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Cytostatic and apoptotic effects of paclitaxel in human breast tumors

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Abstract *Purpose*: We have previously reported incomplete cytotoxic responses of other human solid tumors (bladder, head and neck, ovarian and prostate) to paclitaxel. This finding is qualitatively different from the nearly complete response observed in monolayer cultures of human cancer cell lines. The present study examined the pharmacodynamics of paclitaxel in human breast tumors. *Methods*: Three-dimensional histocultures of patient tumors were used. The cytostatic effect was evaluated by measurement of the inhibition of 48-h cumulative bromodeoxyuridine (BrdUrd) incorporation. The apoptotic effect was evaluated in terms of morphological changes and by in situ DNA end labeling. *Results*: Paclitaxel produced partial cytostasis (~30% maximum) and induced apoptosis (maximum apoptotic index of 3.3% to 29%) in all 15 tumors. More than 95% of apoptotic cells were BrdUrd labeled, but not all BrdUrd-labeled cells were apoptotic. The maximal apoptotic indices in the tumors were significantly correlated with the BrdUrd labeling index of untreated controls ($r^2 = 0.63$, $P < 0.01$). The maximum apoptotic effect was observed at a tenfold lower drug concentration (0.1 μM) compared to the maximum cytostatic effect (1 μM). Neither of these effects was enhanced by increasing the drug concentration to 10 μM. *Conclusions*: The pharmacodynamics of paclitaxel in human breast tumors are comparable to those found in other human solid tumors. The labeling of apoptotic cells by BrdUrd and the correlation between the proliferation index and apoptosis suggest that drug-induced apoptosis is linked to cell proliferation and is completed after DNA synthesis. The finding that maximal cytostatic and apoptotic effects of paclitaxel were achieved at or below the clinically achievable concentration of 1 μM suggests further increasing the dose to elevate plasma concentration beyond 1 μM may not improve treatment outcome.

Key words Paclitaxel · Taxol · Pharmacodynamics · Breast cancer

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Introduction

Paclitaxel shows significant activity against human cancers including ovarian, breast, non-small-cell lung, and head and neck cancers (reviewed in reference 2). Paclitaxel is effective in treating breast cancer with a 20–60% complete and partial response rate (reviewed in reference 6). The significant activity of paclitaxel in previously treated breast and ovarian cancer patients is particularly impressive, as most previously treated patients are refractory to subsequent chemotherapy. Paclitaxel has multiple pharmacologic effects. It promotes the polymerization of microtubules, causes blockade at the G_2/M interphase, inhibits DNA synthesis, and induces apoptosis in tumor cell lines, murine solid tumors, and leukemic cells in patients [5, 8, 13, 19, 22, 24].

The clinical activity of paclitaxel in patients, as a function of drug concentration and exposure time, is a subject of intense interest. Different durations and frequencies of treatments are being evaluated clinically to identify the most efficacious treatment schema. Paclitaxel is usually administered every 3 weeks by infusion over 3, 24 or 96 h, at doses ranging from 135 to 250 mg/m^2 [6]. The 3-h treatment schedule is advocated in part because of its application in an ambulatory setting [26], whereas preclinical data suggest that the therapeutic effect is improved by prolonging treatment to 96 h [15]. Recently, phase I and II trials have been initiated to test the merits of daily and weekly treatment schedules. The maximal tolerated dose is related to treatment duration and frequency. In general, a higher dose is better

tolerated when given over a longer time. Pharmacodynamic data are needed to determine whether increasing the dose or the dosing frequency can increase the drug effects.

We have recently shown that paclitaxel produces cytostatic (i.e. inhibition of DNA synthesis) and apoptotic effects in three-dimensional histocultures of human bladder, head and neck, ovarian and prostate tumors, but neither effect is complete even at drug concentrations that are ten times the clinically achievable concentration [29, 34]. These data are qualitatively and quantitatively different from the data obtained using monolayer cultures of human cancer cell lines, which exhibit nearly complete responses to paclitaxel at lower concentrations [21, 23]. The effects of paclitaxel in three-dimensional cultures of human breast tumor cells are not known.

The goal of the present study was to determine the effects of paclitaxel in human breast tumors as a function of drug concentration. These studies required the evaluation of drug sensitivity in individual patient tumors, and were performed using histocultures of surgical specimens of breast 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 [33]. The clinical relevance of the human tumor histoculture system has recently been demonstrated by Hoffman et al. These investigators have shown in retrospective and semiprospective preclinical and clinical studies that drug responses in human solid tumor histocultures correlate with the sensitivity and resistance of cancer patients to chemotherapy and with patient survival [10, 18, 27].

Materials and methods

Chemicals and supplies

Paclitaxel was a gift from Bristol-Myers Squibb (Wallingford, Ct.). Sterile pig skin collagen (Spongostan standard) was purchased from Health Designs Industries (Rochester, N.Y.), bromodeoxyuridine (BrdUrd) from Sigma (St. Louis, Mo.), cefotaxime sodium from Hoechst-Roussel (Somerville, N.J.), gentamicin from Solo Pak Laboratories (Franklin Park, Ill.), minimal essential medium (MEM) from Life Technologies (Grand Island, N.Y.), antibodies against BrdUrd from BioGenex (San Ramon, Calif.), and a labeled streptavidin-biotin (LSAB) detection kit from Dako (Carpinteria, Calif.). All chemicals and reagents were used as received.

Histoculture of tumor specimens

Specimens of human breast cancer were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Tumor pathology was determined by clinical pathologists. The tumor specimens were placed in medium within 10 to 30 min of surgical excision, and maintained at 4 °C until use.

Histoculture of the tumor specimens was performed as previously described [11]. In brief, the non-necrotic portions of the specimens were cut to about 1 mm^3 pieces under sterile conditions. Four to six tumor pieces were placed on a 1-cm^2 presoaked collagen gel in six-well plates, and cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The culture medium (pH 7.4) consisted of MEM supplemented with 9% heat-inactivated fetal bovine serum, 0.1 m*M* nonessential amino acids, 100 μg/ml gentamicin and 95 μg/ml cefotaxime. The tumor pieces were cultured for 3 or 4 days before use in pharmacodynamic studies.

Pharmacological effects of paclitaxel

Tumor histocultures were incubated with paclitaxel. A 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 antiproliferative effect of paclitaxel was measured by the inhibition of DNA precursor incorporation into tumor cells. Note that this method measured all cells that incorporated the precursor, including the apoptotic cells. Tumor histocultures were exposed to various concentrations of paclitaxel ranging from 0.001 to 10 μ*M* for 24 h. These concentrations are equivalent to about 1% to 1000% of the clinically achievable plasma concentrations [29, 34]. After drug treatment, the medium was exchanged and the tumor pieces were washed three times with 5 ml of drug-free medium. The tumor pieces were incubated with 40 μ*M* BrdUrd for 48 h, washed twice with PBS, 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 LSAB kits and standard immunohistochemical methods. Controls were processed similarly, with the exception of drug treatment. Tissue sections were examined microscopically, the labeled tumor cells were scored, and the fraction of labeled cells (LI) was determined. A typical experiment used a total of 12 to 20 tumor pieces for each drug concentration. A minimum of 200 cells per tumor piece, or >1500 cells, were counted per concentration.

Apoptosis was evaluated by monitoring morphological changes and by the TUNEL method. Apoptotic cells were defined by chromatin condensation and margination, disappearance of nucleoli, formation of membrane blebs, apoptotic bodies and cell shrinkage [16]. TUNEL was performed according to the manufacturer's instruction for ApoTag, the in situ apoptosis detection kit (Oncor), except that slides were pretreated by boiling in 0.01 *M* sodium citrate solution (pH 6.0) for 5 min. We and others have previously shown that this method gives the same results as morphological observation [11, 12].

Pharmacodynamic data analysis

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

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

where E is the LI of drug-treated tissues, C is the drug concentration, E_0 is the LI of untreated controls, K is the drug concentration at one-half E_0, *n* is a curve shape parameter, and R_e is the residual fraction. The maximal extent of inhibition, E_{max}, is $(E_0 - R_e)$. Values for IC_{30} (the drug concentration needed to produce 30% inhibition) were determined, instead of the more customary IC_{50}, because paclitaxel produced less than 50% effect in some tumors. Equation 1 is a modification of the more commonly used equation that describes a sigmoidal concentration-effect relationship that encompasses a spectrum of effects from 0% to 100%. Inclusion of the R_e term was necessary to described the less-than-complete effect, i.e. the average maximum inhibition of DNA synthesis was $<100\%$.

Statistical analysis

Differences in mean values between groups were analyzed using paired or unpaired Student's *t*-tests when the standard deviations were of similar magnitude, and using the Wilcoxon two-sample test

when otherwise. Software for statistical analysis (NPAR1WAY and TTEST procedures) was by SAS (Cary, N.C.). Frequencies were compared using Fisher's exact test (FREQ procedure). Predictive relationships between tumor pathologic parameters and tumor chemosensitivity were evaluated by linear regression analysis using the REG software routine.

Results

Human breast tumor histocultures

Of 22 specimens of breast carcinoma evaluated, 15 were successfully cultured, yielding sufficient viable tumor cells (> 200 cells/fragment) for pharmacodynamic evaluation. In general, these were specimens with little stroma and obtained from the periphery of a tumor. The other 7 tumors either contained excessive stroma or failed to incorporate BrdUrd. Patient and tumor data are listed in Table 1. The range and average values of BrdUrd LI are comparable to the previously reported LI of 31 ± 17% [33]. High-grade (III) tumors and late-stage (III and IV) tumors tended to show a higher LI than the low-grade and early-stage tumors, but the differences were not statistically significant ($P = 0.13$).

Paclitaxel-induced apoptosis

Figure 1 shows the morphological changes and the staining of apoptotic cells by the TUNEL method. Paclitaxel induced apoptosis in all 15 tumors. The maximal apoptotic index occurred at a drug concentration of 0.1 μ*M* in 80% of tumors. The average apoptotic index was not significantly enhanced when the drug concentration was increased by up to 100-fold to 10 μ*M* (Fig. 2). The maximal apoptotic index varied by about

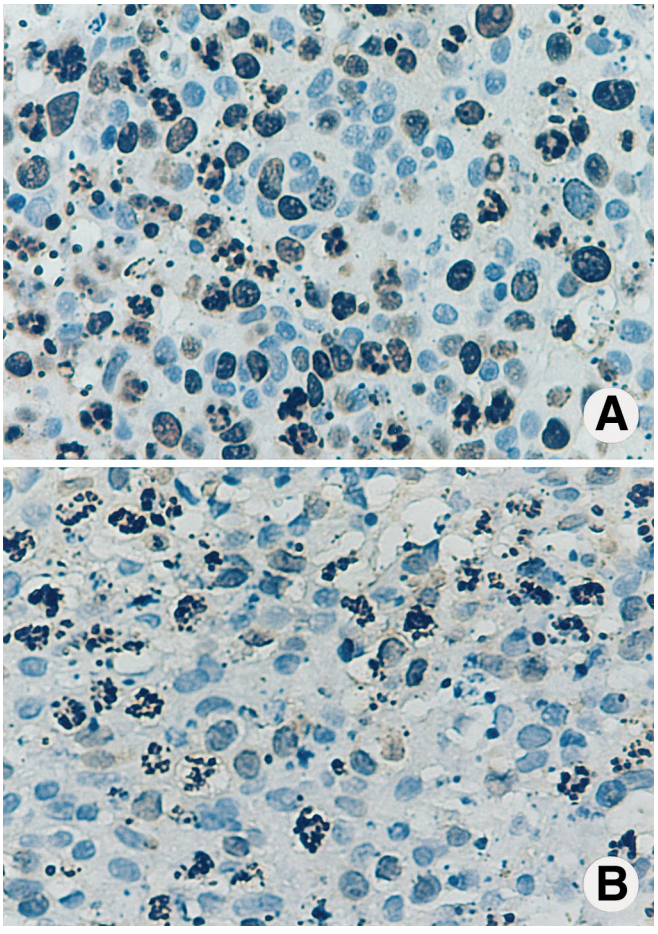

Fig. 1A,B Paclitaxel-induced apoptosis. **A** Apoptotic cells induced by 0.1 μM paclitaxel (tumor no. 5). Note that nearly all apoptotic cells, identified by the presence of apoptotic bodies, were BrdUrd labeled, whereas not all labeled cells were apoptotic (BrdUrd was stained *brown*). **B** Apoptotic cells demonstrated by TUNEL staining (*brown*). Counterstaining hematoxylin (*blue*)

Table 1 Patient and tumor characteristics, and tumor sensitivity to paclitaxel. IC_{30} is the taxol concentration needed to produce 30% inhibition of BrdUrd-labeled cells after a 24-h drug treatment. Emax is the maximal effect

Tumor no.	Patient age (years)	Stage	Grade	Control LI (%)	Antiproliferative effect		Apoptotic effect maximum index (%)[a]
					IC_{30} (m*M*)	Emax (%)	
1	71	IIA	I	19	0.4	50	3.3
2	53	IIA	III	32	0.3	42	14
3	31	IIIA	II	21	0.11	53	3.3
4	35	IIIA	II/III	34	0.61	30	15
5	23	IIIB	III	75	0.13	34	27
6	44	IIIA	III	45	2.5	31	12
7	48	IIIB	III	40	> 10	28	6.9
8	85	IIIB	III	42	3.5	34	9.4
9	70	IIIA	III	45	3.7	33	13
10	70	IV	II	50	> 10	14	29
11	57	IIA	III	60	> 10	27	18
12	42	IV	III	56	> 10	17	10
13	74	IIB	III	22	0.2	64	4
14	49	IIA	III	69	> 10	21	24
15	44	IIIA	III	39	5.9	33	10
Mean	53	NA	NA	43	3.5[b]	34	13
SD	18			17	0.11 to > 10[b]	14	8

[a] The untreated controls showed a maximum apoptotic index of 0–0.8%
[b] Median is presented instead of mean ± SD because IC_{30} in five tumors exceeded 10 μ*M*

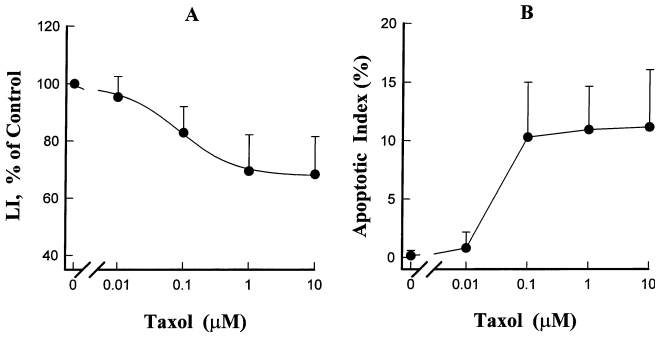

Fig. 2A,B Pharmacodynamics of paclitaxel effects. Human breast tumors were treated with paclitaxel for 24 h. **A** Drug-induced inhibition of 48-h cumulative BrdUrd incorporation. **B** Drug-induced apoptosis. Note that the inhibition of BrdUrd labeling is expressed relative to untreated controls, whereas the apoptotic index represents the actual number (not relative to controls). Also note the different scales for the two effects. Values are means ± SD of 15 tumors

tenfold among the tumors. More than 95% of apoptotic cells were BrdUrd labeled, but not all BrdUrd-labeled cells were apoptotic.

Paclitaxel-induced inhibition of DNA synthesis

Paclitaxel produced a sigmoidal, concentration-dependent inhibition of BrdUrd LI (Fig. 2). The average maximum inhibition was ~30%. The IC_{30} values for the antiproliferation effect varied by >100-fold (Table 1). The antiproliferation effect reached its maximum level at a drug concentration of 1 μ*M*. Further increasing the drug concentration to 10 μ*M* did not enhance the effect.

Tumor pathology and paclitaxel effects

The maximal apoptotic index correlated significantly with BrdUrd LI of untreated controls ($r^2 = 0.63$, $P < 0.001$). There was a tendency for the late-stage and high-grade tumors to show a higher sensitivity to the apoptotic effect and a lower sensitivity to the antiproliferation effect compared with the less malignant tumors, as indicated by the higher apoptotic index and the higher IC_{30} for the first group. However, the differences were not statistically significant (Table 2).

Relationship between apoptotic and cytostatic effects

Individual tumors displayed opposite sensitivity to the two drug effects, e.g. the tumor that was the most sensitive to the cytostatic effect was the least sensitive to the apoptotic effect (tumor 13 in the present study). The maximum antiproliferative effect correlated inversely with the maximum apoptotic effect (Table 1; $r^2 = 0.43$, $P < 0.01$).

Discussion

The results of the present study show that paclitaxel inhibited DNA synthesis and induced apoptosis in all 15 human breast tumors. Several aspects of these pharmacological effects are comparable to the drug effects in human head and neck, bladder, prostate and ovarian tumors, as follows [3, 7, 11, 25]. First, paclitaxel produced incomplete cytostasis and incomplete apoptosis, even at concentrations that were ten times the clinically achievable concentration of 1 μ*M*, in nearly all of the 100 human solid tumors studied. In contrast, paclitaxel produces complete cytotoxicity in monolayers of human cancer cells at much lower concentrations [21, 23]. These differences suggest that three-dimensional human tumors are more resistant to paclitaxel than monolayers. Multilayer structure-related chemoresistance is well documented (reviewed in reference 17). The incomplete inhibition of BrdUrd LI in human breast tumors is

Table 2 Relationship between tumor stages, grade and paclitaxel effects. IC_{30} is the paclitaxel concentration needed to produce 30% inhibition of BrdUrd-labeled cells after a 24-h drug treatment. Emax is the maximal effect. The values are means ± SD, except where noted. Statistical analysis was performed using Student's unpaired *t*-test, except where noted

Tumor status	*n*	Control LI (%)	Antiproliferative effect		Apoptotic effect maximum index (%)
			IC_{30} (μ*M*)[a]	Emax (%)	
Stage I/II	5	34 ± 15	0.47 (0.2 to >10)	41 ± 18	8 ± 5
Stage III/IV	10	48 ± 16	3.6 (0.11 to >10)	31 ± 10	16 ± 9
P-value		0.12	0.62	0.15	0.1
Grade I & II	4	32 ± 15	3.2 (0.11 to >10)	38 ± 18	11 ± 12
Grade III	11	47 ± 16	3.5 (0.13 to >10)	33 ± 12	14 ± 7
P-value		0.13	0.79	0.57	0.63

[a] Median and range are presented because the IC_{30} in five tumors exceeded 10 μ*M*. Statistical analysis by Wilcoxon's nonparametric two-sample test

consistent with literature data showing that paclitaxel-treated cells can proceed with DNA synthesis [21, 23]. The less-than-complete cytostatic and apoptotic effects suggest that repeated treatments may be necessary. Studies to evaluate the effect of more frequent treatments are warranted.

Second, the labeling of nearly all paclitaxel-induced apoptotic cells by DNA precursor and the significant correlation between the maximum apoptotic index and BrdUrd 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 (e.g. Table 1). A similar positive correlation between paclitaxel-induced apoptosis and proliferation has been observed in monolayer cultures of human lung, breast, cervical, colon, ovarian, and astrocytoma cells, and in Chinese hamster ovarian cells [9, 21, 23]. Third, the maximum apoptotic effect was observed at a lower drug concentration than the maximum cytostatic effect. Fourth, because both the cytostatic and apoptotic effects are expected to be a function of intracellular drug concentration, the opposite sensitivity of individual tumors to the two effects indicate that these effects are mainly determined by factors unrelated to and/or in addition to drug concentration. Finally, more malignant head and neck, bladder, breast and prostate tumors appear to be equally or more sensitive to the apoptotic effect than less malignant tumors. In advanced ovarian tumors, apoptosis is the major paclitaxel effect. These findings suggest the efficacy of paclitaxel in treating late-stage and high-grade tumors, since apoptosis is generally considered a major antitumor effect. A notable difference among the different tumor types is that in bladder and head and neck tumors, the more malignant tumors show a significantly lower sensitivity to the paclitaxel-induced cytostatic effect. In contrast, results of the same comparison in breast, ovarian and prostate tumors did not show statistically significant differences, probably because of the limited sample size.

For breast tumors, intertumor variation in sensitivity to the cytostatic effect (>100-fold) of paclitaxel is substantially higher than the variation for the apoptotic effect (10-fold). These variations are presumably a result of biological differences between individual tumors. Studies to evaluate intertumor differences in the expression of the multidrug resistance gene and genes involved in the regulation of apoptosis (e.g. p53, bcl-2), and the relationship between these differences and tumor sensitivity are ongoing.

In breast tumors, the maximum paclitaxel effects for a 24-h treatment were achieved at a concentration of 1 μM, which is comparable to the 0.9 μM steady-state concentration in humans attained during a 24-h infusion of the maximal tolerated dose of 250 mg/m^2 [29, 34]. Because increasing the drug concentration to 10 μM did not enhance either the antiproliferative or the apoptotic effects, we suggest that increasing the drug dose to elevate plasma concentrations is not likely to enhance the therapeutic effect. At present, it is not known whether cytostasis or apoptosis is responsible for the clinical activity of paclitaxel. The results of the present and previous studies, using histocultures of human breast, head and neck, ovarian and prostate tumors, show that for a 24-h treatment, maximum apoptosis is achieved at 0.1 μM in 70–80% of these tumors [7, 11, 25].

If indeed apoptosis is the major effect, our results would imply that it may be unnecessary to use the maximal tolerated dose in these tumor types. Additional studies are also needed to determine the maximally effective concentrations in different treatment schedules, e.g. 96-h treatment and repeated daily treatment. It should be emphasized that these pharmacodynamic 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 [30], (b) depletion of paclitaxel from culture medium is dependent on cell density [14], and (c) uptake of paclitaxel is saturable, and the drug efflux from cells is slow, resulting in a delayed effect [23].

It is noted that the present study used ethanol as the solvent for paclitaxel. The clinical preparation of paclitaxel uses Cremophor as the solvent. Paclitaxel may show less intracellular retention in Cremophor because the highly lipophilic paclitaxel may favor partition in Cremophor. Cremophor has multiple effects that may alter the paclitaxel pharmacodynamics. In mice, Cremophor is responsible for the nonlinear plasma pharmacokinetics of paclitaxel [31]. The data further suggest that Cremophor alters the plasma protein binding, tissue distribution, tissue binding and consequently the intracellular retention of paclitaxel. On the other hand, the small volume of distribution of Cremophor suggests that it is not taken up in tissues [31]. Hence, the intracellular retention of paclitaxel at the tissue level under in vivo conditions may be significantly different from that under in vitro conditions.

Another factor that needs to be considered in developing optimal treatment schedules for paclitaxel is the issue of treatment duration. The kinetics of the time-dependent drug effect are not fully understood; some literature reports indicate a higher activity by prolonging the treatment duration [15, 20, 23, 24, 32] whereas other reports indicate no enhancement [1, 28]. The present study was designed to establish the cytostatic and apoptotic effects of paclitaxel. Owing to the limited size of patient specimens and the labor-intensive nature of the histoculture chemosensitivity evaluation system, we used only one treatment time. The 24-h treatment duration was selected because it is one of the most commonly used schedules.

A separate study in our laboratory using multiple human cancer cell lines to examine the relationship between treatment durations and paclitaxel effects, has found that paclitaxel produces cytotoxicity even

after treatment is terminated, in part because the drug-induced apoptosis occurs slowly with a lag-time of about 24 h, and in part because of the slow drug efflux from cells which in effect extends the treatment duration [4]. Furthermore, because the drug effects are exerted in the M phase, the cell cycle distribution kinetics also play a role in determining the response of tumor cells to paclitaxel treatments. A computational pharmacokinetic model is being developed in our laboratory to incorporate the differences between in vitro and in vivo conditions, and to account for the time-dependent changes in drug concentration in plasma, the kinetics of drug uptake into and efflux from tumor cells, the cell cycle kinetics of tumor cells, and the time-dependent effects of paclitaxel, in order to provide more accurate quantitative analysis and for extrapolating in vitro pharmacodynamic data to in vivo situations.

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References

1. Adler OLM, Herzog TJ, Williams S, Rader JS, Mutch DG (1994) Analysis of exposure times and dose escalation of paclitaxel in ovarian cancer cell lines. Cancer 74: 1891
2. Arbuck SG, Blaylock BA (1995) Taxol: clinical results and current issues in development. In: Suffness M (ed) Taxol – science and applications. Boca Raton, CRC Press, pp 379–415
3. Au JL-S, Kalns J, Gan Y, Wientjes MG (1997) Pharmacologic effects of paclitaxel in human bladder tumors. Cancer Chemother Pharmacol 41: 69–74
4. Au JL-S, Li D, Gao X, Gan Y, Chen CT, Ge S, Johnson AL, Wientjes MG (1997) Time-dependent taxol cytotoxicity. Proc Am Assoc Cancer Res 38: 4
5. Bhalla K, Ilerado AM, Tourkina E, Tang C, Mahoney ME, Huang Y (1993) Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukemia 7: 563
6. Capri G, Tarenzi E, Fulfaro F, Gianni L (1996) The role of taxanes in the treatment of breast cancer. Semin Oncol 23 [Suppl 2]: 68
7. Chen CT, Au JLS, Gan Y, Wientjes MG (1997) Differential time dependency of antiproliferative and apoptotic effects of taxol in human prostate tumors. Urol Oncol 3: 11
8. Crossin KL, Carney DH (1981) Microtubule stabilization by taxol inhibits initiation of DNA synthesis by thrombin and epidermal growth factor. Cell 27: 341
9. Donaldson KL, Goolsby GL, Wahl AF (1994) Cytotoxicity of the anticancer agents cisplatin and taxol during cell proliferation and the cell cycle. Int J Cancer 57: 847
10. Furukawa T, Kubota T, Hoffman RM (1995) The clinical applications of the histoculture drug response assay. Clin Cancer Res 1: 305
11. Gan Y, Wientjes MG, Schuller DE, Au JLS (1996) Pharmacodynamics of taxol in human head and neck tumors. Cancer Res 56: 2086
12. Gold R, Schmied M, Glegerich G, Breltschopf H, Hartung HP, Toyka KV, Lassmann H (1994) Differentiation between cellular apoptosis and necrosis by combined use of in situ tailing and nick translation techniques. Lab Invest 71: 219
13. Horwitz SB (1994) Taxol (paclitaxel): mechanism of action. Ann Oncol 5:53
14. Kang H-J, Tran A, Schuller DE, Au JLS (1996) Taxol uptake and retention in human cancer cells and solid tumors. Proc Am Assoc Cancer Res 37: 367
15. Kelland LR, Abel G (1992) Comparative in vitro cytotoxicity of taxol and taxotere against cisplatin-sensitive and -resistant human ovarian carcinoma cell lines. Cancer Chemother Pharmacol 30: 444
16. Kerbel RS, Kobayashi H, Graham CH (1994) Intrinsic or acquired drug resistance and metastasis: are they linked phenotypes? J Cell Biochem 56: 37
17. Kerr JFR, Clay M, Harmon BV (1994) Apoptosis: its significance in cancer and cancer therapy. Cancer 73: 2013
18. Kubota T, Sasano N, Abe O, Nakao I, Kawamura E, Saito T, Endo M, Kimura K, Hiroshi D, Sasano H, Nagura H, Ogawa N, Hoffman RM, and The Chemosensitivity Study Group For The Histoculture Drug-Response Assay (1995) Potential of the histoculture drug-response assay to contribute to cancer patient survival. Clin Cancer Res 1: 1537
19. Li X, Gong J, Felman E, Seiter K, Traganos F, Darzynkiewicz Z (1994) Apoptotic cell death during treatment of leukemias. Leuk Lymphoma 13: 65
20. Lieberman JE, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB (1993) Cytotoxic studies of paclitaxel (Taxol) in human tumor cell lines. Br J Cancer 68: 1104
21. Liebmann J, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB (1994) The influence of Cremophor EL on the cell cycle effects of paclitaxel (taxol) in human tumor cell lines. Cancer Chemother Pharmacol 33: 331
22. Liu Y, Bhalla K, Hill C, Priest DG (1994) Evidence for involvement of tyrosine phosphorylation in taxol-induced apoptosis in a human ovarian tumor cell line. Biochem Pharmacol 48: 1265
23. Lopes NM, Adams EG, Pitts TW, Bhuyan BK (1993) Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell lines. Cancer Chemother Pharmacol 32: 235
24. Milas L, Hunter NR, Kurdoglu B (1995) Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. Cancer Chemother Pharmacol 35: 297
25. Millenbaugh N, Gan Y, Au JL-S (1998) Pharmacodynamics of paclitaxel-induced cytostatic and apoptotic effects in human ovarian tumors. Pharm Res 15: 123
26. ODAC prefers three-hour taxol infusion (1993) Cancer Lett 19: 38
27. Robbins KT, Connors KM, Storniolo AM, Hanchett C, Hoffman RM (1994) Sponge-gel-supported histoculture drug-response assay for head and neck cancer. Arch Otolaryngol Head Neck Surg 120: 288
28. Roberts JR, Allison DC, Donehower RC, Rowinsky EW (1990) Development of polyploidization in taxol-resistant human leukemia cells in vitro. Cancer Res 50:710
29. Rowinsky EK, Donehower RC (1993) The clinical pharmacology of paclitaxel (Taxol®). Semin Oncol 20:16
30. Song D, Hsu L-F, Au JL-S (1996) Binding of taxol to plastic and glass containers and protein under in vitro conditions. J Pharm Sci 85: 29
31. Sparreboom A, Tellingen O van, Nooijen WJ, Beijnen JH (1996) Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56: 2112
32. Tishler RB, Geard CR, Hall EJ, Schiff PB (1992) Taxol sensitizes human astrocytoma cells to radiation. Cancer Res 52: 3495
33. Vesico RA, Redfern CH, Nelson RJ, Ugoretz S, Stern PH, Hoffman RM (1987) In vivo-like drug responses of human tumors growing in three-dimensional gel-supported primary culture. Proc Natl Acad Sci USA 84: 5029
34. Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, Dutcher JP (1987) Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. J Clin Oncol 8: 1232