Enhancement of Paclitaxel Delivery to Solid Tumors by Apoptosis-Inducing Pretreatment: Effect of Treatment Schedule

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ABSTRACT

The limited penetration of paclitaxel into solid tumors may limit its therapeutic efficacy. We recently showed a correlation between an increase in interstitial space and an enhancement of drug delivery in solid tumors. The present study evaluated whether this observation can be used to develop a treatment strategy, where an apoptosis-inducing pretreatment with paclitaxel is used to enhance its own delivery to solid tumors. In histocultures of human pharynx FaDu xenograft tumors, pretreatment with 1 µM nonradiolabeled paclitaxel, which resulted in ~25% apoptosis and a 25% reduction in cell density, enhanced the penetration rate of [3H]paclitaxel. Likewise, dividing a total drug exposure to two treatments, separated by an interval to allow apoptosis to occur, resulted in higher drug penetration rate and accumulation compared with giving the same drug exposure continuously. Similar results were obtained in rats bearing subcutaneously implanted prostate MAT-LyLu tumors; fractionation of the dose, to include 1) a pretreatment that yielded sufficient and clinically relevant plasma concentration to induce apoptosis and 2) a second dose given at an interval selected to allow apoptosis and reduction in tumor cell density to occur, resulted in higher tumor concentration compared with other treatments using the same total dose but either did not include an apoptosis-inducing pretreatment or did not allow for apoptosis to occur. We conclude that the pharmacological effect of paclitaxel affects its own delivery to solid tumors and that modifications of the paclitaxel treatment schedule can enhance drug delivery in solid tumors.

Paclitaxel, one of the most important anticancer drugs developed in the past two decades, is active against multiple types of human solid tumors (Rowinsky, 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). The intracellular concentration of paclitaxel is critical for its pharmacological effect; drug resistance in several resistant sublines is correlated with reduced intracellular drug accumulation compared with the sensitive parent cell lines (Lopes et al., 1993; Bhalla et al., 1994; Jekunen et al., 1994; Riou et al., 1994; Speicher et al., 1994).

It has been proposed that drug delivery to the tumor core is necessary to prevent tumor regrowth and is important for treatment efficacy (Durand, 1990; Erlanson et al., 1992; Baguley and Finlay, 1995; Jain, 1996). Drug delivery to the tumor core is governed by several factors, which differ for systemic and regional treatments. Following a systemic intravenous injection, drug delivery to cells in solid tumors involves three processes, i.e., distribution through vascular space, transport across microvascular walls, and diffusion through interstitial space in tumor tissue (Jain, 1987). When the drug is directly injected into a tumor, e.g., by intratumoral injection or by direct instillation into peritumoral space such as in intravesical therapy of superficial bladder cancer and in intraperitoneal therapy of ovarian cancer, drug delivery to cells in solid tumors is primarily achieved by diffusion through interstitial space (Markman et al., 1995; Nativ et al., 1997; Song et al., 1997; Markman, 1998).

The diffusion characteristics of paclitaxel, despite its relatively low molecular mass (853 daltons), are likely to be similar to those of a protein because of its extensive binding to proteins in interstitial fluid (Baguley and Finlay, 1995). We have shown that resistance to paclitaxel penetration into tumor tissue is highly dependent on the cell density; paclitaxel penetration in human tumor histocultures is restricted to the periphery until the cell density is reduced due to drug-induced apoptosis, at which time paclitaxel distributes evenly throughout the tumor (Kuh et al., 1999). The slow appearance of apoptosis in tumor histocultures (i.e., at about 24 h) is in agreement with our observations in multiple human tumor cell lines (Au et al., 1998). Based on these findings, we hypothesize that the delivery of paclitaxel to solid tumors can be enhanced by an apoptosis-inducing pre-
treatment. This hypothesis was evaluated in the present study. In vitro studies were performed using histocultures of human pharynx FaDu tumor xenograft maintained in immuno-
nodeficient mice, and in vivo studies were performed using a syngeneic tumor model, i.e., rats bearing subcutaneously im-
planted prostate MAT-LyLu tumors.

Materials and Methods

Chemicals and Reagents. Paclitaxel was obtained from the
Bristol Myers Squibb Co. (Wallington, CT) and the National Cancer
Institute (Bethesda, MD); 3[H]paclitaxel (specific activity, 19.3
Ci/mmol) from the National Cancer Institute; cefotaxime sodium
from Hoechst-Roussel Inc. (Somerville, NJ); gentamicin from Solo
Pak Laboratories (Franklin Park, IL); other tissue culture supplies
were obtained from Life Technologies, Inc. (Grand Island, NY). Solv-
able tissue gel solubilizer and Atomlight scintillation fluid were
obtained from DuPont Biotechnology Systems (Boston, MA), Hyper-
film from Amersham Pharmacia Biotech (Arlington Heights, IL),
autoradiographic supplies from Kodak (Rochester, NY), and Cremp-
ophor EL from Sigma Chemical Co. (St. Louis, MO).

Animals. Male nu/nu BALBc mice, weighing 18 to 21 g, were
purchased from the National Cancer Institute; male Copenhagen
rats, weighing 190 to 210 g, were obtained from Harlan Biomedicals
(Dawely, OH). Animal care was provided by the Laboratory Animal
Resources in our institution.

Tumor Culture and Drug Treatment: In Vitro Studies. FaDu
cells were obtained from the American Type Culture Collection (Ma-
nassas, VA) and maintained in minimum essential medium supple-
mented with 9% heat-inactivated fetal bovine serum, 2 mM L-gluta-
tamine, 0.1 mM nonessential amino acids, 90 μg/ml gentamicin, and
90 μg/ml cefotaxime. Cells were harvested from confluent cul-
tures using trypsin and resuspended in fresh medium before plating.
Cells with greater than 90% viability, as determined by Trypan Blue
exclusion, were used for tumor implantation. Cells were centrifuged
and resuspended in culture medium mixed with equal volume of
Matrigel (Collaborative Biomedical Products, Bedford, MA). Matri-
gel is a solubilized tissue basement membrane preparation extracted
from the Engelbreth-Holmswam mouse tumor and supports the
Tumor cells (10^6 cells in 0.1 ml) were injected subcutaneously in the
left and right sides of the upper back in a mouse, with a 21-gauge
needle. Tumors were removed when they reached a size of about 0.5 g
and dissected into 1-mm^3 pieces under sterile conditions within 2 h
after removal from the host. Five to six tumor pieces were placed on
a 1-cm^2 collagen gel (presoaked with drug-free medium) in a six-well
plate and incubated with 4 ml of culture medium at 37°C in a
humidified atmosphere of 95% air and 5% CO_2, for 3 to 4 days. Drug
treatment was done as previously described (Kuh et al., 1999).
Briefly, the histocultures were transferred to a collagen gel pre-
soaked with drug-containing medium for at least 8 h. The presooking
was to eliminate the delay in drug transport from medium through
the collagen gel (Kuh et al., 1999). Drug treatment was terminated
by carefully transferring histocultures to a collagen gel presoaked
with drug-free medium.

Drug Penetration and Accumulation: In Vitro Studies. The
spatial distribution of [3H]paclitaxel in histocultures was studied using
autoradiography and imaging techniques as described previ-
ously (Kuh et al., 1999). Briefly, frozen cross sections (10 μm) taken
from the middle of the histocultures were placed on glass slides,
followed by exposure to 3H-sensitive film (Hyperfilm 3H) for a week.
The film was developed, and the slide was stained with H&E. Image
analysis was used to capture the autoradiographic image (where the
gains indicated the location of the radiolabeled drug) as well as the
histological image of the tissue section stained with H&E (which
showed the tissue structure and distribution of tumor cells), and to
overlay the two images to visualize the distribution of [3H]paclitaxel
in the tumor tissue.

The total drug concentration in tumor histocultures was measured
as described previously (Kuh et al., 1999). Briefly, after incubating
with 4 ml of culture medium containing a mixture of radiolabeled and
nonradiolabeled paclitaxel (specific activity, 0.301 Ci/mmol), histo-
cultures were removed, blot-dried, weighed, incubated overnight
with Solvable tissue/gel solubilizer at 50°C in an oven, mixed with
Atomlight scintillation fluid, and analyzed for total radioactivity
using a liquid scintillation counter. Thirty to 35 tumor histocultures
were used for each time point. We have shown that 95% of the
radioactivity was represented by paclitaxel and its epimerization
product, 7-epitaxol (Kuh et al., 1999). Because 7-epitaxol has similar
microtubule binding affinity and cytotoxicity to paclitaxel (Riegel
and Horwitz, 1987), total radioactivity was expressed in paclitaxel
equivalents. Drug concentration in the tissue was calculated as the
amount of drug divided by the tissue weight.

Effect of Apoptosis-Inducing Pretreatment on Drug Penet-
ration and Accumulation in Tissues: In Vitro Studies. Three
in vitro studies were performed to examine the effect of pretreat-
ment. The study protocols were as described under Results. Tumor
cell density and the fraction of apoptotic cells were determined by
counting the number of total tumor cells and apoptotic cells in
non-necrotic regions at 400× magnification. Apoptotic cells were
determined based on morphological changes in tumor cells such as
chromatin condensation and margination, disappearance of nucleoli,
formation of membrane blebs, formation of apoptotic bodies, and/or
cell shrinkage (Kerr et al., 1994). Our laboratory and others have
shown that this method yields the same results as the terminal
deoxynucleotidyl transferase-mediated dUTP nick end labeling
method (Gold et al., 1994; Gan et al., 1996).

Effect of Apoptosis-Inducing Pretreatment on Drug Accu-
mulation in Tumors: In Vivo Studies. The in vivo studies were
performed using a rat tumor model because of the ease of adminis-
tering an intravenous infusion and sampling blood repeatedly. The
rat MAT-LyLu tumor cells were originally obtained from Dr. J.
Isaacs (Johns Hopkins University, Baltimore, MD), and have been
maintained in our laboratory in RPMI-1640 medium supplemented
with 9% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 90
μg/ml gentamicin, and 90 μg/ml cefotaxime sodium. Tumor cells (5 × 10^6
cells in 0.1 ml medium, >90% viability as determined by Trypan
Blue exclusion) were injected subcutaneously in the right and left
upper backs of a male Copenhagen rat with a 21-gauge needle.
Experiments were initiated when the tumor was between 0.3 to 0.5 g
in size.

The jugular vein and carotid artery of tumor-bearing rats were
catheterized, under light ether anesthesia, with polyethylene tubing
(PE-50, Becton Dickinson Co., Sparks, MD) for drug administration
and for blood sampling, respectively. Each catheter was exteriorized
to the dorsal side of the neck and attached to a second length of
polyethylene tubing (PE-50). The catheters and tubing were covered
with metal coil tubing. Animals were allowed to recover for 4 to 5 h
and then given an intravenous infusion of paclitaxel using an infu-
sion pump (Harvard Apparatus, South Natick, MA). Paclitaxel was
dissolved in Cremophor EL/ethanol (1:1, v/v) and diluted with 0.9% NaCl.
The total infusion volume was 2 to 3 ml and contained 10 to 15
Cremophor EL. Five groups of animals were treated as de-
scribed under Results. Blood samples (0.12 or 0.22 ml) were obtained
at predetermined times, and the plasma fraction were stored at
−70°C for analysis by HPLC. At the end of the experiment, tumors
were harvested, and one-quarter of each tumor was fixed in 10%
neutralized formalin solution and embedded in paraffin. Tumor cross
sections (5 μm) taken from the middle of tumor pieces were used for
evaluation of tumor cell density and the fraction of apoptotic cells.
The remainder of the tumor was stored at −70°C until HPLC anal-
ysis.

HPLC Analysis. The concentration of nonradiolabeled paclitaxel
in plasma and tumor was analyzed using our previously reported
methanol). The extraction yield was 92%

... 6

... limit of 1 ng of paclitaxel per injection.

... of paclitaxel and cephalomanine was at 229 nm, with a detection

... column onto the analytical column. Detec-

... 12 min, which contained paclitaxel and cephalomanine, was trans-

... analyt-

ical column (Bakerbond Octadecyl, 250- x 4.6-mm i.d., 5-µm particle

... size, J. T. Baker, Phillipsburg, NJ). Samples were injected onto the

... and eluted with the clean-up mobile phase (49% ace-

... nitrile in water) at 1 ml/min. Concurrently, the analytical mobile

... phase (49% acetonitrile in water) was directed through the

... analytical column at a flow rate of 1.2 ml/min. The fraction from 5 to

... min, which contained paclitaxel and cephalomanine, was trans-

... of paclitaxel and cephalomanine was at 229 nm, with a detection

... limit of 1 ng of paclitaxel per injection.

Statistical Analysis. The statistical significance of the differences

... between treatment groups was analyzed using the two-tailed

unpaired Student’s t test.

Results

Effect of Apoptosis-Inducing Pretreatment on Drug Delivery in Tumor: In Vitro Studies. Three in vitro stud-
ies were performed using the FaDu tumor histocultures. The first study evaluated the importance of apoptosis on pacli-

taxel penetration. Figure 1 shows the apoptotic cells induced by paclitaxel. This study used two concentrations of [3H]paclitaxel that differed in their ability to induce apoptosis and reduce tumor cell density, with the 50 nM concentration being more effective than the 10 nM concentration (Fig. 2). Tumor penetration of [3H]paclitaxel at the 10 nM concentration was limited to the periphery of the histocultures throughout 72 h. In comparison, drug penetration at the 50 nM concentration was limited to the periphery in the first 24 h but subsequently increased, along with an increase in the apoptotic fraction and a reduction of the cell density, such that the radiolabeled drug was evenly distributed throughout the tumor by 72 h (Figs. 2 and 3). These data confirm our previous finding that apoptosis enhances the penetration of paclitaxel in solid tumors (Kuh et al., 1999).

The second study examined the effect of apoptosis-inducing pretreatment on paclitaxel penetration. Tumor histocultures were treated for 72 h with 10 or 50 nM [3H]paclitaxel. At each concentration, we used a control group and a pretreated group, which received an additional exposure of 3000 nM · h nonradiolabeled paclitaxel (i.e., 1000 nM nonradiolabeled paclitaxel for 3 h), initiated at 24 h before the treatment with the radiolabeled paclitaxel. Note that the nonradiolabeled paclitaxel is not detected by autoradiography and does not interfere with the detection of [3H]paclitaxel. Figure 2 shows the paclitaxel pretreatment-induced apoptosis (average of ~25% throughout the histocultures) and reduced the cell density by the time the [3H]paclitaxel treatment was applied. The pretreatment enhanced the rate and extent of [3H]pacl-
taxel penetration at both 10 and 50 nM concentrations (Fig.

... 3), such that 1) the 10 nM treatment resulted in penetration of

... histoculture into the tumor core and even drug distribu-

... in tumors at 72 h, and 2) even drug distribution in tumors was attained more rapidly for the 50 nM treatment

... h without pretreatment). Based on these findings, we hypothesized that a treatment schedule, which allows for the induction and occurrence of apoptosis can enhance drug delivery to solid tumors.

To evaluate the effect of treatment schedule on paclitaxel delivery in tumors, a third study examined the drug pene-

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Fig. 1. Morphological changes in paclitaxel-induced apoptosis. FaDu tumor histocultures were treated with 0 and 1 µM paclitaxel for 3 h. At 21 h after drug treatment, tumors were frozen and cut into 10-µm sections using a cryotome. The sections were evaluated for drug-induced apoptosis. Apoptotic cells were indicated by morphological changes, i.e., formation of apoptotic bodies and/or cell shrinkage (arrow 1) and chromatin condensation and margination (arrow 2); 400× magnification.

Fig. 2. Effect of pretreatment and paclitaxel concentration on the drug-induced apoptosis and cell density. FaDu tumor histocultures were treated with 10 and 50 nM [3H]paclitaxel continuously, without (closed symbols) or with (open symbols) pretreatment by 1 µM nonradiolabeled paclitaxel for 3 h. The pretreatment was initiated 24 h before the [3H]paclitaxel treatment. Fraction of apoptotic cells (circles) and tumor cell density (squares) were determined by counting the number of total and apoptotic tumor cells per 400× microscopics field (three random fields per tumor). Mean ± S.D. (n = 3 histocultures).
etration increased simultaneously with an increase in apoptotic fraction and reduction in cell density during the next 12 h, drug penetration during the 36-h treatment remained confined to less than 20 cell layers in the periphery (Fig. 4). In comparison, the dose-fractionation group showed a higher apoptotic fraction, a lower cell density, and a more rapid penetration, resulting in an even drug distribution throughout the histocultures at 36 h (Fig. 4 and Table 1). A comparison of total drug accumulation in the two groups showed 40 to 115% higher drug accumulation in the dose fractionation group at 24 and 36 h (Table 1). The results of this study indicate that fractionation of a dose to include a pretreatment to induce apoptosis resulted in greater drug penetration and accumulation in tumors, as compared with a single continuous treatment.

Collectively, these results indicate that under in vitro conditions, 1) an apoptosis-inducing pretreatment enhances the drug penetration during subsequent treatments, 2) treatments at higher drug concentrations that induce appreciable apoptosis and reduce tumor cell density result in more rapid drug penetration compared with treatments at lower concentration, and 3) different treatment schedules, because of their different ability to induce apoptosis, result in different delivery of paclitaxel in solid tumors.

Effect of Apoptosis-Inducing Pretreatment on Drug Accumulation in Tumor: In Vivo Studies. The selection of treatment schedules in rats was based on three considerations. First, the dose and treatment duration of the pretreatment should be sufficient to induce apoptosis in tumors. Second, the interval between the pretreatment and the sub-
Concentrations of [3H]paclitaxel in tumor histocultures were determined by liquid scintillation counting. To correct for the difference in the dosing schedules, drug concentrations should be additive. Hence, in the absence of

\[ C_{T-Adjusted} = \frac{C_T \times T}{C_{T-Adjusted}} \]

where \( C_T \) is the total concentration and \( T \) is the time of concentration.

The second schedule used a continuous treatment at a lower concentration that induced a lower extent of apoptosis compared with the pretreatment of the first schedule. The second schedule used a continuous treatment at a lower concentration that induced a lower extent of apoptosis compared with the pretreatment of the first schedule. The second schedule used a continuous treatment at a lower concentration that induced a lower extent of apoptosis compared with the pretreatment of the first schedule.

Three studies were performed. The first study examined the effect of pretreatment on drug delivery to tumor tissue, in three groups of animals. Group 1 received a pretreatment of 5 mg/kg over 1 h and, 23 h later, a second dose infused at a slower rate (i.e., 5 mg/kg over 6 h). To determine the drug delivery to the tumors at each of the two doses, group 2 received only the pretreatment dose as in group 1, and group 3 received only the second treatment as in group 1. Tissue concentrations should be additive. Hence, in the absence of an enhanced drug delivery due to apoptosis, the immediate post-treatment drug concentration in group 1 at 30 h should be less than the sum of those concentrations in group 2 at 24 h and group 3 at 6 h. This is because the duration between the administration of the first dose and the time of concentration measurement was longer in group 1 than in group 2 (30 versus 24 h), which, due to the concentration decline with time, should result in a lower concentration in group 1. Conversely, in the presence of a substantially enhanced drug delivery due to apoptosis, the concentration in group 1 should exceed the sum of concentrations in groups 2 and 3. The results show the latter to be the case; the post-treatment concentration in group 1 was >50% higher than the sum of the concentrations in groups 2 and 3 (i.e., 3.94 \( \mu g/g \) versus <2.61 \( \mu g/g \), see Table 2). These results support our hypothesis that apoptosis induced by the pretreatment enhances drug delivery.

To confirm the role of apoptosis and reduced cell density on drug delivery and to establish the importance of the time interval between the pretreatment and the subsequent dose, the second study compared the tissue morphology and tumor concentration in group 1 with those in group 4, which received the same two doses by the same infusion schedules as group 1, with the exception that the two doses were separated by only 10 min (time required to change and reset the

![Image](https://example.com/image1.png)

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment Schedule</th>
<th>Time after Initiation of Treatment</th>
<th>Apoptotic Fraction</th>
<th>Cell Density</th>
<th>Total Paclitaxel in Tumor</th>
<th>C-T-Adjusted Paclitaxel in Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>% cells/field</td>
<td>( \mu g/g )</td>
<td></td>
<td>( h^{-1} )</td>
</tr>
<tr>
<td>600 nM × 1 h + 50 nM × 12 h</td>
<td>24</td>
<td>20.3 ± 4.3</td>
<td>60.9 ± 1.3</td>
<td>2.68 ± 0.31</td>
<td>5.23 ± 0.61</td>
</tr>
<tr>
<td>Initiated 23 h later</td>
<td>36</td>
<td>25.4 ± 3.4</td>
<td>62.4 ± 2.2</td>
<td>4.33 ± 0.29</td>
<td>4.23 ± 0.28</td>
</tr>
<tr>
<td>50 nM × 24 h</td>
<td>24</td>
<td>15.2 ± 3.34</td>
<td>71.3 ± 2.9</td>
<td>3.11 ± 0.31</td>
<td>3.04 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>17.0 ± 3.94</td>
<td>72.6 ± 3.2</td>
<td>2.01 ± 0.32</td>
<td>1.96 ± 0.31</td>
</tr>
</tbody>
</table>
infusion syringe and pump) as opposed to the 23 h in group 1. At the end of treatment (30 h for group 1 and 7.2 h for group 4), group 4 showed a 60% lower apoptosis, 30% higher cell density, and 20% lower drug concentration, compared with group 1 (Table 2). These results indicate the importance of timing on drug-induced changes in tissue morphology and on drug delivery.

To address the importance of dose fractionation on drug delivery, the third study compared the tumor concentration in group 1 to that in group 5, which received the same total dose as group 1 with the exception that the dose was delivered continuously at a slower rate over a longer duration, (i.e., without dose fractionation). At the end of treatment (30 h for group 1 and 12 h for group 5), group 5 showed a 35% lower apoptosis, 25% higher cell density and 25% lower drug concentration, compared with group 1 (Table 2). These results support the concept of using dose fractionation to enhance drug delivery.

Another measurement of drug penetration in tumor is the tumor-to-plasma concentration ratio. The postinfusion
plasma pharmacokinetic data in group 1 (after the first dose) showed a major half-life of 1.8 h for paclitaxel in rats, indicating that drug concentrations in plasma and tumor will be, theoretically, within 90% of a steady state following an infusion for 6 h or longer (i.e., ≥3.3 half-lives). This is confirmed by the results shown in Fig. 6; the average difference in the plasma concentrations at the last two time points, separated by 1 or 2 h, in groups 3, 4, 5, and 5 was ~10%. Under the steady-state condition, the tumor-to-plasma concentration ratio represents the drug partition from plasma to tumor and a higher concentration ratio indicates a higher drug partition. A comparison of the tumor-to-plasma concentration ratio shows that the ratio in group 1 was 62, 130, and 40% higher than in groups 3, 4, and 5, respectively (Table 2). Because of the unusually high accumulation of paclitaxel in tumor cells, with an intracellular-to-extracellular ratio of 2.5:1 and a concentration of 0.034 ± 0.003 mg/g in the remaining two rats. Not calculated because tumors were harvested when the plasma concentrations were not at a steady state.

In summary, results of the present study indicate that the delivery of paclitaxel to tumor can be enhanced by using a paclitaxel pretreatment that induces apoptosis and reduction in cell density and by using treatment schedules designed to take advantage of these drug-induced changes in tumor tissue composition. The enhancement in paclitaxel delivery was observed in tumor histocultures where drug transport is by diffusion through the interstitial space as well as in tumors grown in animals where blood flow contributes to drug transport. Hence, our approach of using apoptosis-inducing pretreatment to enhance drug delivery to solid tumors applies to regional therapy where the drug is supplied from the extra-cellular fluid surrounding the tumor as well as systemic therapy where the drug is supplied through the circulation from within the tumor. It is also noteworthy that induction of apoptosis was achieved at paclitaxel concentrations (i.e., 600–6000 nM) that are within the plasma concentration range attained in patients given intravenous paclitaxel therapy (Sonnholm and Relling, 1994), indicating the potential clinical utility of the pretreatment approach.

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Infusion Rate (mg/kg/h)</th>
<th>Total Dose</th>
<th>Time for Harvesting Tumor</th>
<th>Tumor Concentration (μg/ml)</th>
<th>Plasma Concentration at the Time of Tumor Harvesting (μg/ml)</th>
<th>Tumor-to-Plasma Concentration Ratio</th>
<th>Apoptotic Fraction (%)</th>
<th>Cell Density (cells/field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 6 h at 0 h</td>
<td>5</td>
<td>30</td>
<td>3.04 ± 0.35b</td>
<td>1.42 ± 0.11</td>
<td>2.80 ± 0.29</td>
<td>14.5 ± 2.5</td>
<td>0.034 ± 0.003</td>
</tr>
<tr>
<td>2</td>
<td>1.0 6 h at 0 h</td>
<td>1.0 6 h</td>
<td>6</td>
<td>2.26 ± 0.13</td>
<td>Not detected</td>
<td>1.21 ± 0.12</td>
<td>1.73 ± 0.18</td>
<td>85 ± 4.52</td>
</tr>
<tr>
<td>3</td>
<td>1.0 6 h at 0 h</td>
<td>1.0 6 h</td>
<td>6</td>
<td>3.24 ± 0.31</td>
<td>Not applicable</td>
<td>2.98 ± 0.60</td>
<td>1.20 ± 0.30</td>
<td>108 ± 5.78</td>
</tr>
<tr>
<td>4</td>
<td>1.0 6 h at 0 h</td>
<td>1.0 6 h</td>
<td>6</td>
<td>2.5 ± 0.34</td>
<td>1.50 ± 0.07</td>
<td>1.98 ± 0.23</td>
<td>6.5 ± 1.6</td>
<td>115 ± 5.18</td>
</tr>
<tr>
<td>5</td>
<td>0.33 12 h at 0 h</td>
<td>1.0 12 h</td>
<td>12</td>
<td>2.05 ± 0.34</td>
<td>1.50 ± 0.07</td>
<td>1.98 ± 0.23</td>
<td>6.5 ± 1.6</td>
<td>115 ± 5.18</td>
</tr>
</tbody>
</table>

* p < 0.05, compared with all other groups.
* p < 0.01, compared with all other groups except group 2.
* Drug concentrations in the four rats were 0.35 and 0.25 μg/g in two rats and below the detection limit (0.2 μg/g) in the remaining two rats.
* Not calculated because tumors were harvested when the plasma concentrations were not at a steady state.
* p < 0.01, compared with all other groups except group 1.

**Discussion**

Inadequate delivery of anticancer drugs to the tumor core is an obstacle to successful chemotherapy (Jain, 1987, 1996; Durand, 1990; Erlanson et al., 1992). We have shown an inverse relationship between paclitaxel penetration in solid tumors and tumor cell density (Kuh et al., 1999). The present study was designed to test the hypotheses that an apoptosis-inducing pretreatment with paclitaxel enhances its delivery to solid tumors and that the paclitaxel treatment schedule can affect the drug delivery to solid tumors. The results show that 1) modification of paclitaxel treatment schedules by including an apoptosis-inducing pretreatment reduces cell density and enhances the penetration and accumulation of a properly timed subsequent dose in solid tumors, and 2) dose fractionation into two doses separated by an interval to allow for apoptosis to occur results in greater drug delivery into solid tumors compared with a single treatment. For paclitaxel, the drug-induced apoptosis occurs after a delay of about 16 to 24 h, as shown earlier (Cheng et al., 1995; Au et al., 1998, 1999) and confirmed in the present study.

The apoptosis-induced enhancement of drug delivery to tumors is likely the result of an increased interstitial space. For example, the diffusion coefficient in a gel structure is a function of interstitial space and tortuosity (Schultz and Armstrong, 1978; Fox and Wayland, 1979). Hence, apoptosis, by creating a larger fraction of interstitial space and/or a decrease in tortuosity, would result in a more rapid drug diffusion. Drug-induced apoptosis may also, by decreasing the interstitial fluid pressure and decompressing the tumor microvesSEL, result in enhanced drug permeability across the tumor microvasculature and drug diffusion in interstitial tissue (Griffon-Etienne et al., 1999).

**References**


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