Cytostatic and Apoptotic Effects of Paclitaxel in Human Ovarian Tumors

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Purpose. The present study evaluated the cytostatic and apoptotic effects of a 24-hr paclitaxel treatment in ovarian tumors.
Methods. Three-dimensional histocultures of surgical specimens from patients (n = 17) were used. The cytostatic effect was measured by inhibition of 96-hr cumulative DNA precursor incorporation and induction of apoptosis was determined by morphological changes.
Results. Paclitaxel produced partial inhibition of DNA precursor incorporation in about 40% of tumors (maximum inhibition of ~30%) and induced apoptosis in about 90% of tumors (maximum apoptotic index of ~15%). In responsive tumors, maximum cytostatic and apoptotic effects were achieved at ≤1 μM with no further enhancement by increasing the drug concentration to 10 μM. In individual tumors, the apoptotic effect inversely correlated with cytostatic effect (r^2 = 0.27, p = 0.031), and the maximal apoptotic index correlated with the LI for the untreated controls (r^2 = 0.38, $p < 0.01$). More than 95% of apoptotic cells after paclitaxel treatment were labeled with DNA precursor. The incomplete cytostatic and apoptotic effects of paclitaxel and the link between DNA synthesis and apoptosis in ovarian tumors are similar to our previous findings in other human solid tumors.
Conclusions. These findings suggest that (a) apoptosis is the major paclitaxel effect in advanced ovarian tumors, (b) tumor sensitivity to drug-induced cytostatic effect is opposite to sensitivity to apoptotic effect, (c) paclitaxel-induced apoptosis increases with increased cell proliferation and is completed after DNA synthesis, and (d) further increasing the dose to elevate plasma concentration beyond 1 μM may not improve treatment outcome.

KEY WORDS: paclitaxel; taxol; ovarian cancer; cytostasis; apoptosis.

INTRODUCTION

Ovarian cancer is the fifth most common type of cancer and the fourth leading cause of cancer mortalities in women in the United States, with 26,800 new cases and 14,200 deaths estimated for 1997 (1). Most patients are asymptomatic in the early stages of ovarian cancer; 65% of cases are diagnosed at an advanced stage (2). Although the overall 5-year survival rate has increased from 38% to 44% in the last 10 years, tumor recurrence in patients with advanced disease is common and usually fatal (3). In early clinical trials, single-agent paclitaxel therapy produced significant responses in previously treated and platinum-resistant patients (4). A subsequent trial of paclitaxel combined with cisplatin in previously untreated patients yielded an overall response rate of 73%, leading to the use of this combination as standard first-line therapy for advanced ovarian cancer (3).

Paclitaxel has been shown to have multiple pharmacologic effects in human cancer cells, including increased polymerization and stabilization of microtubules, blockade of cells at the G2/M phase of the cell cycle, inhibition of DNA synthesis, and induction of apoptosis (5–10). We have recently shown that paclitaxel produces cytostatic (i.e., inhibition of DNA synthesis) and apoptotic effects in 3-dimensional histocultures of human head and neck, breast, prostate and bladder tumors, but neither effect is complete even at a drug concentration that exceeds the clinically achievable concentration by 10-fold (11–14). These data are qualitatively different from the data obtained using monolayer cultures of human cancer cell lines, which exhibit a complete response to paclitaxel at lower concentrations (15,16).

The goals of the present study were to determine the pharmacodynamics of drug-induced cytostatic and apoptotic effects and whether the response of human ovarian tumors to paclitaxel is similar to other solid tumors. These studies required the evaluation of drug sensitivity in individual patient tumors, and were performed using histocultures of surgical specimens of ovarian tumors. The major advantages of the histoculture system over monolayer cultures are the maintenance of a 3-dimensional tissue structure and organization, co-existence of tumor and stromal cells, cell-cell interaction, and inter- and intra-tumor heterogeneity (17). The use of tumors from individual patients allows evaluation of the relationship between tumor characteristics and chemosensitivity. The clinical relevance of the histoculture system is supported by the findings of Hoffman and coworkers in retrospective and semiprospective preclinical and clinical studies, which showed correlations between in vitro chemosensitivity and patient response and resistance to treatment by other chemotherapeutic agents (18–20).

Paclitaxel is currently administered to patients as a 3-, 24-, or 96-hr infusion. The most effective exposure time for paclitaxel has not been identified, but preclinical studies showed that cytotoxicity was increased for prolonged exposures of 16–48 hr (21). Our previous studies on human solid tumors have indicated a greater cytostatic effect when treatment is prolonged from 2 to 24 hr but no increase in apoptosis when treatment is prolonged from 24 to 96 hr (11–13). The present study evaluated the 24-hr treatment.

MATERIALS AND METHODS

Chemicals and Supplies

Paclitaxel was a gift from Bristol-Myers Squibb Co. (Wallingford, CT). Sterile pigskin collagen (Spongostan Standard) was purchased from Health Designs Industries (Rochester, New

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ABBREVIATIONS: MEM, minimum essential medium; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate buffered saline; BrdUrd, bromodeoxyuridine; IC_{30}, drug concentration needed to produce a 30% inhibition of DNA precursor incorporation; LI, labeling index; E_{max}, maximal inhibition of thymidine labeling index; $AI_{Control}$, apoptotic index in untreated controls; AI_{Total}, apoptotic index in drug-treated samples; AI_{Net}, net increase in apoptotic index due to drug treatment.

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York), cefotaxime sodium form Hoescht-Roussel Co. (Somerville, NJ), ^{3}H-thymidine (specific activity, 65 Ci/mmole) from Moravek Biochemicals, Inc. (Brea, CA), NTB-2 nuclear track emulsion from Eastman Kodak Chemicals (Rochester, NY), bromodeoxyuridine (BrdUrd) from Sigma Chemical Co. (St. Louis, MO), BrdUrd antibody from BioGenex (San Ramon, CA), and LSAB detection kit from Dako, Inc. (Carpiteria, CA). All other tissue culture medium and supplies were purchased from GIBCO Laboratories (Grand Island, NY). All chemicals and supplies were used as received.

Tumor Specimens

Surgical specimens of human ovarian tumors were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center, and from the neighboring Riverside and Grant Hospitals. Tumor pathology was determined by the pathology department. Tumor specimens were placed in minimum essential medium (MEM) within 10 to 30 min after surgery, stored at 4°C and prepared for culturing within one hour from excision. Patient and tumor characteristics are listed in Table I. Most tumors are at late stage (≥ III) because 65% of ovarian cancers are at an advanced stage at the time of diagnosis.

Culture Conditions

Histoculture of tumors was performed as previously described (22). In brief, fat, connective tissue, and necrotic portions of the tumor were trimmed off and the remaining portions were cut into 1 mm^3 fragments. Four to six tumor pieces were placed on a 1 cm^2 presoaked collagen gel, and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The culture medium consisted of a 1:1 mixture of MEM and Dulbecco's modified Eagle's medium, supplemented with 9% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 0.1 mM MEM nonessential amino acids, 100 μg/ml gentamicin, 95 μg/ml cefotaxime sodium, and concentrated MEM vitamin solution (100 fold concentration, 10 ml per liter). The pH of the medium was 7.4. After culture for 2 or 3 days, the tumors were used for pharmacodynamic studies.

Pharmacodynamic Studies

Paclitaxel stock solution was prepared in ethanol. Sufficient volume of stock solution was added to the culture medium so that the final ethanol concentration was <0.1%.

The cytostatic effect of paclitaxel was measured by the inhibition of DNA precursor incorporation in tumor cells. Initial experiments used ^{3}H-thymidine as the DNA precursor. Because of our finding that the two DNA precursors, BrdUrd and ^{3}H-thymidine, labeled the same cells resulting in identical labeling indices in other human solid tumors (11), later experiments used BrdUrd to reduce the use of radioisotopes.

Tumor histocultures were exposed to various concentrations of paclitaxel ranging from 0.01 to 10 μM for 24 hr. The clinical achievable paclitaxel concentration for a 24-hr infusion at 250 mg/m^2 is 0.9 μM (23). After drug treatment, medium was exchanged and tumors were washed 3 times with drug-free medium. Tumors were incubated with 40 μM BrdUrd or

Table I. Patient and Tumor Characteristics and Tumor Sensitivity to Paclitaxel

					Cytostatic effect		Maximum apoptotic index (%)		
No.	Age	Stage	Grade	Control LI (%)	IC_{30} (μM)	E_{max} (%)	$AI_{Control}$	AI_{Total}	AI_{Net}
1	66	IIIC	II	61	>5	17[a]	7.98	32.2	24.2
2	70	IIIC	II	20	0.04	53	0.00	1.49	1.49
3	80	IIIC	I	42	>5	0[a]	0.13	6.41	6.28
4	55	IIIC	III	86	>5	8[a]	1.92	19.5	17.6
5	54	IIIC	III	68	>6	0[a]	3.67	17.8	14.1
6	56	IV	III	91	>6	9[a]	3.14	23.5	20.3
7	62	IIIC	II	54	0.60	33	0.32	4.54	4.23
8	60	IV	II	64	>6	0[a]	5.68	15.4	9.70
9	51	IIIB	III	62	0.01	35	6.32	14.8	8.47
10	58	IIIB	III	60	>6	17[a]	3.95	10.1	6.15
11	59	IIIC	II	52	>10	16[a]	10.40	14.9	4.49[a]
12	72	IIIC	II	27	>10	20[a]	1.30	9.95	8.64
13	67	IIC	III	50	0.10	34	6.49	13.3	6.82[a]
14	65	IV	II	50	>10	25	1.31	9.66	8.35
15	37	IC	II	58	>10	22	3.60	14.6	11.0
16	86	III	I	32	>10	7[a]	3.64	18.5	14.8
17	56	III	III	72	>10	14	2.49	23.2	20.1
Mean	NA	NA	NA	56	0.19[b]	31[b]	3.67	14.8[c]	11.7[c]
SD	NA	NA	NA	19	0.28	12	2.92	8.10	6.60

Note: All tumors were ovarian carcinomas. IC_{30} is the drug concentration needed to produce a 30% inhibition of labeling index. Maximal apoptotic indices in untreated controls ($AI_{Control}$), in drug-treated samples (AI_{Total}). AI_{Net} is the net increase in apoptotic index due to drug treatment (i.e. difference between AI_{Total} and $AI_{Control}$).

[a] Not significantly different from control.

[b] Mean and SD were calculated using the tumors with measurable IC_{30} or E_{max}, respectively.

[c] Data of tumors 11 and 13 were excluded in the calculation of Mean and SD.

1 μM ^{3}H-thymidine for 96 hr, washed 3 times with PBS, and 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 immunohistochemical methods, and for ^{3}H-thymidine labeling by autoradiography as previously described (12,22). Controls were processed similarly with the exception of drug treatment. Tissue sections were examined microscopically, the BrdUrd- or ^{3}H-thymidine-labeled tumor cells were scored, and the fraction of labeled cells (LI) was determined. A typical experiment used 10 to 20 tumor pieces for each drug concentration. A minimum of 50 cells per tumor piece, or >500 cells were counted per concentration.

The fraction of apoptotic cells was determined microscopically, using the established apoptotic features of chromatin condensation and margination, disappearance of nucleoli, formation of membrane blebs, apoptotic bodies and/or cell shrinkage (24). Our laboratory and others have shown that this method gives the same results as the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method (12,25).

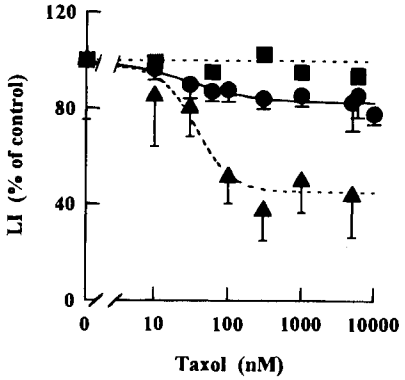

Fig. 1. *Relationship between paclitaxel concentration and inhibition of DNA precursor incorporation.* Human ovarian tumors were treated with paclitaxel for 24 hr. Inhibition of 96 hr cumulative precursor incorporation was expressed as a percent of untreated controls. Data represent the average of 17 tumors (mean ± SEM) including the 10 non-responsive tumors (●), and the least (■, tumor 2) and the most sensitive (▲, tumor 6) tumors. Lines are computer fitted according to equation 1.

Pharmacodynamic Data Analysis

The relationship of paclitaxel-induced inhibition of DNA synthesis and drug concentration was analyzed by computer fitting the following equation to the experimental data:

$$E = (E_0 - R_e) \cdot \left(1 - \frac{C^n}{K^n + C^n}\right) + R_e \qquad (1)$$

where E is the LI of drug-treated tissues, E_0 is the LI of untreated controls, C is the drug concentration, K is the drug concentration at one-half of $(E_0 - R_e)$, n is a curve shape parameter, and R_e is the residual fraction. E_{max} equals $(E_0 - R_e)$. Values for IC_{30} (the drug concentration needed to produce 30% inhibition), instead of the more commonly used IC_{50}, were determined because 50% inhibition was not achieved in nearly all tumors. Equation 1 is a modification of the more commonly used equation that describes a sigmoidal concentration-effect relationship that encompasses a spectrum of effect from 0% to 100%. Inclusion of the R_e term is necessary to describe the less than complete effect (see Results).

Statistical Analysis

Software for statistical analysis was by SAS (Cary, NC). Differences in mean and median values between groups were analyzed using unpaired Student's t test and/or Wilcoxon nonparametric two-sample test. Relationships among tumor chemosensitivity parameters, and between these parameters and tumor grade were evaluated by linear correlation using the CORR software routine.

RESULTS

Histocultures

Seventeen of the 34 tumors processed for histoculture showed sufficient numbers of cells, i.e. ≥50 cells per microscopic field, to enable pharmacologic evaluation. The 96 hr cumulative thymidine LI of these 17 histocultures was 56 ± 19% (Table I), which is higher than the previously reported growth fraction index of 40 ± 17% for advanced ovarian tumors (26). This difference may be due to the longer pretreatment culture period used in the earlier study (i.e. up to 11 days) compared to the present study (up to 3 days). The LI was inversely correlated with the age of patients ($r^2 = 0.41$, $p < 0.006$), i.e. tumors from younger patients had a higher LI.

Paclitaxel-Induced Inhibition of DNA Synthesis

There was considerable intertumor variation in the cytostatic effect of paclitaxel (Figure 1 and Table I). In 10 of the 17 tumors, paclitaxel did not significantly decrease the fraction of DNA synthesizing cells, and the maximal inhibition of DNA synthesis (E_{max}) was indistinguishable from 0%. The remaining 7 tumors showed significant and concentration-dependent inhibition of the LI, with E_{max} values between 14 and 53%. Only 4 of the 7 tumors in this latter group had measurable IC_{30} values, which ranged from 0.01 to 0.6 μM, or a 60-fold variation (Table I). There was no correlation between LI of controls and IC_{30} ($p = 0.94$) or between LI of controls and E_{max} ($p = 0.17$).

Paclitaxel-Induced Apoptosis

Figure 2 shows the apoptotic morphology of cells after paclitaxel treatment. Fifteen tumors showed significant increases in apoptotic index after paclitaxel treatment ($p < 0.05$), whereas 2 tumors did not show a significant response (Table I). For the responsive tumors, the enhancement in apoptotic index became significant at paclitaxel concentrations of 0.01 μM for 1 tumor, 0.03 μM for 4 tumors, 0.06 μM for 2 tumors, 0.1 μM for 3 tumors, 0.3 μM for 4 tumors, and 1 μM for 1 tumor; and the maximal apoptotic index occurred at 0.1 μM for 3 tumors, 0.3 μM for 6 tumors, 1 μM for 4 tumors, and 10 μM for 2 tumors.

In drug-treated tumors, about 95% of apoptotic cells were labeled by DNA precursor, whereas not all labeled cells were apoptotic. The maximum apoptotic index correlated significantly with the LI of untreated controls ($r^2 = 0.38$, $p < 0.01$,

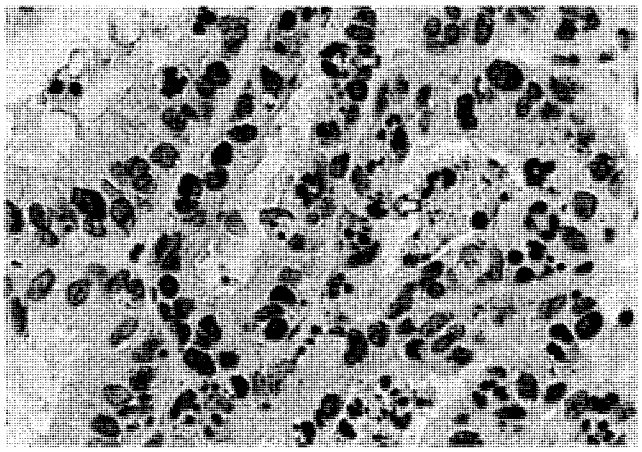

Fig. 2. *Paclitaxel-induced apoptosis.* Apoptotic tumor cells induced by 1 μM paclitaxel (tumor 17). Arrows show condensation of nuclear chromatin with loss of nuclear membrane, disappearance of nucleoli, formation of apoptotic bodies and/or cell shrinkage.

Figure 3). Collectively, these findings suggest that drug-induced apoptosis occurred in cells that were undergoing DNA synthesis.

In general, tumors showed a greater response to the apoptotic effect than to the cytostatic effect. For example, only 40% of tumors gave a cytostatic response whereas 90% of tumors underwent treatment-induced apoptosis. Comparison of cytostatic and apoptotic effects of paclitaxel showed that the maximum apoptotic index negatively correlated with E_{max} (r^2 = 0.27, p = 0.031, Figure 3), but had no correlation with IC_{30} (p = 0.13).

Tumor Pathology and Chemosensitivity

Results of statistical analysis indicate no significant relationships between grade and fraction of tumors resistant to cytostatic effect, IC_{30}, E_{max}, and maximal apoptotic index (p values of 1.0, 0.54, 0.96, and 0.17, respectively). The relationship between tumor sensitivity and stage could not be evaluated because only of the limited sample size of early stage tumors (2 of 17).

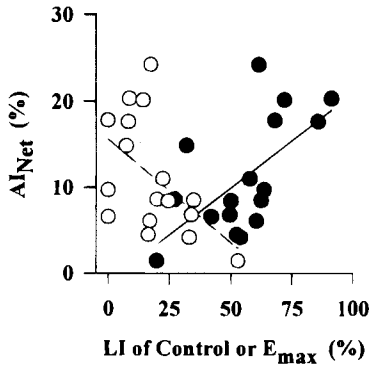

Fig. 3. *Relationships of AI_{Net} with LI and E_{max}.* Statistically significant correlations were found between LI of untreated controls and AI_{Net} (maximal apoptotic index corrected for apoptosis in untreated controls) induced by paclitaxel (●, solid regression line, r^2 = 0.38, p < 0.01) and between E_{max} and AI_{Net} (○, dashed regression line, r^2 = 0.27, p = 0.031).

DISCUSSION

Results of the present study show that paclitaxel inhibited DNA synthesis in about 40% and induced apoptosis in about 90% of 17 human ovarian tumors. A comparison of these results with those of our previous studies in other human tumors, i.e. bladder, breast, head and neck, and prostate tumors (11–14), indicates that the ovarian tumors were most resistant to the cytostatic effect of paclitaxel. The reasons for this difference are not apparent, but may be related to the LI of tumors. Seven of the 10 resistant tumors had a LI of greater than 50%. We have shown in human bladder tumors that sensitivity to the cytostatic effect of mitomycin C is inversely correlated with the tumor LI (27).

The effects of paclitaxel are qualitatively comparable to the effects in human head and neck, bladder, breast and prostate tumors in several aspects, as follows (11–14). (a) Paclitaxel produced incomplete cytostasis and incomplete apoptosis, even at concentrations that were 10 times the clinically achievable concentration of 1 μM, in nearly all of the 100 human solid tumors that were studied. In contrast, paclitaxel produced complete cytotoxicity in monolayers of human cancer cells at much lower concentrations (15,16). In a separate study, we examined the rate of ^{3}H-paclitaxel penetration in histocultures of human pharynx and ovarian tumors (28). The results showed that paclitaxel was evenly distributed throughout the histocultures at 24 hr, at which time the intracellular drug concentration also reached its maximum level. The intratumoral drug concentration obtained after incubating the histocultures with an extracellular drug concentration of 10 μM was ~100 μM. This intracellular concentration exceeded the concentration that caused complete cytotoxicity in monolayer cultures of human pharynx FaDu cells. Hence, the different drug sensitivity among the monolayer cultures and histocultures is not due to inadequate drug penetration into histocultures, but is likely a result of multilayer structure-related chemoresistance (29). Other investigators have shown that human ovarian cancer cells cultured as 3-dimensional spheroids were more than 5-fold less sensitive to paclitaxel than the same cells cultured in monolayers (30). The incomplete inhibition of DNA synthesis in human ovarian tumors is also consistent with literature data showing that paclitaxel-treated cells can proceed with DNA synthesis (31,32). The less-than-complete cytostatic and apoptotic effects suggest that repeated treatments may be necessary. (b) The labeling of nearly all paclitaxel-induced apoptotic cells by DNA precursor and the significant correlation between maximum apoptotic index and LI indicate that apoptosis is linked to proliferation and is completed after DNA synthesis. Consistent with this hypothesis is the finding that the maximum apoptotic index in individual tumors never exceeded the LI (Table 1). A similar positive correlation between paclitaxel-induced apoptosis and proliferation was observed in monolayer cultures of human and rodent cancer cells (15,31,33). (c) The inhibition of DNA synthesis showed a greater inter-tumor variability than apoptosis, and the maximum apoptotic effect was achieved at 1 μM in a higher percentage of tumors than the maximum cytostatic effect. (d) Individual tumors displayed opposite sensitivity to the two drug effects, e.g. the tumor that was the most

sensitive to the apoptotic effect was the least sensitive to the cytostatic effect (e.g. tumors 1 and 13 in the present study), and the maximum apoptotic index correlated negatively with E_{max}. Because both effects are expected to be a function of intracellular drug concentration, the unparallel sensitivity of individual tumors to the two effects indicate that these effects are determined by factors unrelated to and/or in addition to drug concentration.

For ovarian tumors, inter-tumor variation in sensitivity to the cytostatic effect (>100 fold difference in IC_{30} values) of paclitaxel is substantially higher than the variation for the apoptotic effect (16 fold difference in maximum apoptotic index). These variations are presumably due to biological differences among individual tumors. Studies to evaluate inter-tumor differences in the expression of multidrug resistance gene and genes that are involved in the regulation of apoptosis (e.g. p53, bcl-2), and the relationship between these differences and tumor sensitivity are ongoing.

At present, it is not known whether cytostasis or apoptosis is responsible for the clinical activity of paclitaxel. In view of the favorable response of advanced ovarian cancer to paclitaxel (3,4), our finding that a high fraction (60%) of ovarian tumors was resistant to the cytostatic effect of paclitaxel suggests that this effect may not be critical for its antitumor activity. A recent study in mice shows that the antitumor activity of paclitaxel in 16 tumor types (seven adenocarcinomas, two squamous cell carcinomas, six sarcomas, and one lymphoma) is correlated with drug-induced apoptosis but not with mitotic arrest, suggesting that apoptosis is responsible for the drug effect in vivo (34). If the same holds true for humans, the higher apoptotic effect in the more rapidly proliferating tumors and the higher LI in tumors from the younger patients would suggest that paclitaxel treatment is more effective in this patient population. Furthermore, the maximum apoptotic effect for a 24 hr treatment occurring at $\leq$ 1 μM in 70–80% of breast, head and neck, prostate, bladder, and ovarian tumors (11–14) suggests that escalating the dose to elevate the drug concentration may not enhance the apoptotic effect. Additional studies are needed to determine the maximally effective concentrations at different treatment schedules, e.g. 96 hr treatment and repeated daily treatment. It should be emphasized that the above comparisons are done using the assumption that the in vitro concentrations are equal to in vivo concentrations. There are several known differences for paclitaxel pharmacokinetics under in vitro and in vivo conditions. For example, we have shown that (a) there are differences in the extent of protein binding in culture medium and in plasma, (b) depletion of paclitaxel from culture medium is dependent on cell density, and (c) uptake of paclitaxel is saturable (27,35). Our laboratory is developing a pharmacokinetic model to incorporate these differences among in vitro and in vivo conditions, in order to provide more accurate quantitative analysis and for extrapolation of in vitro pharmacodynamic data to in vivo situations.

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REFERENCES

1. Cancer Facts and Figures-1997, American Cancer Society, 1997.
2. R. C. Young, C. A. Perez, and W. J. Hoskins. In V. T. DeVita, Jr., S. Hellman, and S. A. Rosenberg (eds.), *Cancer: Principles & practice of oncology, Fourth edition,* J. B. Lippincott Co., Philadelphia, pp. 1226–1263, (1993).
3. R. F. Ozols and J. B. Vermorken. *Semin. Oncol.* **24(1, Suppl 2)**:S2-1–S2-9 (1997).
4. R. C. Donehower and E. R. Rowinsky. *Cancer Treat. Rev.* **19(Suppl C)**:63–78 (1993).
5. S. B. Horwitz. *Annals. Oncol.* **5**:53–56 (1994).
6. K. L. Crossin and D. H. Carney. *Cell* **27**:341–350 (1981).
7. Y. Liu, K. Bhalla, C. Hill, and D. G. Priest. *Biochem. Pharmacol.* **48**:1265–1272 (1994).
8. K. Bhalla, A. M. Ilerado, E. Tourkina, C. Tang, M. E. Mahoney, and Y. Huang. *Leukemia (Baltimore)* **7**:563–568 (1993).
9. L. Milas, N. R. Hunter, and B. Kurdoglu. *Cancer Chemother. Pharmacol.* **35**:297–303 (1995).
10. X. Li, J. Gong, E. Felman, K. Seiter, F. Traganos, and Z. Darzynkiewicz. *Leuk. Lymphoma* **13**:65–70 (1994).
11. J. L.-S. Au, J. E. Kalns, Y. Gan, and M. G. Wientjes. *Cancer Chemother. Pharmacol.* (in press).
12. Y. Gan, M. G. Wientjes, D. E. Schuller, and J. L.-S. Au. *Cancer Res.* **56**:2086–2093 (1996).
13. C.-T. Chen, J. L.-S. Au, Y. Gan, and M. G. Wientjes. *Urol. Oncol.* in press (1997).
14. Y. Gan, J. Liu, and J. L.-S. Au. *Cancer Chemother. Pharmacol.* Submitted (1997).
15. J. E. Liebmann, J. A. Cook, C. Lipschultz, D. Teague, J. Fisher, and J. B. Mitchell. *Br. J. Cancer* **68**:1104–1109 (1993).
16. J. L.-S. Au, D. Li, X. Gao, Y. Gan, C.-T. Chen, S. Ge, A. L. Johnson, and M. G. Wientjes. *Proc. Amer. Assoc. Cancer Res.* **38**:4 (1997).
17. R. A. Vescio, C. H. Redfern, R. J. Nelson, S. Urogetz, P. H. Stern, and R. M. Hoffman. *Proc. Natl. Acad. Sci. USA* **84**:5029–5033 (1987).
18. K. T. Robbins, K. M. Connors, A. M. Storniolo, C. Hanchett, and R. M. Hoffman. *Arch. Otolaryngol. Head & Neck Surg.* **120**:288–292 (1994).
19. T. Furukawa, T. Kubota, and R. M. Hoffman. *Clin. Cancer Res.* **1**:305–311 (1995).
20. T. Kubota, N. Sasano, O. Abe, I. Nakao, E. Kawamura, T. Saito, M. Endo, K. Kimura, D. Hiroshi, H. Sasano, H. Nagura, N. Ogawa, R. M. Hoffman, and the chemosensitivity study group for the histoculture drug-response assay. *Clin. Cancer Res.* **1**:1537–1543 (1995).
21. S. G. Arbuck, R. Canetta, N. Onetto, and M. C. Christian. *Semin. Oncol.* **20(No. 4, Suppl 3)**:31–39 (1993).
22. T. D. Schmittgen, J. L.-S. Au, M. G. Wientjes, R. A. Badalament, and J. R. Drago. *J. Urol.* **145**:203–207 (1991).
23. E. K. Rowinsky, M. Wright, B. Monsarrat, G. J. Lesser, and R. C. Donehower. *Cancer Surveys Volume 17: Pharmacokinetics and Cancer Chemotherapy.* **17**:283–304 (1993).
24. J. F. R. Kerr, M. Clay, and B. V. Harmon. *Cancer* **73**:2013–2026 (1994).
25. R. Gold, M. Schmied, G. Giegerich, H. Breitschopf, H. P. Hartung, K. V. Toyka, and H. Lassman. *Lab. Invest.* **71**:219–225 (1994).
26. R. A. Vescio, K. M. Connors, T. Youngkin, G. M. Bordin, J. A. Robb, J. N. Umbreit, and R. M. Hoffman. *Proc. Natl. Acad. Sci. USA* **87**:691–695 (1990).
27. T. D. Schmittgen, J. M. Weaver, R. A. Badalament, M. G. Wientjes, E. A. Klein, D. C. Young, and J. L.-S. Au. *J. Urol.* **152**:1632–1636 (1994).
28. H.-J. Kang, A. Tran, D. E. Schuller, and J. L.-S. Au. *Proc. Am. Assoc. Cancer Res.* **37**:367 (1996).
29. A. Frankel, R. Buckman, and R. S. Kerbel. *Cancer Res.* **57**:2388–2393 (1997).
30. P. L. Olive and R. E. Durand. *Cancer Metastasis Rev.* **13(2)**:121–138 (1994).

31. N. M. Lopes, E. G. Adams, T. W. Pitts, and B. K. Bhuyan. *Cancer Chemother. Pharmacol.* **32**:235–242 (1993).
32. B. H. Long and C. R. Fairchild. *Cancer Res.* **54**:4355–4361 (1994).
33. K. L. Donaldson, G. L. Goolsby, and A. F. Wahl. *Int. J. Cancer* **57**:847–855 (1994).
34. C. G. Milross, K. A. Mason, N. R. Hunter, W. K. Chung, L. J. Peters, and L. Milas. *J. Natl. Cancer Inst.* **88**:1308–1314 (1996).
35. D. Song, L.-F. Hsu, and J. L.-S. Au. *J. Pharm. Sci.* **85**:29–31 (1996).