td>
<td>45</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>94004</td>
<td>3.0 ± 0.55</td>
<td>242 ± 15</td>
<td>81</td>
<td>ND</td>
</tr>
<tr>
<td>94045</td>
<td>1.7 ± 0.15</td>
<td>35 ± 4.3</td>
<td>21</td>
<td>ND</td>
</tr>
<tr>
<td>94042</td>
<td>ND</td>
<td>68 ± 16</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>95049</td>
<td>4.4 ± 0.3</td>
<td>159 ± 9.0</td>
<td>36</td>
<td>ND</td>
</tr>
<tr>
<td>95048</td>
<td>2.7 ± 0.13</td>
<td>342 ± 23</td>
<td>127</td>
<td>ND</td>
</tr>
</tbody>
</table>

*S†Cells are lines are matched pairs consisting of the parental drug-sensitive (S) line and the corresponding drug-resistant derivative (e.g., AdrR, MDR1, DX5, and DR4). IC_{50} = the nM concentration of agent that causes 50% inhibition of cell growth; S = drug-sensitive member of each pair of tumor cell lines; R/S values represent the IC_{50} of the drug-resistant (R) cell line divided by the IC_{50} of the drug-sensitive (S) cell line from which the resistant cell line was derived (e.g., R/S for MCF-7 cells = IC_{50} for MCF-7AdrR cells/IC_{50} for MCF-7 cells); Dox = doxorubicin; PACTx = paclitaxel; Docetx = docetaxel; ND = not determined. All assays consisted of at least six replicate wells and were performed on three separate occasions. IC_{50} values are presented as the mean ± standard deviation.

†Cells were exposed to each agent for 72 hours (approximately 3.3 cell doublings).

‡Cells were exposed to each agent for 100 hours (approximately 3.5 cell doublings).

fewer than 1000 arbitrary units of fluorescence intensity (Fig. 3, A). The multiple peaks of fluorescence intensity displayed by the vehicle-treated DLD_4 cells and SW620 cells after the efflux period suggest that these cell lines contain a mixed population of cells with different amounts of mdr1 mRNA. In all three cell lines, the addition of the Pgp modulator verapamil during the uptake and efflux periods led to an increase in the uptake and retention of Rh-123. However, cells exposed to either tRA96023 or IDN-5109 accumulated and retained more Rh-123 than did cells treated with either vehicle or verapamil. Surprisingly, the toxic taxane IDN-5109 was nearly as potent as the nontoxic multidrug resistance reversal agent tRA96023 in increasing Rh-123 retention, demonstrating that IDN-5109 shows a strong inhibitory effect on the activity of Pgp. In contrast, cells treated with either paclitaxel or docetaxel accumulated and retained only slightly higher levels of Rh-123 than did cells treated with vehicle, and they accumulated and retained much less Rh-123 than did cells treated with IDN-5109 or tRA96023. Among the three multidrug-resistant colon carcinoma cell lines, tRA96023 and IDN-5109 blocked the efflux of Rh-123 more effectively in SW620 cells, which express the lowest amount of mdr1 mRNA, than in HCT-15 cells, which express the highest amount of mdr1 mRNA. From these results, we conclude that IDN-5109 modulates the activity of Pgp, resulting in the greater intracellular uptake and retention of the Pgp substrate, Rh-123, as compared with either paclitaxel or docetaxel.

We also found that MDA435/LCC6^{mdr1} breast carcinoma cells, which express high levels of Pgp, and the Pgp-nonexpressing MDA435/LCC6 cells from which they were derived displayed patterns of Rh-123 uptake and efflux similar to those of the colon carcinoma cell lines when they were treated with the different taxanes (Fig. 3, B). For example, MDA435/LCC6 cells accumulated and retained over 200-fold more Rh-123 than MDA435/LCC6^{mdr1} cells when treated with vehicle. In addition, tRA96023 and IDN-5109 prevented the efflux of Rh-123 from the Pgp-expressing cells to a greater extent than did verapamil, paclitaxel, or docetaxel. More specifically, based on integration of the Rh-123 fluorescence peak (Fig. 3, B), the addition of 1 μM tRA96023 or IDN-5109 increased MDA435/LCC6^{mdr1} cellular retention of Rh-123 9.4- and 8.1-fold, respectively, in comparison to paclitaxel, and 24- and 20.7-fold, respectively, in comparison to docetaxel. Of interest, the taxane analogue 94004, which differs from IDN-5109 only by having a hydroxy group instead of an acetyl group at the C-10 position, yielded an Rh-123 uptake and efflux profile similar to that of docetaxel. These data are consistent with the tumor cell growth inhibition (IC_{50}) results discussed earlier, and they further suggest that the C-10 acetyl group is important for IDN-5109 to block Pgp and modulate the uptake and retention of itself and Rh-123.

Doxorubicin, another Pgp substrate, is a standard treatment of breast cancer. We, therefore, measured the effects of tRA96023 and IDN-5109 on doxorubicin uptake and retention in MDA435/ LCC6 and MDA435/LCC6^{mdr1} breast tumor cell lines. Cells treated with tRA96023 and IDN-5109 accumulated and retained higher levels of doxorubicin than cells treated with verapamil, paclitaxel, or docetaxel, providing further evidence that these two taxanes can modulate Pgp activity (Fig. 3, B). More specifically, based on integration of the doxorubicin fluorescence peak, the addition of 1 μM tRA965023 or IDN-5109 increased MDA435/LCC6^{mdr1} cellular retention of doxorubicin 1.9- and 1.7-fold, respectively, in comparison to paclitaxel.

Because both tRA96023 and IDN-5109 were effective in maintaining high intracellular concentrations of Pgp substrates in cells that expressed high levels of Pgp, we tested whether these drugs if used in combination with each other or with other taxanes would have synergistic effects. MDA435/LCC6^{mdr1} cells treated simultaneously with tRA96023 and either IDN-5109, paclitaxel, or docetaxel retained more Rh-123 than cells treated with each agent alone (Fig. 4). As expected, cells treated with the combination of IDN-5109 and tRA96023 retained the most Rh-123, suggesting that this combination of drugs could be more effective against Pgp-expressing tumors than IDN-5109 alone.
Accumulation and Retention of $[^3\text{H}]$Paclitaxel and $[^3\text{H}]$IDN-5109

We compared the uptake and retention of IDN-5109 and paclitaxel by use of radiolabeled derivatives of each drug to ascertain whether the observed inhibition of Pgp activity noted with IDN-5109 was associated with increased intracellular levels of IDN-5109 in Pgp-expressing cells. We measured the radioactivity in tumor cells that were incubated for 2 hours in the presence of $[^3\text{H}]$paclitaxel and $[^3\text{H}]$IDN-5109 and then in fresh medium lacking the radiolabeled compounds for up to 10 hours. Both $[^3\text{H}]$paclitaxel and $[^3\text{H}]$IDN-5109 accumulated to similarly high levels in MDA435/LCC6 cells, which have no detectable Pgp expression (Fig. 5). In contrast, Pgp-expressing MDA435/LCC6-mdr1 cells accumulated substantially more $[^3\text{H}]$IDN-5109 than $[^3\text{H}]$paclitaxel, but the levels of $[^3\text{H}]$IDN-5109 did not approach those observed to accumulate in MDA435/LCC6 cells. This suggests that the ability of IDN-5109 to decrease Pgp activity may result in its increased retention by Pgp-expressing cells compared with the retention of paclitaxel, which does not modulate Pgp activity. After the 2-hour uptake period, MDA435/LCC6-mdr1 cells had accumulated 10-fold lower intracellular concentrations of $[^3\text{H}]$paclitaxel than MDA435/LCC6 cells and, after 2 hours of efflux, $[^3\text{H}]$paclitaxel was eliminated completely from the Pgp-expressing cells but not from the Pgp-nonexpressing cells.

The addition of tRA96023 greatly enhanced uptake of $[^3\text{H}]$IDN-5109 and $[^3\text{H}]$paclitaxel in MDA435/LCC6-mdr1 cells, as compared with the uptake observed without tRA96023. Both $[^3\text{H}]$paclitaxel and $[^3\text{H}]$IDN-5109 were retained at elevated levels in MDA435/LCC6-mdr1 cells through 10 hours of efflux when those cells were treated with either 500 nM or 1 $\mu$M tRA96023. These results support the hypothesis that, by blocking Pgp, IDN-5109 increases its own uptake and chemotherapeutic activity as compared with paclitaxel in Pgp-expressing, multidrug-resistant tumor cell lines and that this effect can be further increased by the addition of tRA96023.

Effects of IDN-5109 and Paclitaxel on Xenograft Tumors Derived From Human Colon Cancer Cells

Because the in vitro results suggested that IDN-5109 might be an effective chemotherapeutic agent in vivo, we tested IDN-5109 in a mouse xenograft model of tumors derived from the subcutaneous injection of SW-620 human colon carcinoma cells. SW-620 cells were chosen for this study because they expressed sufficient levels of Pgp to allow rapid efflux of Rh-123 (Fig. 3, A), despite having the lowest level of Pgp mRNA of the three colon carcinoma cell lines studied. Intrinsic Pgp expression in the gut limits paclitaxel uptake and, thus, its bioavailability, when administered orally. However, we considered the possibility that IDN-5109 might have better oral bioavailability than paclitaxel because we had observed the inhibitory effects of IDN-5109 on Pgp activity in vitro (Fig. 3). We, therefore, compared the chemotherapeutic activities of IDN-5109 and paclitaxel administered either orally or intravenously to mice bearing xenograft tumors derived from SW-620 cells. Table 2 shows that established SW-620 tumors were resistant to paclitaxel administered intravenously at its maximum tolerated cumulative dose of 100 mg/kg. In contrast, IDN-5109 administered intravenously at a cumulative dose of 240 mg/kg was effective in retarding the growth of tumors derived from SW-620 cells, resulting in a 79-day delay (95% confidence interval [CI] = 51 to 107 days) in tumor growth compared with the growth of tumors in control animals. One animal that received intravenous IDN-5109 was considered to be cured because its tumor completely regressed and no tumor was palpable at the conclusion of the experiment on day 150 after tumor cell implantation.

When administered orally to mice bearing established xenograft tumors, paclitaxel and IDN-5109 also differed in their antitumor activities. Mice that received paclitaxel orally at a cumulative dose of 300 mg/kg showed no statistically significant difference ($P = .194$) in tumor growth delay compared with control mice treated orally with vehicle. In contrast, it took 79
Fig. 3. Rhodamine 123 (Rh-123) and doxorubicin retention in P-glycoprotein (Pgp)-expressing human colon and breast carcinoma cell lines. Panel A: Colon tumor cell lines SW620, DLD1, and HCT-15, which expressed increasing levels of Pgp, respectively, were incubated with 5 \( \mu \)g/mL of Rh-123 and 0.001% of dimethyl sulfoxide (DMSO) (vehicle), 10 \( \mu \)M verapamil, 1 \( \mu \)M tRA96023, 1 \( \mu \)M IDN-5109, 1 \( \mu \)M paclitaxel, or 1 \( \mu \)M docetaxel for 1 hour at 37 °C. Cells were washed with phosphate-buffered saline (PBS) and incubated with 0.001% DMSO (vehicle), 10 \( \mu \)M verapamil, 1 \( \mu \)M tRA96023, 1 \( \mu \)M IDN-5109, 1 \( \mu \)M paclitaxel, or 1 \( \mu \)M docetaxel for a 4-hour efflux period at 37 °C. Panel B: MDA435/LCC6MDR (LCC6 MDR; Pgp-expressing) and MDA435/LCC6 (LCC6 WT; Pgp-nonexpressing) human breast carcinoma cells were incubated with either 5 \( \mu \)g/mL Rh-123 or 10 \( \mu \)M doxorubicin and either 1 \( \mu \)M verapamil (doxorubicin experiment), 10 \( \mu \)M verapamil (Rh-123 experiment), 1 \( \mu \)M tRA96023, 1 \( \mu \)M IDN-5109, 1 \( \mu \)M paclitaxel, or 1 \( \mu \)M docetaxel for an uptake period of 1 hour (Rh-123 experiment) or 2 hours (doxorubicin experiment) at 37 °C. Cells were then washed with PBS and incubated with 1 \( \mu \)M verapamil (doxorubicin experiment), 10 \( \mu \)M verapamil (Rh-123 experiment), 1 \( \mu \)M tRA96023, 1 \( \mu \)M IDN-5109, 1 \( \mu \)M 94004, 1 \( \mu \)M paclitaxel, or 1 \( \mu \)M docetaxel for a 4-hour efflux period at 37 °C. Flow cytometry was used to generate histograms representing Rh-123 or doxorubicin fluorescence within a population of cells. Separate histograms were generated for each cell line/treatment group before incubation with Rh-123 or doxorubicin (autofluorescence), immediately after incubation with Rh-123 or doxorubicin (uptake), and after the 4-hour efflux period (uptake and efflux). Those histograms were then superimposed to generate one histogram per drug treatment group for each cell line. As a histogram peak shifts to the right on the x-axis, the intracellular fluorescence increases. Broad peaks indicate a mixture of cells that have accumulated varying amounts of fluorescence (Rh-123 or doxorubicin). ND = not determined.
As a histogram peak shifts to the right on the x-axis, the intracellular fluorescence increases. Broad peaks indicate a mixture of cells that have accumulated varying amounts of fluorescence (Rh-123).

DISCUSSION

Paclitaxel has proven to be a valuable chemotherapeutic agent against a variety of solid tumors. However, multidrug resistance imposes substantial limits on tumor responsiveness to paclitaxel that are often observed in the clinical setting. Several mechanisms have been proposed to account for the resistance of tumors to taxanes in general, including altered microtubule assembly or stability and overexpression of the Pgp efflux pump.

Aspects concerning the three-dimensional nature of tumors, such as immature vascularization, reduced blood flow, tissue hypoxia, and junctional complexes, limit drug access to tumor cells growing as a solid tumor. Such resistance mechanisms have been demonstrated in studies on tumor spheroids in vitro (61). Our laboratory has taken two approaches to overcome the resistance of tumors to paclitaxel. In the first, we developed drug resistance reversal agents (e.g., tRA96023), which act solely by blocking Pgp action, thereby increasing the potency of other drugs such as paclitaxel (26). In the other approach, we developed a series of control group, and three were cured. However, when administered orally at cumulative doses below 240 mg/kg IDN-5109 was not effective in retarding tumor growth (data not shown). We estimate that IDN-5109 has a bioavailability of approximately 50% when it is administered orally because roughly twice as much IDN-5109 was required to inhibit tumor growth when it was administered orally as when it was administered by intravenous injection.

To improve on the antitumor effect demonstrated by IDN-5109 alone, we compared the effects of orally administered IDN-5109 given with and without the taxane-reversal agent, tRA96023, on the growth of xenograft tumors derived from paclitaxel-resistant DLD1 human colon carcinoma cells. DLD1 cells express approximately 7.6-fold more Pgp mRNA than SW-620 cells (60) and, presumably, more Pgp. When given at its maximum tolerated cumulative dose, paclitaxel did not inhibit the growth of tumors derived from DLD1 cells (data not shown). In contrast, mice that received IDN-5109 administered orally at a cumulative dose of 360 mg/kg had a statistically significant increase (P = .002) in the number of days (23 days; 95% CI = 15.2 to 30.8 days) that tumor growth was delayed compared with control mice that received the Tween vehicle (Table 3). Mice that received lower cumulative doses of IDN-5109 (120 or 240 mg/kg) administered orally had little or no delay in the growth of their tumors compared with control mice that received vehicle. However, mice that received the combination of IDN-5109 at 240 mg/kg and tRA96023 at 80 mg/kg had a statistically significant delay (P = .002) in the growth of their tumors (26 days; 95% CI = 18 to 70 days) compared with control mice that received the combination of Tween and vehicles. Of interest, increasing the dose of tRA96023 to 120 mg/kg in this combination treatment decreased the number of days that tumor growth was delayed to 10 days (95% CI = 5.8 to 14.2 days). Mice treated with the combination of IDN-5109 at 360 mg/kg and tRA96023 at 40 mg/kg had the longest delay in tumor growth (29 days; 95% CI = 20.6 to 37.4 days). All of the animals treated with the various drug combinations exhibited minimal weight loss (Table 3). These results suggest that the combination of IDN-5109 and the nontoxic reversal agent tRA96023 may be an effective treatment to inhibit or delay the growth of tumors that express higher levels of Pgp.
potent semisynthetic 14β-hydroxy-10-deacetylbaccatin III taxanes, including the taxane now referred to as IDN-5109, that were able to inhibit directly the growth of the Pgp-expressing MCF-7Adr tumor cell line at much lower concentrations than paclitaxel (24). IDN-5109 is a semisynthetic taxane that is a highly effective growth-inhibitory agent against paclitaxel-resistant tumor cell lines in vitro (24) and human tumor xenografts in vivo (30,31). IDN-5109 also demonstrated improved growth-inhibitory properties compared with paclitaxel in two xenograft tumor models derived from cells that were moderately responsive to paclitaxel (32). Although a preliminary study (29) has indicated that IDN-5109 induces a strong G2/M arrest and induces apoptosis in Pgp-expressing CEM VBLr cells, the mechanism(s) responsible for the increased activity of IDN-5109 in paclitaxel-resistant, Pgp-expressing tumors has remained a mystery.

Fig. 5. Accumulation and retention of [3H]paclitaxel and [3H]IDN-5109 in human breast carcinoma cells treated with various taxanes. MDA435/LCC6 (a and b; Pgp-nonexpressing) and MDA435/LCC6mdr1 (c-h; Pgp-expressing) human breast carcinoma cells were incubated for 2 hours with either [3H]paclitaxel (white bars) or [3H]IDN-5109 (gray bars) in the absence (a-d) or presence (e and f) of 500 nM or 1 μM (g and h) tRA96023. The cells were then washed to remove unincorporated radiolabeled drug and incubated for 8 hours in medium lacking both the radiolabeled drug and tRA96023 (a-d) or in medium lacking the radiolabeled drug and containing either 500 nM (e and f) or 1 μM (g and h) tRA96023. The cells were harvested, and radioactivity associated with the cells was measured at the indicated times through scintillation counting and expressed as mean counts per minute (cpm) per milligrams of protein. Data bars include 95% confidence intervals.

Table 2. Effects of IDN-5109 and paclitaxel on xenograft tumors derived from SW-620 human colon carcinoma cells*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Cumulative dose, mg/kg</th>
<th>Individual dose, mg/kg</th>
<th>Days to reach 600 mm³, median (range)</th>
<th>Growth delay, days</th>
<th>95% CI</th>
<th>Maximum weight loss, mean % 95% CI</th>
<th>Cures, per group</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>PO</td>
<td>0</td>
<td>0</td>
<td>25 (23–36)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>IDN-5109</td>
<td>PO</td>
<td>720</td>
<td>180</td>
<td>71 (57–134)</td>
<td>0.003</td>
<td>46</td>
<td>11 to 81</td>
<td>22</td>
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<tr>
<td>IDN-5109</td>
<td>PO</td>
<td>480</td>
<td>120</td>
<td>104 (72–150+)</td>
<td>0.003</td>
<td>79</td>
<td>46 to 112</td>
<td>7</td>
</tr>
<tr>
<td>IDN-5109</td>
<td>PO</td>
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<td>80</td>
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<td>0.027</td>
<td>&gt;125</td>
<td>70 to 180</td>
<td>5</td>
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<tr>
<td>Vehicle</td>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>26 (20–36)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
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<td>IV</td>
<td>240</td>
<td>60</td>
<td>105 (73–150+)</td>
<td>0.003</td>
<td>79</td>
<td>51 to 107</td>
<td>7</td>
</tr>
<tr>
<td>IDN-5109</td>
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<td>160</td>
<td>40</td>
<td>57 (48–77)</td>
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<td>31</td>
<td>21 to 41</td>
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<td>3 to 25</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>22 (18–27)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Paclitaxel</td>
<td>PO</td>
<td>300</td>
<td>75</td>
<td>24 (20–55)</td>
<td>0.194</td>
<td>2</td>
<td>14 to 18</td>
<td>4</td>
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<td>34 (24–57)</td>
<td>0.010</td>
<td>6</td>
<td>1.8 to 13.8</td>
<td>3</td>
</tr>
</tbody>
</table>

*Groups of five mice bearing palpable xenograft tumors derived from SW-620 human colon carcinoma cells began oral paclitaxel treatment on day 7, intravenous paclitaxel treatment on day 10, oral IDN-5109 treatment on day 11, or intravenous IDN-5109 treatment on day 13 after tumor implantation, with four doses given at 4-day intervals for all four treatments. Tumor volume was measured every 3–4 days with calipers, and animals were killed when their tumors reached a target tumor volume of 600 mm³ or at the predetermined end of the study (150 days). CI = confidence interval; PO = oral; IV = intravenous.

†Median number of days for tumor to reach predetermined target volume (600 mm³), along with the shortest and longest duration of time taken to reach 600 mm³ for each drug administration group.

‡Statistical significance comparing median number of days required for tumors to reach 600 mm³ in drug-treatment group with median number of days required for tumors to reach 600 mm³ in the corresponding vehicle-treated group by use of the Cox–Mantel test (59).

§Designates difference in median number of days to reach target tumor volume of 600 mm³ between vehicle-treated control group and drug-treated groups.

‖Designates complete regression of tumor at the conclusion of the experiment (day 150). Number of cures/group.
Table 3. Effects of combination treatment with IDN-5109 and tRA96023 on xenograft tumors derived from DLD1 human colon carcinoma cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative dose, mg/kg</th>
<th>Individual dose, mg/kg</th>
<th>Days to reach 600 mm³ (median)</th>
<th>P† control</th>
<th>P§ (IDN-5109)</th>
<th>Growth delay, days</th>
<th>95% CI</th>
<th>Maximum weight loss, mean %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween</td>
<td>0</td>
<td>0</td>
<td>21 (21–26)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>Tween/Crem</td>
<td>0</td>
<td>0</td>
<td>21 (21–21)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IDN-5109</td>
<td>120</td>
<td>30</td>
<td>21 (21–25)</td>
<td>.26</td>
<td>0</td>
<td>1.5 to 1.5</td>
<td>0</td>
<td>15.0 to 3.0</td>
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</tr>
<tr>
<td>IDN-5109 + tRA96023</td>
<td>120 + 80</td>
<td>30 + 20</td>
<td>27 (18–32)</td>
<td>.15</td>
<td>.15</td>
<td>1.1 to 10</td>
<td>0</td>
<td>8</td>
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<tr>
<td>IDN-5109 + tRA96023</td>
<td>120 + 120</td>
<td>30 + 30</td>
<td>25 (21–31)</td>
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<td>.02</td>
<td>0.9 to 7.1</td>
<td>0</td>
<td>7</td>
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<tr>
<td>IDN-5109 + tRA96023</td>
<td>240 + 80</td>
<td>60 + 20</td>
<td>47 (29–150)</td>
<td>.006</td>
<td>.006</td>
<td>18.0 to 70</td>
<td>0</td>
<td>19.1 to 2.9</td>
<td>—</td>
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<tr>
<td>IDN-5109 + tRA96023</td>
<td>240 + 120</td>
<td>60 + 30</td>
<td>31 (28–40)</td>
<td>.002</td>
<td>.002</td>
<td>5.8 to 14.2</td>
<td>2</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>IDN-5109 + tRA96023</td>
<td>360 + 40</td>
<td>90 + 10</td>
<td>50 (44–69)</td>
<td>.002</td>
<td>.021</td>
<td>20.6 to 37.4</td>
<td>9</td>
<td>13.4 to 4.6</td>
<td>—</td>
</tr>
</tbody>
</table>

*Groups of five mice bearing palpable xenograft tumors derived from DLD1 human colon carcinoma cells began receiving oral doses of IDN-5109 with or without tRA96023 on day 7 after tumor implantation, every 4 days up to day 19. Tumor volume was measured every 3–4 days, and statistical analyses were completed when tumors reached the target volume of 600 mm³ or on reaching the conclusion of the experiment (day 150). CI = confidence interval.
†Median number of days for tumor to reach a volume of 600 mm³, along with the shortest and longest duration of time taken to reach a volume of 600 mm³ for each drug-administration group.
‡Statistical significance comparing median number of days required for tumors to reach 600 mm³ in each drug-treatment group with median number of days required for tumors to reach 600 mm³ in the corresponding vehicle-treated group using the Cox–Mantel test (59).
§Statistical significance comparing median number of days required for tumors to reach 600 mm³ in each combination-treatment group with the median number of days of required for tumors to reach 600 mm³ in the group receiving the corresponding dose of IDN-5109 alone using the Cox–Mantel test (59).
|§Designates difference in median number of days to reach target tumor volume of 600 mm³ between Tween-treated control group and IDN-5109-treated groups or between Tween/Cremophor-treated control group and IDN-5109 + tRA96023-treated groups.

Our findings from in vitro growth-inhibition assays confirm that IDN-5109 is a novel taxane that is effective against human tumor cell lines that are resistant to paclitaxel and docetaxel. However, differences in chemical structure confer on IDN-5109 an advantage, relative to paclitaxel, against Pgp-expressing tumors (24). This study sought to understand why IDN-5109 is much more effective than paclitaxel at inhibiting the growth of Pgp-expressing human tumor cells. Increased activity of IDN-5109 could be explained by numerous possibilities, including increased cellular uptake, enhanced apoptotic induction, enhanced tubulin polymerization capabilities, and/or increased cellular retention of drug.

It is unlikely that IDN-5109’s enhanced growth-inhibitory properties, as compared with the growth-inhibitory properties of paclitaxel, against Pgp-expressing cells is due to direct effects on microtubule stability. We have shown that IDN-5109 and paclitaxel have similar effects on tubulin polymerization in vitro and on colony formation in vivo by use of the tubulin mutant-containing Tax-18 CHO cell line. These results suggest that the differences in molecular mechanism(s) of action between paclitaxel and IDN-5109 involve a target other than tubulin.

It was suggested previously that IDN-5109 is not a substrate for Pgp, since IDN-5109 is active in Pgp-expressing cell lines (29). In this study, we explored an alternative possibility, that the modulation of Pgp activity, as measured by the accumulation and retention of various Pgp substrates by tumor cells that express different amounts of Pgp, could represent a potential mechanism to explain the superior growth-inhibitory activity of IDN-5109 against Pgp-expressing tumors. Our data suggest that, in Pgp-expressing cells, IDN-5109 modulates Pgp action, as demonstrated by increased levels of Pgp substrate (Rh-123 and doxorubicin) retention in these cells. The acetyl group at the C-10 position of the taxane nucleus appears to be important in conferring IDN-5109 with increased Pgp modulation characteristics. This structural feature may explain the enhanced in vitro activity of taxanes with an acetyl group at this position, as compared with taxanes containing a hydroxyl group at the C-10 position.

Our current model for IDN-5109’s mechanism of action involves IDN-5109 binding to Pgp and inhibiting the efflux activity of the pump, thus allowing IDN-5109 and other compounds to accumulate in cells. This would explain the superior antitumor effect of IDN-5109, compared with that of paclitaxel, on tumors that express Pgp. The results demonstrating increased intracellular accumulation and retention of [3H]IDN-5109 versus [3H]paclitaxel in a Pgp-expressing human breast carcinoma cell line are consistent with increased tumor growth inhibition of Pgp-expressing tumor cell lines by IDN-5109 as compared with paclitaxel. [3H]IDN-5109 was retained in MDA435/LCC6/mdr1 cells for up to 10 hours, while paclitaxel was effluxed rapidly from the cells. These findings lend support to our hypothesis that IDN-5109, by directly affecting Pgp action, is a self-modulating agent capable of blocking its own efflux and increasing its accumulation in Pgp-expressing tumor cells. Future studies will explore the physical nature of the interaction of IDN-5109 with Pgp. The ability of IDN-5109 to inhibit the efflux action of Pgp opens possibilities for synergistic interaction between IDN-5109 and other drugs (e.g., doxorubicin) that demonstrate resistance in Pgp-positive, multidrug-resistant tumors.

The taxane-based drug resistance reversal agent tRA96023 also modulated Pgp pump activity, and the combination of IDN-5109 and tRA96023 appeared to increase inhibition of Pgp activity, allowing for an increase in retention of Pgp substrates and increased tumor cell growth inhibition. The use of this nontoxic, drug resistance reversal agent with other Pgp-excludable drugs (e.g., doxorubicin and vinca alkaloids) will be explored in future studies. An increase in antitumor activity with such drug combinations against Pgp-expressing tumors is a distinct possibility, especially considering the results demonstrating tRA96023’s ability to enhance retention of doxorubicin, [3H]paclitaxel, and [3H]IDN-5109.
A previous study (31) demonstrated that IDN-5109 is an orally bioavailable taxane. In this study, we compared the antitumor effects of IDN-5109 and paclitaxel, using both intravenous and oral administration, in nude mice bearing tumor xenografts derived from Pgp-expressing cells. Experimental therapeutic studies in mice strongly indicated that IDN-5109 when administered either intravenously or orally is superior to paclitaxel in inhibiting the growth of paclitaxel-resistant tumors when administered either intravenously or orally. The increased oral bioavailability of IDN-5109, as compared with paclitaxel, may be a result of modulatory effects by IDN-5109 on the action of the Pgp pump inherently expressed in the gut. Of interest, lower doses (320 and 480 mg/kg) of orally administered IDN-5109 were more effective against tumors than a higher dose (720 mg/kg). This result could reflect experimental error associated with the small test groups of mice used (n = 5) for these studies. Alternatively, IDN-5109 uptake from the gut into the bloodstream may have been affected by the increase in gut toxicity elicited by higher doses of drug. Nevertheless, we observed that all of the oral doses of IDN-5109 described herein demonstrated an antitumor effect. It is also important to note that oral doses of IDN-5109 equaling intravenous doses (≤240 mg/kg) showed little tumor-growth inhibition against Pgp-expressing SW-620 colon tumor xenografts in mice. When administered orally, paclitaxel showed little antitumor activity suggesting that it is excluded from the bloodstream by the natural expression of the Pgp pump in the intestinal lining. IDN-5109 formulated in Tween 80/ethanol proved to be less toxic than paclitaxel formulated in Cremaphor/ethanol, allowing for the administration of higher doses of IDN-5109 to mice.

The combination of IDN-5109 with tRA96023 was more effective that IDN-5109 alone against tumors derived from paclitaxel-resistant DLD₁ colon carcinoma cells, suggesting that the combination of the two agents may be of therapeutic value. The in vitro flow cytometric data suggested that the combination’s enhanced activity as compared with each agent alone might be due to increased intracellular drug retention within the tumor cell. The combination of 240 mg/kg of IDN-5109 with 80 mg/kg of tRA96023 produced a statistically significant (P = 0.006) antitumor effect in vivo compared with IDN-5109 alone. Of interest, increasing the dose of tRA96023 to 120 mg/kg in combination with IDN-5109 produced no statistically significant difference in tumor-growth inhibition compared with IDN-5109 alone. This finding may suggest that, depending on the dose, tRA96023 can either increase IDN-5109 uptake when it is administered orally or, at high doses, can compete for IDN-5109 uptake, thus compromising the oral bioavailability of IDN-5109. Pharmacokinetic studies are necessary to better understand how tRA96023 acts when given in combination with IDN-5109.

Preclinical toxicology studies conducted at the Roswell Park Cancer Institute indicated that the toxicity profile of IDN-5109 in rats and dogs was similar to the toxicity profiles of other clinically useful, antineoplastic agents (Kanter P: unpublished results). In both species, gastrointestinal toxicity was the dose-limiting toxicity. Myelosuppression, primarily decreased neutrophils, was observed at high doses of IDN-5109, with little observed effect on platelets. Other toxic effects included anorexia and weight loss, hind-limb weakness (rats), hair loss, diarrhea, and vomiting. Neuropathic toxicity was also noted in rats but was reversible. No neurotoxicity was evident in dogs. IDN-5109’s effectiveness against drug-resistant tumors, as demonstrated here and in preclinical testing, has facilitated its entrance into clinical trial, where it may prove to be more effective than paclitaxel or docetaxel against a variety of tumors, especially those exhibiting a multidrug resistance phenotype. Currently, drug therapies for colon cancer treatment are severely limited. IDN-5109 may be able to broaden the spectrum of taxane use to include colon tumors, thus providing an alternative to the current regimens of treatment.

References

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NOTES

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