Pharmacodynamics of Taxol in Human Head and Neck Tumors

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ABSTRACT

The pharmacodynamics of taxol in human head and neck squamous cell carcinoma were studied using histocultures of surgical specimens from patients (n = 22). Tumors were treated with taxol for 24 h. The inhibition of DNA synthesis was determined by 48 h cumulative bromodeoxyuridine (BrdUrd) incorporation. The induction of apoptosis was measured by morphological changes, in situ DNA end labeling, post-ethanolase III-BrdUrd labeling, and DNA fragmentation. Inhibition of the BrdUrd labeling index (LI) by taxol was incomplete, with 11 tumors showing a maximal inhibition (ELmax) of 30-50% and the remaining 11 tumors showing an ELmax of 50-80%. For both groups, the inhibition approached maximum values at 1 μM taxol concentration; an additional 10-fold increase in drug concentrations did not significantly enhance the inhibition. The taxol concentrations required for a 30% inhibition (IC50) were 4.2 and 0.3 μM for the first and second groups, respectively. The IC50 correlated with the ELmax (r² = 0.39; P < 0.001). Taxol induced apoptosis in all tumors; 11 tumors showed a maximal fraction of apoptotic tumor cells between 3 and 10% and 11 tumors between 13 and 28%, whereas untreated controls showed a maximal apoptotic index of <1%. For individual tumors, the maximal apoptotic index occurred between 0.1 and 3 μM, and correlated with the BrdUrd LI for the untreated control (r² = 0.37; P < 0.01). It is interesting that >95% of apoptotic cells were BrdUrd labeled, whereas not all BrdUrd-labeled cells were apoptotic. To investigate the basis of the variable tumor response to taxol, we determined the expression of multidrug resistance P-glycoprotein (Pgp), p53, and bcl-2 proteins, using immunohistochemical staining and Western blot analysis. Eleven (50%), 10 (45%), and 7 (32%) tumors expressed Pgp, p53, and bcl-2, respectively. Patients with Pgp-positive tumors showed a higher number of affected lymph nodes than those with Pgp-negative tumors (P < 0.05). Compared with moderately and well differentiated tumors, the poorly differentiated tumors expressed p53 and Pgp more frequently and showed a lower maximum inhibition of DNA synthesis and a higher apoptotic fraction after taxol treatment (P < 0.05 in both cases). Pgp expression correlated differently with taxol-induced inhibition of DNA synthesis than with apoptosis; Pgp-positive tumors showed a significantly higher ELmax (63%) and IC50 (4.2 μM) but also a higher apoptotic index (17%) than Pgp-negative tumors (ELmax, 36%; IC50, 0.3 μM; and apoptotic index, 6%; P < 0.05 for all cases). p53 and bcl-2 expression did not correlate with taxol-induced inhibition of DNA synthesis or apoptosis. The data indicate that taxol acts through apoptosis and inhibition of proliferation in human head and neck cancer. Pgp overexpression appears to protect cells from the antiproliferative effect of taxol but correlates with a higher apoptosis.

INTRODUCTION

Taxol has shown significant activity in human cancers including ovarian, breast, non-small cell lung, and head and neck carcinoma (reviewed in Ref. 1). A recently completed Phase II trial in recurrent head and neck cancer showed a 40% complete and partial response, suggesting taxol as the most active single agent for the treatment of this disease (2). The biological and pharmacological basis of taxol action and resistance has been a subject of intense interest. Taxol has multiple pharmacological effects. It promotes polymerization and stabilizes the microtubules; causes blockade at the G2-M interphase; inhibits DNA synthesis; and induces apoptosis in tumor cell lines, murine solid tumors, and leukemic cells in patients (3-8).

The MDR1 gene, coding for Pgp, has been implicated in tumor resistance to chemotherapeutic agents. The structural features of taxol, i.e., natural product, high molecular weight, and hydrophobicity, favor it as a substrate for Pgp (9). Overexpression of Pgp has been associated with taxol resistance. Decreased intracellular taxol accumulation was observed in several Pgp-positive and taxol-resistant tumor cell lines (10-12). Transgenic mice expressing the transferred MDR1 gene in their bone marrow cells were about 10-fold more resistant to taxol-induced leukopenia compared with mice with normal bone marrow (13). Furthermore, the taxol resistance can be reversed by MDR1-reversal drugs, supporting the role of MDR1 in taxol resistance (14, 15). Additional mechanisms of taxol resistance have been identified in animal and human cells. These include overproduction of tubulin, acetylation of α-tubulin, mutation of the α-tubulin gene, and alteration of β-tubulin subunits (9). It is not known whether these mechanisms are operative in human solid tumors and lead to taxol resistance.

p53 and bcl-2 are also involved in chemosensitivity. A relationship between p53 gene mutation and chemoresistance has been shown in a number of human malignancies, including small cell lung carcinoma, head and neck squamous cell carcinoma, acute myeloid leukemia, myelodysplastic syndrome, and B-cell chronic lymphocytic leukemia (16-18). The expression of bcl-2 is known to correlate with chemoresistance (reviewed in Ref. 19). Transfection of lymphocytic leukemia cells, neuroblastoma cells, and malignant glioma cells with the bcl-2 gene confers resistance to chemotherapeutic drugs (20-22), whereas antisense-mediated reduction of bcl-2 gene expression in non-Hodgkin's lymphoma cells increases chemosensitivity (23).

p53 and bcl-2 are also among the factors known to modulate cancer-related apoptosis (reviewed in Ref. 24). Recent studies have demonstrated that induction of apoptosis by chemotherapeutic drugs or ionizing radiation may be related to the status of the p53 gene (25-28). Restoration of functional p53 in myeloid leukemia cells transfected with temperature-sensitive mutant p53 gene produced massive and sudden apoptosis (25). The loss of p53 function has been reported to enhance cellular resistance to a number of chemotherapeutic agents (26, 28). bcl-2, on the other hand, has been shown to promote cell survival and inhibit apoptosis, including that induced by anticancer drugs (reviewed in Ref. 29).

The first goal of the present study was to determine the effects of taxol in human head and neck tumors. The relationship between the extracellular taxol concentration and the drug effect on inhibiting...
ond goal was to examine the relationship between tumor sensitivity to taxol with expression of Pgp, p53, and bcl-2 proteins. These studies required the evaluation of drug sensitivity in individual patient tumors and were performed using histocultures of surgical specimens of head and neck tumors. The major advantages of the histoculture system are the maintenance of a three-dimensional tissue architecture, cell-cell interaction, and inter- and intratumoral heterogeneity (30). The use of tumors from individual patients allows evaluation of the relationship between tumor characteristics and chemosensitivity, and the simultaneous evaluation of Pgp, p53, and bcl-2 for each tumor enabled the ranking of their relative contribution to taxol sensitivity and resistance.

MATERIALS AND METHODS

Chemicals and Supplies. Taxol was a gift from Bristol-Myers Squibb (Wallingford, CT). Sterile pigskin collagen (Spongostan Standard) was purchased from Health Designs Industries (Rochester, NY); BrdUrd and collagenase type II from Sigma Chemical Co. (St. Louis, MO); cefotaxime sodium from Hoechst-Roussel (Somerville, NJ); gentamicin from Solo Pak Laboratories (Franklin Park, IL); DMEM and MEM from Life Technologies, Inc. (Grand Island, NY); TdT, biotinylated dUTP, and exonuclease III from Boehringer Mannheim (Indianapolis, IN); and a nonradioactive Western blot kit from Amersham (Arlington Heights, IL). Antibodies against BrdUrd, p53 (DO7), and Pgp (JSB-1) were obtained from BioGenex (San Ramon, CA), bcl-2 antibody and a Labelled Streptavidin-Biotin detection kit from DAKO Corp. (Carpinteria, CA), and Pgp polyclonal antibody (ab-1) from Oncogene (Cambridge, MA). All chemicals and reagents were used as received.

Tumor Specimens. Specimens of human head and neck squamous cell carcinoma were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Tumor pathology was determined by the Pathology Department. Tumor specimens were placed in HBSS within 10-30 min after surgery and maintained at 4°C until use. Patient and tumor pathology data are listed in Table 1.

Histocultures. Histoculture of tumors was performed as described previously (31). In brief, tumor specimens were cut to about 1 mm² and placed in six-well plates. Four to six tumor pieces were placed on a 1-cm² presoaked collagen gel, and cultured in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. The culture medium consisted of DMEM/MEM (1:1) supplemented with 9% heat-inactivated fetal bovine serum, 0.1 mm nonessential amino acids, 100 µg/ml gentamicin, and 95 µg/ml cefotaxime. The pH of the medium was 7.4. After culture for 3 or 4 days, the tumors were used for pharmacodynamic studies.

Pharmacological Effects of Taxol. Taxol stock solution was prepared in ethanol. Sufficient volume of stock solution was added to the culture medium so that the final ethanolic concentration was <0.1%. Two effects of taxol in head and neck tumors were determined, i.e., inhibition of DNA synthesis and apoptosis. Inhibition of DNA synthesis was measured by the inhibition of BrdUrd incorporation in tumor cells. Note that this method measured all cells that incorporated BrdUrd, including the apoptotic cells. Tumor histocultures were exposed to various concentrations of taxol ranging from 0.01 to 10 µM for 24 h. These concentrations are equivalent to about 1-1000% of the clinically achievable concentrations (32). After drug treatment, the medium was exchanged and the tumors were washed three times with 5 ml of drug-free medium. Tumors were incubated with 40 µM BrdUrd for 48 h, then fixed in 10% neutralized formalin and embedded in paraffin. The embedded tissues were cut into 5 µm sections using a microtome, deparaffinized, and analyzed for BrdUrd labeling using the LSAB kit and standard immunohistochemical methods. Controls were processed similarly, with the exception of drug treatment. Tissue sections were examined microscopically, the BrdUrd-labeled tumor cells were scored, and the fraction of labeled cells (LI) was determined. A typical experiment used a total of 12-20 tumor pieces for each drug concentration. A minimum of 200 cells/tumor piece, or >1500 cells, was counted per concentration.

Apoptosis was measured by four methods. The first method was monitoring morphological changes using light microscopy; apoptosis was monitored by chromatin condensation and margination, disappearance of nucleoli, formation of membrane blebs, apoptotic bodies, and cell shrinkage (33). The second method was TUNEL, using previously reported procedures (34) with minor modifications. Briefly, tissue sections after deparaffinization and rehydration were treated with 5 µg/ml proteinase K for 20 min at room temperature and then washed with distilled water four times each for 5 min. The sections were immersed in TdT buffer (200 mM potassium cacodylate, 2.5 mM Tris-HCl (pH 6.0), 2.5 mM CoCl₂, 0.25 mg/ml BSA, and 0.1 mM gentamicin) and 0.5 µM biotinylated dUTP at 37°C for 30 min. The slides were rinsed with PBS and washed with 2X SSC (1X buffer contained 0.15 M sodium chloride and 0.015 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.4).

Table 1. Patient and tumor characteristics and tumor sensitivity to taxol

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<th>IC₅₀ (µM)</th>
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<th>Maximal apoptotic index (%)</th>
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Mean NA NA NA NA 16 3.4 15 6.2 NA NA NA NA

NA NA NA NA

Table 1. Patient and tumor characteristics and tumor sensitivity to taxol

All tumors were squamous carcinoma. Staining ranged from negative (—) to weakly positive (+), positive (+ +), and strongly positive (+ + +). IC₅₀ is the concentration of taxol needed to produce a 30% inhibition of BrdUrd labeling index. Only one patient (no. 18) received prior taxol treatment.

*Maximal apoptotic index after taxol treatment. The index in untreated controls in all tumors was <1%.

Changed from Pgp-negative to Pgp-positive after taxol treatment.

Mean and SD were calculated using 10 µM as IC₅₀ for the two tumors with IC₅₀ greater than 10 µM.
covered with 2% BSA for 10 min, incubated with hydrogen peroxidase-conjugated streptavidin in 1.5% agarose gel containing ethidium bromide (0.4 μg/ml), visualized by Tris-HCl and 10 mM EDTA (pH 8.0). DNA was separated electrophoretically (1:1) and precipitated by adding 0.2 volume 10 M NH₄Cl and 2 volume tricon-30 (Amicon, Beverly, MA). For the detection of p53 and bcl-2, cells were harvested by centrifugation and washed twice with PBS. The membrane- and cytoplasm-enriched fraction was harvested by centrifugation and washed twice with PBS. For Pgp detection, the membrane- and cytoplasm-enriched fraction was harvested by centrifugation and washed twice with PBS. For Pgp detection, the membrane- and cytoplasm-enriched fraction was harvested by centrifugation and washed twice with PBS. The membrane- and cytoplasm-enriched fraction was harvested by centrifugation. The supernatants were concentrated by Cen-
Pharmacodynamics of Taxol-induced Apoptosis. Initial studies used four methods to measure apoptosis. Fig. 2, a–c, shows the detection of apoptotic cells by morphological changes, TUNEL, and post-exonuclease III-BrdUrd labeling. Results in three tumors showed an identical fraction of apoptotic cells measured by these three methods with a variation of <3%. Subsequent studies evaluated the apoptotic index by morphological changes, with 20% of tissue slides evaluated additionally by the exonuclease III-BrdUrd labeling or TUNEL for verification.

Taxol induced apoptosis in all 22 tumors (Table 1). The maximal apoptotic index in taxol-treated tumors was significantly higher than that in untreated controls. Taxol at concentrations as low as 0.01 μM induced apoptotic cells in two-thirds of the tumors. In individual tumors, the maximal apoptotic index occurred at between 0.1 and 3 μM and remained relatively constant with additional increases in concentration (Table 2). More than 95% of apoptotic cells were BrdUrd-labeled (Fig. 2), but not all BrdUrd-labeled cells were apoptotic. The fraction of apoptotic cells not labeled by BrdUrd was <5% in taxol-treated tumors, similar to controls. The maximum apoptotic index correlated significantly with the BrdUrd LI of untreated controls (Fig. 3; r² = 0.37; P < 0.01).

Kinetics of Apoptosis. The DNA synthesis inhibition and apoptotic effects of taxol described above were observed at a fixed time point (72 h after initiation of drug treatment). To characterize the kinetics of taxol-induced apoptosis, six head and neck tumors were exposed to 1 μM taxol and examined at different time points from 0 to 144 h. Apoptotic cells, detected by morphological changes and TUNEL, appeared at 24 h after initiation of taxol treatment. DNA fragmentation was detected at 24 h after initiation of drug treatment (Fig. 4). The apoptotic index peaked at 48 ± 12 h and then decreased slightly for longer treatment (Fig. 5).

Pgp, p53, and bcl-2 Expression. The positive control, i.e., the cortex of human adrenal gland, was stained positive by both the monoclonal antibody JSB-1 and the polyclonal antibody ab-1, whereas the negative control (mouse IgG) showed no staining. Fig. 2, d and e, shows the staining of Pgp by JSB-1 and ab-1, respectively. The two antibodies recognize different internal epitopes (36, 37). Both antibodies showed mixed membranous and cytoplasmic staining. In general, the JSB-1 antibody gave a more intense staining than the ab-1 antibody. Eleven tumors were stained positive by both antibodies. Two additional tumors were weakly positive by either JSB-1 or ab-1 antibody. Because of the possible cross-reactivity of JSB-1 antibody to myosin and to blood group A carbohydrate determinants (36, 39) and because of the unknown cross-reactivity of polyclonal ab-1 antibody to other proteins, only the tumors that were stained by both antibodies were considered positive. Tumors from nine chemothera-py-naive patients and one patient treated previously with taxol were Pgp-positive before taxol treatment and remained Pgp-positive afterward. One additional tumor that was stained by ab-1 but not by JSB-1 before taxol treatment was stained by both antibodies after taxol treatment.

p53 was detected in 10 tumors (Fig. 2f) and bcl-2 in six tumors (Fig. 2g). There was no change in the expression of these two proteins after taxol treatment, suggesting that the expression of these proteins is not inducible by taxol. The human head and neck FaDu cells (positive control for p53) showed a strong p53 signal, whereas the mouse IgG (negative control) showed negative results (the results not shown). The infiltrating lymphocytes in all bcl-2-negative tumors were stained positive for bcl-2, indicating that the negative staining of the tumor cells was not due to a lack of immunoreactivity to the antibody.

Five tumors provided sufficient materials for both immunohistochemical and Western blot analysis. Three of these tumors were Pgp-positive, two were p53-positive, and two were bcl-2-positive, as defined by immunohistochemical detection. Results of the Western blot analysis (Fig. 6) agreed with the immunohistochemical staining.

Tumor Pathology and Expression of Tumor Markers. Poorly differentiated tumors behaved differently from moderately and well differentiated tumors: the poorly differentiated tumors expressed more frequently p53 (8/11 versus 2/11; P = 0.010) and Pgp (8/11 versus 3/11; P = 0.033) and showed a lower maximum inhibition of DNA synthesis (41.7 ± 11.2% versus 56.5 ± 15.6%; P = 0.019) and a higher apoptotic fraction after taxol treatment (15.3 ± 8.1% versus 7.9 ± 7.1%; P = 0.034). bcl-2 expression did not differ between tumor grades.

No statistically significant degree of coexpression of tumor markers was found in poorly differentiated tumors, whereas the coexpression of Pgp and bcl-2 was significant in low-grade tumors (P = 0.024). There was no relationship between the expression of Pgp, p53, or bcl-2 and nodal metastasis (P > 0.3).

Compared with the Pgp-negative tumors, the Pgp-positive tumors showed a higher number of affected nodes (1.8 ± 1.1 versus 0.9 ± 0.9 nodes; P = 0.048).

Relationship between Taxol Effects and Expression of Pgp, p53, and bcl-2. Table 3 summarizes the results. All 11 Pgp-negative tumors showed a >50% maximal inhibition of BrdUrd LI with a mean IC₃₀ of 0.3 μM, whereas the 11 Pgp-positive tumors showed a <50% maximal inhibition with a mean IC₃₀ of 4.2 μM. The differences in the maximal inhibition of BrdUrd LI and the IC₃₀ for the two groups were statistically significant (P < 0.05 in each case), suggesting that Pgp-expressing tumors were less sensitive to the inhibition of DNA synthesis by taxol. Tumor stage showed a weakly positive correlation with IC₃₀ (r² = 0.15). There

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![Fig. 1. Relationship between taxol concentration and drug-induced inhibition of BrdUrd incorporation. Human head and neck tumor histocultures were treated with taxol for 24 h. Inhibition of 48 h cumulative BrdUrd incorporation was expressed as a percentage of the untreated controls. Data represent the means of 22 tumors; bars, SE. Inset, the least (O, tumor 17) and the most sensitive tumors (□, tumor 5). Lines are computer fitted according to Eq. A. Data included the apoptotic cells that were BrdUrd labeled.](image-url)
was no relationship between IC₅₀ and p53 expression (P = 0.96) or between IC₅₀ and bcl-2 expression (P = 0.26).

The Pgp-positive tumors showed more apoptotic cells than the Pgp-negative tumors, with a 2-3-fold and significantly higher maximal apoptotic index. Differences in apoptotic indices among the two groups became significant at taxol concentrations ≥ 0.1 μM (Tables 2 and 3). p53 and bcl-2 expression did not affect the apoptotic index (Tables 2 and 3).

DISCUSSION

Microtubules are required for mitosis and interphase functions such as maintenance of cell shape, motility, intracellular transport, and transmembrane signaling (9). Dynamic instability where tubulins exchange at microtubule ends is critical for microtubule functions (40). Taxol promotes the assembly and prevents depolymerization of microtubules and thereby suppresses its dynamic instability. The stabi-
Table 2. Correlation between taxol-induced apoptosis and Pgp, p53, and bcl-2 expressions

<table>
<thead>
<tr>
<th>Protein Status</th>
<th>n</th>
<th>Concentration (μM)</th>
<th>0</th>
<th>0.01</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgp +</td>
<td>11</td>
<td>0.2 ± 0.5</td>
<td>3.7 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2 ± 0.3</td>
<td>10.8 ± 6.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.8 ± 9.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5 ± 7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.8 ± 6.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pgp −</td>
<td>11</td>
<td>0.2 ± 0.2</td>
<td>1.2 ± 1.1</td>
<td>0.2 ± 0.1</td>
<td>3.7 ± 1.9</td>
<td>6.0 ± 5.3</td>
<td>4.3 ± 3.6</td>
<td>3.6 ± 3.3</td>
<td>3.9 ± 3.8</td>
</tr>
<tr>
<td>p53&lt;sup&gt;+&lt;/sup&gt;</td>
<td>10</td>
<td>0.1 ± 0.2</td>
<td>2.9 ± 3.7</td>
<td>0.2 ± 0.1</td>
<td>9.2 ± 5.9</td>
<td>8.0 ± 4.1</td>
<td>10.3 ± 9.0</td>
<td>10.2 ± 9.0</td>
<td>8.3 ± 7.1</td>
</tr>
<tr>
<td>p53&lt;sup&gt;−&lt;/sup&gt;</td>
<td>12</td>
<td>0.2 ± 0.5</td>
<td>1.8 ± 2.1</td>
<td>0.2 ± 0.1</td>
<td>5.9 ± 5.4</td>
<td>10.9 ± 9.5</td>
<td>6.8 ± 4.4</td>
<td>5.8 ± 4.4</td>
<td>7.1 ± 5.7</td>
</tr>
<tr>
<td>bcl-2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>0.2 ± 0.6</td>
<td>2.6 ± 2.9</td>
<td>0.2 ± 0.1</td>
<td>8.4 ± 4.9</td>
<td>11.6 ± 9.6</td>
<td>11.0 ± 6.8</td>
<td>9.9 ± 8.7</td>
<td>9.9 ± 6.4</td>
</tr>
<tr>
<td>bcl-2&lt;sup&gt;−&lt;/sup&gt;</td>
<td>15</td>
<td>0.1 ± 0.2</td>
<td>1.7 ± 1.5</td>
<td>0.1 ± 0.1</td>
<td>6.3 ± 5.1</td>
<td>8.7 ± 7.5</td>
<td>7.1 ± 6.8</td>
<td>7.2 ± 6.1</td>
<td>6.0 ± 5.8</td>
</tr>
<tr>
<td>Overall</td>
<td>22</td>
<td>0.1 ± 0.4</td>
<td>2.5 ± 3.1</td>
<td>0.1 ± 0.1</td>
<td>7.3 ± 5.8</td>
<td>10.4 ± 8.6</td>
<td>8.4 ± 7.1</td>
<td>7.9 ± 6.8</td>
<td>7.6 ± 6.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are expressed as maximal apoptotic index (mean ± SD).
<sup>b</sup> P < 0.01 compared with Pgp-negative tumors.
<sup>c</sup> No difference between p53-positive and p53-negative tumors.
<sup>d</sup> No difference between bcl-2-positive and bcl-2-negative tumors.

Localization of spindle microtubules leads to blockade of cells in the G<sub>2</sub>-M phase (3, 40). In interphase cells, taxol also induces formation of microtubule bundles, prevents quiescent cells from entering the cell cycle, and inhibits DNA synthesis (4).

Data from the present study show that taxol inhibited DNA synthesis and induced apoptosis in all 22 human head and neck tumors. But neither effect was complete. The varied extent of drug-induced inhibition of DNA synthesis and apoptosis among individual tumors is probably due to patient-related tumor heterogeneity. Inhibition of DNA synthesis showed a greater variability among individual tumors than apoptosis. On average, the maximal apoptotic effect was achieved at a lower extracellular taxol concentration (0.3 μM) than the maximal inhibition of DNA synthesis (1-3 μM). The incomplete inhibition of DNA synthesis by taxol is different from the effect of other drugs; we reported complete inhibition of DNA precursor incorporation in human head and neck tumors by 5-fluorouracil, mitomycin C, and cisplatin (31). These differences suggest the necessity of using different pharmacodynamic end points in evaluating drugs with different action mechanisms in the solid tumor histoculture system.

The present study was designed to study the pharmacological effects of taxol in human head and neck tumors. Not all the patients from whom tumor specimens were obtained received taxol monotherapy. Hence, it was not possible to determine the correlation of in vitro drug effects with patient response. Robbins et al. (41), Furukawa et al. (42), and Kubota et al. (43) have shown in retrospective and semiprospective preclinical and clinical studies that drug response in human tumor histocultures, using inhibition of DNA precursor incorporation or inhibition of metabolic reduction of tetrazolium dye as the end point, correlates with the sensitivity, resistance, and survival of head and neck, colorectal, and gastric cancer patients in response to treatment by mitomycin, doxorubicin, 5-fluorouracil, or cisplatin. Additional studies are needed to determine the in vitro-in vivo correlation of taxol sensitivity and to determine whether the inhibition of DNA synthesis and/or apoptosis is the critical cytotoxic event in patients.

Sarcoma is a less frequently occurring type of head and neck tumor.
Taxol induces apoptosis in tumor cell lines and murine solid tumors by unknown mechanism (5–8). In the current study, essentially all apoptotic cells were BrdUrd-labeled, indicating that proliferation and apoptosis are linked. Possibilities include that traversal of the S-phase is needed for susceptibility to taxol-induced apoptosis, or conversely, that the taxol-induced apoptotic signal forces the cell into a round of DNA synthesis. The first possibility is supported by the positive correlation between taxol-induced apoptosis and BrdUrd LI (Fig. 5), suggesting that higher cell proliferation predisposes to apoptosis. A similar positive correlation between taxol-induced apoptosis and proliferation was observed in human lung N417 and A549 cells, breast MCF7 cells, cervical HeLa cells, colon HT29 cells, ovarian A2780 cells, astrocytoma U373 cells, and Chinese hamster ovarian cells (44–46). Our data further show that the taxol-induced apoptosis in human head and neck tumors did not follow the classical concentration-dependent relationship in which the effect increases with concentrations to reach a complete response. Maximal apoptotic index occurred at between 0.1–3 μM drug concentration; additional increase in drug concentration did not significantly enhance the effect.

Pgp-positive tumors were more sensitive to the apoptotic effect of taxol but less sensitive to its inhibition of DNA synthesis. The reason for the opposing effects is not clear. Our data indicate that apoptotic cells were also labeled by BrdUrd. Assuming that apoptotic cells cannot undergo DNA synthesis, our data would suggest that the taxol-induced apoptosis occurs after DNA synthesis, i.e., after cells pass through the G1-S checkpoint that is the critical site for the p53-dependent apoptosis pathway (47, 48). These data, together with the finding of no relationship between p53 protein expression and taxol effect, suggest that the taxol-induced apoptosis in human head and neck tumors is via p53-independent pathways, as observed by others in tumor cell lines (49). However, because the antibody used to measure p53 protein does not distinguish the functional wild type from the nonfunctional mutated gene products, and because wild-type p53 has been detected immunohistochemically in head and neck tumors, our data do not rule out the involvement of p53.

bcl-2 has been shown to block apoptosis (19, 29), but the effect is not universal. For example, human prostate cancer cells overexpressing bcl-2 enter apoptosis when exposed to β-lapachone (50). Taxol-induced apoptosis in some tumor cell lines has been suggested to act through bcl-2-independent pathways (12, 51, 52). Haldar et al. (53) found recently in taxol-treated lymphoid cells that bcl-2 was phosphorylated, and the phosphorylated bcl-2 did not protect cells from apoptosis. Our data indicate no relationship between taxol-induced apoptosis with bcl-2 expression in human head and neck cancer.

In conclusion, our data indicate (a) inhibition of DNA synthesis and induction of apoptosis by taxol in head and neck squamous cell carcinoma, with substantial variation among individual patient tumors; (b) correlation of taxol-induced apoptosis with BrdUrd LI; (c) that Pgp expression had an opposing effect on the tumor sensitivity to taxol-induced inhibition of DNA synthesis and apoptosis; and (d) the Pgp and p53 bands in human head and neck FaDu cells, used as positive controls. bcl-2 is not expressed in these cells.

We evaluated the taxol effect in one orbital Meckel cell sarcoma, and found that taxol induced apoptosis (maximal apoptotic index of 25%) but did not inhibit the incorporation of BrdUrd. Additional studies are needed to determine whether sarcoma of the head and neck responds differently to taxol compared with squamous carcinoma.

Our finding of an incomplete inhibition of DNA synthesis in human head and neck squamous carcinoma is consistent with literature data showing that taxol-treated cells, including Chinese hamster ovarian, human breast cancer (MCF-7), and human lung cancer (A549) cells, can proceed with DNA synthesis (44–45). These DNA-synthesizing cells did not complete cytokinesis, yielding hyperdiploid cells with up to 16 n DNA. Although the fate of these hyperdiploid cells is unknown, it is reasonable to assume that these cells are perturbed. Additional studies to define the DNA ploidy and the fate of the taxol-treated cells in human head and neck tumors are needed.

**Table 3** Relationship between tumor sensitivity to taxol and expression of Pgp, p53, and bcl-2 proteins

| Protein | Status | Number (%) | Control BrdUrd LI (mean ± SD) | IC<sub>30</sub> (μM; mean ± SD) | Maximal inhibition of BrdUrd LI | Maximal apoptotic index (%)<sup>a</sup>
<table>
<thead>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgp</td>
<td>+</td>
<td>11 (50)</td>
<td>62 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>p53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>10 (45)</td>
<td>53 ± 19</td>
<td>2.2 ± 3.5</td>
<td>46 ± 13</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>bcl-2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>15 (68)</td>
<td>50 ± 19</td>
<td>3.5 ± 4.3</td>
<td>40 ± 12</td>
<td>14 ± 9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Maximum apoptotic index occurred at 0.1–3 μM concentrations in individual tumors.

<sup>b</sup>P < 0.01 compared with Pgp-negative tumors. Mean and SD were calculated using 10 μM as IC<sub>30</sub> for the two tumors with IC<sub>30</sub> greater than 10 μM.

<sup>c</sup>No difference between p53-positive and p53-negative tumors.

<sup>d</sup>No difference between bcl-2 positive and bcl-2-negative tumors.
REFERENCES


Pharmacodynamics of Taxol in Human Head and Neck Tumors

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