Paclitaxel Cytotoxicity against Human Lung Cancer Cell Lines Increases with Prolonged Exposure Durations

Mark S. Georgiadis, Edward K. Russell, Adi F. Gazdar, and Bruce E. Johnson

INTRODUCTION

Paclitaxel is the first member of a new class of chemotherapeutic agents which interact with polymerized tubulin to both promote the formation of microtubules and to prevent their disassembly (1). This mechanism of action causes cells to be blocked in G2-M, and this cell cycle specificity may, in turn, result in a schedule-dependent effect on cytotoxicity (2).

Preliminary in vitro data demonstrate that paclitaxel cytotoxicity may be a function of the duration of paclitaxel exposure to cancer cells. Rowinsky et al. (3) used a clonogenic assay system to evaluate the cytotoxicity of paclitaxel against four leukemia cell lines. They found increasing cytotoxicity in three of the four cell lines as the duration of exposure to paclitaxel (0.1–10 μM) increased from 2 to 22 h. A second clonogenic based system was used to evaluate a human ovarian cancer cell line and also showed increasing cytotoxicity as the duration of paclitaxel exposure increased from 2 to 18 h (4). Unfortunately, these studies evaluated the schedule effect only to a maximum exposure duration of 22 h. Liebmann et al. (5) evaluated paclitaxel cytotoxicity in a clonogenic assay with three adenocarcinoma cell lines. In the lung adenocarcinoma cell line A549, they noted a 100-fold increase in cytotoxicity as the duration of exposure to paclitaxel (≥0.05 μM) increased from 24 to 72 h. A similar effect was seen with both a breast and a pancreatic adenocarcinoma cell line.

In clinical trials, paclitaxel has been administered for infusion durations of 1, 3, 6, 24, 96, and 120 h (6–10). Initial trials utilized infusion durations of 24 h to minimize hypersensitivity reactions from one component of the paclitaxel vehicle, Cremophor EL (1). More recent clinical trials have begun to evaluate both shorter and longer infusion durations, but the most effective paclitaxel schedule has yet to be determined (11). The NCI4-Navy Medical Oncology Branch maintains a large number of human lung cancer cell lines. Clinical information is available for many of the patients from whom the cell lines were established, and previous study has determined that lung cancer cell lines exhibit significant heterogeneity with in vitro chemosensitivity testing (12, 13). However, only one human lung cancer cell line has been previously evaluated with respect to paclitaxel exposure duration (5). Prior to evaluating paclitaxel in clinical trials for patients with lung cancer, we sought to expand upon the concept of paclitaxel schedule dependency by testing the in vitro cytotoxicity of different paclitaxel exposure durations against 28 lung cancer cell lines. We believed that this would enable us to evaluate for the degree of heterogeneity of paclitaxel's cytotoxicity within a series of cell lines of similar tumor sensitivity.

ABSTRACT

Paclitaxel blocks cells in G2-M, and this may result in a schedule-dependent effect on paclitaxel cytotoxicity. To test this hypothesis, we evaluated paclitaxel cytotoxicity in 28 human lung cancer cell lines. Fourteen of the cell lines were derived from patients with non-small cell lung cancer (NSCLC), and 14 were from patients with small cell lung cancer (SCLC). All cell lines were exposed to a range of paclitaxel concentrations for durations of 3, 24, and 120 h, and cytotoxicity was measured with a tetrazolium-based assay. The median IC50 values for all 28 cell lines at exposure durations of 3, 24, and 120 h were >32 μM, 23 μM, and 0.38 μM, respectively. The median IC50 values for the NSCLC cell lines were >32 μM, 9.4 μM, and 0.027 μM at exposure durations of 3, 24, and 120 h, respectively. For the 14 SCLC cell lines, the median IC50 values were >32 μM, 25 μM, and 5.0 μM, respectively. Five of the 14 SCLC cell lines had IC50 values at 120 h of paclitaxel exposure that were 1000-fold less than the remaining SCLC cell lines. The median IC50 values for these five sensitive SCLC cell lines at 3-, 24-, and 120-h exposures were >32 μM, 23 μM, and <0.0032 μM, respectively. These in vitro cytotoxicity results were independent of the paclitaxel diluent, a 1:1 solution of ethanol and Cremophor EL. We conclude that longer durations of paclitaxel exposure result in an increase in the chemosensitivity of some human lung cancer cell lines and that this phenomenon is more consistent within NSCLC cell lines than in SCLC cell lines.
**MATERIALS AND METHODS**

**Cell Lines.** We evaluated 28 human lung cancer cell lines that were previously established at the NCI-Naval Medical Oncology Branch (12). Of these, 14 were derived from patients with NSCLC, and 14 were from patients with SCLC. Cell cultures were grown in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) and penicillin-streptomycin (Life Technologies, Inc.). Cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C and were in logarithmic growth phase at the time of analysis. Cell lines were identified as being established from patients who had previously received or not received chemotherapy for lung cancer (12). Of note, none of the cell lines were obtained from patients previously or subsequently treated with paclitaxel. In addition, the growth rate of many of the cell lines was previously determined, and this information was included in the present study (12, 14). Finally, many cell lines were previously evaluated for MDRI gene expression via RNA slot blot analysis, and these data were also incorporated into the present study (15).

**Drugs.** Paclitaxel (Taxol) was obtained from the Division of Cancer Treatment, NCI, and Bristol-Myers Squibb (Wallington, CT). Paclitaxel was supplied as the clinical formulation containing 6 mg/ml in 50% (v/v) ethanol and 50% Cremophor EL (polyoxyethylated castor oil). The ethanol/Cremophor EL diluent. Diluent 12 (NSC 614387), was obtained from the Division of Cancer Treatment, NCI. Both paclitaxel and the ethanol/Cremophor EL vehicle were diluted with PBS (Life Technologies, Inc.) to 10× the final concentrations and stored frozen at −70°C.

**MTT Assay.** The details of this assay have been described previously (13, 14). For each of the 28 cell lines, 180 μl of a single-cell suspension (10,000–20,000 cells) was plated into each well of three 96-well microtiter plates. Within each column of wells, 20 μl of the appropriate drug dilution or control were added. Thus, eight replicates were simultaneously evaluated for each cell line, drug concentration, and exposure duration. The final paclitaxel concentrations ranged from 0.0032 to 32 μM, and the control was PBS. After 3 h of incubation, one of the three plates was centrifuged, the media/drug supernatant was removed, and the cells were washed twice with PBS. The cells were then suspended in fresh media and incubated. At 24 h, the cells in the second plate were centrifuged, washed, resuspended, and incubated. At 120 h, 100 μg of MTT substrate (Sigma Chemical Co., St. Louis, MO) were added to each well of the three plates. Following 4 h of incubation with MTT, the plates were centrifuged, the supernatant was removed, and the cells were resuspended in 150 μl of 100% DMSO (Sigma Chemical Co.). After agitation, the absorbance at 570 nm was measured for each well with a scanning multiwell spectrophotometer (Microplate Bio-Kinetics Reader Model EL312; Bio-Tek Instruments, Inc., Winooski, VT). The mean absorbance for the eight replicates at each drug concentration was normalized to the control absorbance, and the relative optical densities were plotted. Thus, the absorbance relative to control was a measure of the fractional cell survival. The drug concentration which caused a 50% decrease in the relative absorbance was defined as the IC₅₀. The entire experiment was performed in triplicate for four NSCLC (NCI-H838, NCI-H1299, NCI-H1355, and NCI-H1734) and five SCLC (NCI-H69, NCI-H449, NCI-H510, NCI-H526, and NCI-H719) cell lines.

**Variant MTT Assays.** In the above assay, after the cells were exposed to paclitaxel for either 3 or 24 h, they were incubated in drug-free media until 120 h when all of the plates were evaluated using the MTT assay. A variant of the above method explored the possibility that there might have been significant cytotoxicity at either 3 or 24 h but that this was coupled with renewed cell proliferation after the paclitaxel was removed. The net result of such an effect would be the false appearance of a lack of cytotoxicity at the shorter exposure durations. In the first variant assay, NCI-H838 and NCI-H449 were exposed to paclitaxel (0.0032 to 32 μM) for 3 or 24 h, and the MTT assay was performed immediately after drug exposure.

A second variation investigated the possibility that cytotoxicity at prolonged exposures was more a function of the ethanol/Cremophor EL vehicle rather than the paclitaxel. In this series of experiments, single-cell suspensions of two cell lines (NCI-H838 and NCI-H526) were each plated into two 96-well plates. In one plate, the cells were exposed to paclitaxel and PBS control as described above. In the second plate, diluent alone replaced the paclitaxel. The ethanol/Cremophor EL vehicle was diluted with PBS to yield v/v identical dilutions as the paclitaxel. Thus, each ethanol/Cremophor EL concentration contained the same amount of diluent as was contained in the corresponding paclitaxel concentration. MTT assays were performed at the end of a 96-h continuous exposure.

**RESULTS**

**MTT Assay.** The results for NCI-H526 are plotted and show that at paclitaxel concentrations ≥0.01 μM, cytotoxicity increases with increasing exposure duration, and cytotoxicity is independent of paclitaxel concentration (Fig. 1). The median (range) IC₅₀ values at each paclitaxel exposure duration for all 28 cell lines were >32 μM (0.027–>32 μM) at the 3-h exposure, 23 μM (0.0037–>32 μM) at the 24-h exposure, and 0.38 μM (<0.0032–>32 μM) at the 120-h exposure. The characteristics of the 14 NSCLC and the 14 SCLC cell lines and the results of the individual MTT assays are tabulated (Tables 1 and 2, respectively). The median (range) IC₅₀ values for the 14 NSCLC cell lines were >32 μM (0.28–>32 μM) at the 3-h exposure, 9.4 μM (0.0092–>32 μM) at the 24-h exposure, and 0.027 μM (0.0091–>32 μM) at the 120-h exposure (Table 1). Data on the treatment status of the patients from whom each NSCLC cell
line was established were available for 11 of the 14 cell lines. Five of these 11 NSCLC cell lines were derived from patients previously treated with chemotherapy, and 6 were derived from chemotherapy-naïve patients. Their appeared to be no relationship between treatment status and sensitivity to paclitaxel in the MTT assay. Data regarding the cell line growth rate were available for six cell lines; three cell lines grew at a slow rate, and three cell lines grew at a fast rate. There was no apparent relationship between growth rate and sensitivity to prolonged exposures of paclitaxel. Information regarding MDRI gene expression was available for 13 of the NSCLC cell lines: 7 cell lines expressed low levels of MDRI mRNA, 5 cell lines expressed intermediate levels, and 1 cell line expressed high levels. There appeared to be no relationship between MDRI gene expression and sensitivity to paclitaxel in this assay.

The median (range) IC50 values for the 14 SCLC cell lines were >32 µM (0.027->32 µM) at 3-h, 25 µM (0.0037->32 µM) at 24-h, and 5.0 µM (<0.0032->32 µM) at 120-h exposure duration (Table 2). Five of the SCLC cell lines appeared to be very sensitive to paclitaxel in this assay: NCI-H209, NCI-H524, NCI-H526, NCI-H719, and NCI-H1870. The median (range) IC50 values for these five cell lines were >32 µM (0.027->32 µM), 23 µM (0.0037->32 µM), and <0.0032 µM (<0.0032-0.0061 µM) for exposure durations of 3, 24, and 120 h, respectively. One-half of the 14 SCLC cell lines were derived from patients previously treated with chemotherapy. In addition, the cellular growth rate was available for eight cell lines, and in six of these cell lines, the growth rate was fast. Data regarding MDRI gene expression were available for 10 cell lines. As for the NSCLC cell lines, there appeared to be no relationship between previous treatment with chemotherapy, in vitro growth rate, or degree of MDRI gene expression and sensitivity to paclitaxel in this assay.

**Variant MTT Assays.** In the above studies, the cells exposed to paclitaxel for either 3 or 24 h were not evaluated with the MTT assay until after reincubation in drug-free media for an additional 117 or 96 h. In the first variant assay, cytotoxicity was assessed immediately after drug exposure. These variant assays did not show evidence of initial cytotoxicity that abated after renewed incubation. The IC50 values for NCI-H838 and NCI-H449 in this variant assay were >32 and >32 µM, respectively, immediately after a 3-h paclitaxel exposure and 24 and >32 µM, respectively, after a 24-h exposure.

In the second variant assay, the cytotoxicity of paclitaxel was compared to that of v/v identical dilutions of the paclitaxel diluent. Thus, IC50 values could be calculated for exposure to the ethanol/Cremophor EL vehicle and expressed as the concentration of paclitaxel containing that amount of diluent. Representative dose-response curves for concurrent 96-h exposures to both paclitaxel and ethanol/Cremophor EL for NCI-H526 found an IC50 for the diluent of 3.1 µM (0.043%, v/v) and an IC50 for paclitaxel of 0.026 µM (Fig. 2). For NCI-H838, the IC50 for ethanol/Cremophor EL was 2.4 µM (0.034%, v/v), and the simultaneous IC50 for paclitaxel was 0.074 µM. Thus, the IC50 values for diluent were 100-fold greater than the corresponding IC50 values for paclitaxel exposure, and these results suggest that the ethanol/Cremophor EL vehicle effects the results of in vitro studies in the MTT assay only at very high concentrations of paclitaxel.

**DISCUSSION**

In this report, we describe the in vitro cytotoxicity of varying durations of paclitaxel exposure against 28 human lung cancer cell lines. There was a >1000-fold decrease in the median IC50 values for the 14 NSCLC cell lines as the duration of exposure to paclitaxel increased from 3 h to 120 h, and there was a <10-fold decrease in the median IC50 values for the 14 SCLC cell lines as the exposure duration increased. Five of the SCLC cell lines were very sensitive to prolonged exposures to paclitaxel, and in this subset of sensitive SCLC cell lines, there was a >10,000-fold decrease in the median IC50 values as the duration of exposure increased to 120 h. Thus, these in vitro studies confirm that paclitaxel exhibits a schedule-dependent effect on cytotoxicity.

In the current analysis, we used the semiautomated MTT assay to study paclitaxel schedule dependency, and this allowed us to evaluate for heterogeneity within the multiple cell lines. The schedule dependency of paclitaxel was consistently detected in 10 of the 14 NSCLC cell lines. However, there was a significant degree of variability in the responsiveness to prolonged exposures of paclitaxel within the SCLC cell lines. This preclinical model predicts that responses to prolonged infusions of paclitaxel in patients with lung cancer may be more common in patients with NSCLC than in patients with SCLC. In addition, we showed that paclitaxel was active in this in vitro system in cell lines established from patients who were both previously treated and untreated. This may have clinical implications since prolonged infusions of paclitaxel have been shown to have activity in patients with chemotherapy-resistant breast cancer (9, 17).

The mechanism responsible for the schedule-dependent effect of paclitaxel is unknown, but we theorize that longer durations of drug exposure may allow a greater proportion of cells to cycle into the G2-M, or susceptible, phase. However, this theory implies that cell lines with the longest doubling times should be most affected by prolonged paclitaxel exposure, and...
Paclitaxel against Human Lung Cancer Cell Lines

Cremophor EL can be detected in the plasma of patients treated with paclitaxel and this was antagonistic to paclitaxel cytotoxicity. Webster et al. (18) measured Cremophor EL concentrations of 0.0058 and 0.015 μM in the second variant MU assay, NCI-H526 and NCI-H838 after 120-h exposure to paclitaxel. In the present study, the paclitaxel IC50 values for NCI-H526 and NCI-H838 after 120-h exposure were 0.0058 and 0.015 μM, respectively. By comparison, in the second variant MTT assay, NCI-H526 and NCI-H838 were exposed to paclitaxel for 96 h, and the IC50 values were 0.026 and 0.074 μM, respectively. These values suggest the absence of a marked difference in cytotoxicity after 96-h versus 120-h paclitaxel exposure, but we believe further evaluation of this issue is warranted.

The present in vitro observations served as the basis for our recently completed Phase I clinical trial of a prolonged paclitaxel infusion schedule. At the recommended Phase II dose of this regimen, 2 complete and 16 partial remissions (response rate, 55%) were noted in 33 patients enrolled in this clinical trial. Our goal will be to incorporate the in vivo and in vitro activity of paclitaxel against lung cancer and to evaluate in vitro paclitaxel exposure durations beyond 96–120 h in cell lines from patients with refractory disease. At the recommended Phase II dose of this regimen, median response durations even longer than 120 h may be required to fully evaluate this issue, and we plan to perform these studies in the future. Others have suggested that increased cytotoxicity with prolonged exposure durations to natural products may in part relate to the ability of longer exposures to overcome the multidrug-resistant phenotype (16). In our series of cell lines, there was no apparent relationship between the cytotoxicity of paclitaxel at any exposure duration and the expression of the MDR1 gene. There were, however, very few lung cancer cell lines that had significant levels of MDR1 expression (15).

The paclitaxel cytotoxicity in our assay was independent of the ethanol/Cremophor EL diluent, since the diluent resulted in significant cytotoxicity only at concentrations above 0.014% (equivalent to paclitaxel concentrations above 1.0 μM). This finding, however, may have potential clinical relevance since Cremophor EL can be detected in the plasma of patients treated with paclitaxel. Webster et al. (18) measured Cremophor EL levels of ≥0.1% (v/v) immediately after a 3-h paclitaxel infusion (dose, 175 mg/m2) in 15 of 17 treated patients. Other investigators have evaluated the paclitaxel diluent for cytotoxicity and have found results similar to ours. Fjällskog et al. (19) studied two human breast cancer cell lines, ZR 75-1 and HS 578T, with a semiautomated fluorometric assay. They found IC50 values for the ethanol/Cremophor EL combination of 0.09% and 0.04%, respectively, for the two cell lines. In contrast, Liebmann et al. (2, 5) showed that higher concentrations of Cremophor EL were antagonistic to paclitaxel cytotoxicity. They showed that exposure of the lung cancer cell line A549 to Cremophor EL (0.135%, v/v) resulted in a block in G1 (2). More importantly, they found a paradoxical decrease in paclitaxel cytotoxicity in this cell line at paclitaxel concentrations ≥10 μM, and they showed that this decrease in cytotoxicity was likely related to the paclitaxel diluent (5). The basis for these discrepant findings may relate to differences in the assay systems (20).

The schedule-dependent effect of paclitaxel cytotoxicity suggests that clinical trials with paclitaxel in patients with lung cancer should evaluate prolonged i.v. infusion schedules. The goal of these schedules would be to maintain a minimal paclitaxel plasma level for several days rather than attempting to reach high peak plasma concentrations. Thus far, clinical trials have evaluated prolonged paclitaxel infusions with either 96-h or 120-h infusion schedules (9, 10, 17, 21). There is little in vitro data in lung cancer cell lines that compare 96-h paclitaxel exposure to 120-h exposure. In the present study, the paclitaxel IC50 values for NCI-H526 and NCI-H838 after 120-h exposure were 0.0058 and 0.015 μM, respectively. By comparison, in the second variant MTT assay, NCI-H526 and NCI-H838 were exposed to paclitaxel for 96 h, and the IC50 values were 0.026 and 0.074 μM, respectively. These values suggest the absence of a marked difference in cytotoxicity after 96-h versus 120-h paclitaxel exposure, but we believe further evaluation of this issue is warranted.

The present in vitro observations served as the basis for our recently completed Phase I clinical trial of a prolonged paclitaxel infusion schedule in patients with advanced lung cancer (21). Patients were treated with paclitaxel by a 96-h continuous infusion (doses, 100–180 mg/m2/96 h) followed by cisplatin (doses, 60–80 mg/m2). The regimen was active in NSCLC with 2 complete and 16 partial remissions (response rate, 55%) in 33 patients with measurable disease. In addition, we have been prospectively establishing tumor cell lines from many of the patients enrolled in this clinical trial. Our goal will be to correlate the in vivo and in vitro activity of paclitaxel against lung cancer and to evaluate in vitro paclitaxel exposure durations beyond 96–120 h in cell lines from patients with refractory disease.

### Table 1
Characteristics of 14 NSCLC cell lines and corresponding IC50 values in the MTT assay at varying durations of paclitaxel exposure

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Therapy</th>
<th>Growth</th>
<th>MDR1</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H23</td>
<td>N</td>
<td>Slow</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H157</td>
<td>N</td>
<td>unk</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H322</td>
<td>Y</td>
<td>unk</td>
<td>intermed</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>unk</td>
<td>Fast</td>
<td>intermed</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H522</td>
<td>N</td>
<td>unk</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H650</td>
<td>unk</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H676</td>
<td>Y</td>
<td>unk</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H727</td>
<td>N</td>
<td>Slow</td>
<td>High</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H838</td>
<td>N</td>
<td>unk</td>
<td>intermed</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H1155</td>
<td>N</td>
<td>Fast</td>
<td>intermed</td>
<td>0.31</td>
</tr>
<tr>
<td>NCI-H1299</td>
<td>Y</td>
<td>unk</td>
<td>intermed</td>
<td>0.28 ± 0.12</td>
</tr>
<tr>
<td>NCI-H1355</td>
<td>Y</td>
<td>unk</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H1373</td>
<td>Y</td>
<td>Slow</td>
<td>Low</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H734</td>
<td>unk</td>
<td>unk</td>
<td>unk</td>
<td>&gt;32</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>&gt;32</td>
<td>9.4</td>
<td>0.027</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent the mean of eight simultaneous replicates. Means ± SD of three independent experiments are provided for four cell lines.
*Cell line established from patient previously treated with chemotherapy. Y, yes; N, no; unk, data unknown (12).
*In vitro growth rate of tumor cell line. intermed, intermediate growth rate; unk, data unknown (12, 14).
*MDR1 gene expression as compared to a cell line expressing high levels of MDR1 mRNA. Low, low levels of expression; intermed, intermediate levels; High, high level of MDR1 mRNA expression (15).
was administered as a 24-h rather than a 3-h infusion (7). Cancer showed significantly more neutropenia when paclitaxel
sent the mean of two independent experiments.

Fig. 2

Table 2 Characteristics of 14 SCLC cell lines and corresponding IC_{50} values in the MTT assay at varying durations of paclitaxel exposure

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Therapy</th>
<th>Growth</th>
<th>MDR1</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H69</td>
<td>Y</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H123</td>
<td>Y</td>
<td>unk</td>
<td>unk</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H128</td>
<td>Y</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H209</td>
<td>N</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H220</td>
<td>N</td>
<td>unk</td>
<td>unk</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H249</td>
<td>Y</td>
<td>Slow</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H449</td>
<td>Y</td>
<td>unk</td>
<td>unk</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H510</td>
<td>Y</td>
<td>intermed</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H524</td>
<td>Y</td>
<td>unk</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H526</td>
<td>N</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H660</td>
<td>N</td>
<td>Fast</td>
<td>Low</td>
<td>&gt;32 ± 0</td>
</tr>
<tr>
<td>NCI-H719</td>
<td>N</td>
<td>unk</td>
<td>intermed</td>
<td>0.027 ± 0.019</td>
</tr>
<tr>
<td>NCI-H889</td>
<td>N</td>
<td>Fast</td>
<td>intermed</td>
<td>&gt;32</td>
</tr>
<tr>
<td>NCI-H1870</td>
<td>N</td>
<td>unk</td>
<td>unk</td>
<td>&gt;32 ± 0</td>
</tr>
</tbody>
</table>

Median >32

Values represent the mean of eight simultaneous replicates. Means ± SD of three independent experiments are provided for five cell lines.

In vitro growth rate of tumor cell line. intermed, intermediate growth rate; unk, data unknown (12).

MDR1 gene expression as compared to a cell line expressing high levels of MDR1 mRNA. Low, low levels of expression; intermed, intermediate levels; High, high level of MDR1 mRNA expression (15).

Pharmacokinetic studies in a subset of these randomized patients determined that the incidence of grade 3 or 4 neutropenia correlated with the duration of exposure to paclitaxel plasma concentrations ≥0.05 μM and did not correlate with the paclitaxel dose, area under the concentration versus time curve, or peak plasma concentration (22). The randomized study found no statistically significant differences in response rates or survival between the 3- or 24-h administration schedules, but both schedules were of relatively short duration. In addition, the study population included only patients who were previously treated with chemotherapy, and this may have lowered the likelihood of being able to detect a response or survival difference between the treatment regimens. The second study evaluated paclitaxel in 26 evaluable women with metastatic breast cancer who were refractory to both anthracyclines and short (24-h) infusions of taxanes (17). These patients were treated with paclitaxel as a 96-h continuous infusion, and 7 of 26 patients (27%) responded to the prolonged paclitaxel infusion after previously failing the shorter infusion.

The in vitro schedule dependency of paclitaxel has now been confirmed by multiple techniques and in several different types of cancer cell lines. Clinical trials have begun to show efficacy with prolonged paclitaxel infusion schedules, and the preliminary results suggest that longer infusion schedules may result in both greater hematological toxicity and greater cancer cell cytotoxicity. We believe that the administration schedule of paclitaxel to cancer patients may have an impact on the cytotoxic effect of this agent and that this issue of dose schedule needs to continue to be studied in clinical trials.

REFERENCES


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