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Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell lines

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Abstract. Taxol is a clinically active anticancer drug, which exerts its cytotoxicity by the unique mechanism of polymerizing tubulin monomers into microtubules and stabilizing microtubules. Our studies with ovarian (hamster CHO and human A2780) cells showed that taxol is a phase-specific agent that is much more cytotoxic to mitotic cells than interphase cells. First, the dose-survival pattern of taxol resembled that of other phase-specific agents, in which cell-kill reached a plateau at a certain concentration. This suggests that the asynchronous cell population consists of a taxol-sensitive (presumably mitotic) fraction and a taxol-resistant fraction. Second, the cells were more responsive to increased exposure time than to increased dose above the plateau concentration. Third, in both asynchronous and synchronous cultures taxol was much more cytotoxic to mitotic than interphase (G_1, S and G_2) cells. Fourth, the taxol concentration needed to kill cells corresponded to the dose needed to block cells in mitosis. Although taxol blocked cells in mitosis, the mitotic block was of short duration. Cells escaped the mitotic block, without cytokinesis, and entered the next round of DNA synthesis to form multinucleated polyploid cells. Taxol was 15- to 25-fold more toxic to A2780 (human ovarian carcinoma) cells compared to CHO cells. This difference in sensitivity correlated with a higher intracellular taxol concentration in A2780 as compared to CHO as determined by either an ELISA assay or by [H^3]-taxol uptake.

Introduction

Taxol, a diterpenoid plant product [28], has shown significant activity against human tumors in the clinic. In phase I studies, taxol has demonstrated activity in malignant melanoma [16] and non-small-cell lung cancer. Phase II clinical trials showed excellent activity against refractory ovarian carcinoma [20] and in metastatic breast cancer [11]. The excellent review by Rowinsky et al. [24] can be consulted for further information on taxol's clinical activity.

Taxol, colchicine, vincristine, and vinblastine are all microtubule poisons. However, unlike taxol, all the other agents act by inducing the disassembly of microtubules. Taxol inhibits cell division by promoting the assembly of microtubules and stabilizing the tubulin polymers by preventing their depolymerization [19, 25, 27]. Taxol-treated cells exhibit characteristic bundles of microtubules that are usually not associated with the microtubule organizing center (centrosome). Mitosis is inhibited because the two centrosomes, which form the two spindle poles during metaphase, do not have the necessary microtubules associated with them. Taxol promotes assembly of microtubules in the absence of factors needed for microtubule assembly, such as GTP or microtubule-associated proteins [26]. Taxol-treated microtubules resist depolymerization by calcium or low temperature [27].

Among the spindle poisons the mechanism of action of vinca alkaloids has been studied in great detail, and yet the correlation between their effect on microtubule function and cytotoxicity remains uncertain. An agent that inhibits microtubule function and blocks cells in mitosis would be expected to be most cytotoxic to mitotic cells. However, Madoc-Jones and Mauro [18] have reported that vincristine is most toxic to cells in S phase and vinblastine is most toxic to both mitotic and S phase cells. In a recent study, Jordan et al. [13, 14] have compared the effect of four vinca alkaloids on microtubule depolymerization and cell proliferation and observed that cell growth is inhibited and cells are blocked in mitosis at drug concentrations that do not depolymerize microtubules. The lack of correlation between the drug's ability to depolymerize microtubules and inhibit cell growth could be due to the fact that vinca alkaloids also affect many other cellular processes, such as RNA, DNA synthesis [9], and cyclic nucleotide metabolism [12], which impact on cell proliferation.

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Taxol is also an antimicrotubule agent. However, in view of the above results with vinca alkaloids, we cannot assume that the major effect of taxol would be on mitotic cells. We report here several types of evidence that show that taxol behaves as expected of a phase-specific agent, killing cells mainly in mitosis. Parts of this paper were previously presented as an abstract [17].

Materials and methods

Materials. Taxol was obtained from the National Cancer Institute, dissolved in DMSO at 1 mg/ml and stored frozen.

Cell culture. CHO/WBL (Chinese hamster ovary, clone of CHO-K1) and A2780 (human ovarian carcinoma) cells were obtained from Roger Yu (Upjohn, Kalamazoo, Mich.) and Dr. R. Ozols (National Cancer Institute, Bethesda, Md.), respectively. CHO cells were maintained as monolayer culture in Ham's F10 medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. A2780 cells were maintained in monolayer culture in RPMI 1640 medium supplemented with 11% heat inactivated (56° C, 45 min) fetal calf serum, 2 mM L-glutamine and 0.25 U/ml of insulin (bovine pancreas). CHO and A2780 grew with doubling times of about 14 h and 16 h, respectively. Cells were maintained in logarithmic growth by harvesting with trypsin-EDTA solution (Irvine Scientific, containing 0.5 mg/ml of trypsin and 0.2 mg/ml of EDTA) and replanting before cells reached confluency. Cells were planted 48 h before an experiment to ensure exponential growth during drug exposure. For each drug concentration tested, two separate cultures were used.

Growth inhibition assay. Cell monolayers (about 2×10^4/25 cm^2) were incubated with varying amounts of taxol for 72 h, harvested with trypsin-EDTA at different times, and cells counted in a Coulter counter. The percent of growth inhibition was calculated as:

$$100 - 100 \times \left(\frac{\text{Cell no. in expt. flask} - \text{cells planted}}{\text{Cell no. in control} - \text{cells planted}} \right)$$

Cell survival determination. Taxol blocks cells in mitosis, which are easily detached from cell monolayer. Therefore, after taxol exposure, we combined the supernatant containing detached cells and the cells harvested from the monolayer. Cells were centrifuged and washed and then planted ($2 \times 10^2 - 10^4$ cells) in 6-well Corning plates. Cell survival was determined by a colony-forming assay as previously described [3]. Cloning efficiency of exponentially growing CHO cells ranged between 50 and 60%, and A2780 between 25 and 40%. Cloning efficiency of the untreated (control) cells was normalized to 100%, and the cloning efficiency of treated cells was expressed as a percentage of control survival.

Mitotic harvest and determination of phase-specific toxicity. Mitotic cells were selectively harvested [4] either without any pretreatment [21] or after pretreatment with 1.6 μg/ml taxol for 2 h. Mitotic cells were planted and the cells incubated until they reached G_1 or S phases of the cell cycle. Cells in M, G_1, or S were exposed to taxol and cell survival was determined. Position of cells in the cell cycle was determined by flow cytometry.

Mitotic index. Chromosome preparations were made by the method described previously [1]. In brief, cells were centrifuged at 1000 rpm in a clinical centrifuge for 4 min and the cell pellet was fixed by gently adding fixative (ethanol : glacial acetic acid, 3 : 1) to the pellet without disturbing it. After fixation for about 10 min, the fixative was decanted, and the cells resuspended in fresh fixative. The cells were centrifuged and the pellet was suspended in a small amount of fresh fixative. A few drops of the cell suspension were placed on a clean glass slide and dried by blowing on it. Two slides were prepared per sample and 1000 cells were counted to determine mitotic index.

Flow cytometry. Progression of cells through the cell cycle was based on DNA histograms determined by flow cytometry. Cells were fixed in ethanol and stained for DNA with mithramycin and the mithramycin stained cells were analyzed for DNA content on a Becton Dickinson FACStarPLUS [Becton Dickinson Immunocytometry Systems (BDIS), San Jose, Calif.] [5, 10]. The following data were collected for about 4×10^4 cells from each sample: forward scatter pulse height, wide-angle scatter pulse height, mithramycin fluorescence pulse area and mithramycin fluorescence pulse width. Subcellular debris was eliminated by gating raw list mode data on forward versus wide-angle light scatter. Cell clumps were eliminated by gating scatter-gated list mode data on mithramycin fluorescence pulse area versus pulse width. Single-parameter DNA histograms from gated list mode data were analyzed for cell cycle phase distribution using the program MODFIT (Verity Software House Inc. Topsham, Me.) running on a Compaq 386/33 computer.

Taxol uptake. [H^3]-taxol was obtained from Amersham (Arlington Heights, ILL.) (specific activity 3.4 mCi/mg) and stored at –30° C. It was purified by HPLC over a 25×0.5 cm column on $CH_3CN : H_2O$ (1 : 1) at 1.0 ml/min. Fractions were collected and absorbance at 227 nm, radioactivity, and immunoreactivity of taxol were measured. Significant amount of contaminant eluted early (3 min). No immunoreactive taxol was seen in this peak. The specific activity of [H^3]-taxol in the second peak (8 min) was 3.47 mCi/mg when measured by ELISA and by radioactivity counting. The specific activity of [H^3]-taxol in ethanol decreased from 3.4 mCi/mg to 0.57 mCi/mg when stored at about 4° C for 5 months. Therefore, [H^3]-taxol stock stored at –30° C was purified by HPLC just prior to use.

We followed the procedure of Manfredi et al. [19] to determine the total nonspecific and specific binding of taxol in the cells. The amount of taxol bound to CHO and A2780 cells was determined after 1 h exposure to varying amounts of [H^3]-taxol (81 μCi/81 μg/ml). To determine nonspecific binding, cells were incubated with [H^3]-taxol in the presence of 0.3 μg/ml colcemid (added 30 min before taxol). Colcemid and taxol bind at different sites and do not affect each other's binding to tubulin. Colcemid was used here to cover up the non-specific binding sites. Specific binding was the difference between total and nonspecific binding. After the 1 h incubation, medium was aspirated and the cell monolayer was washed three times with cold phosphate-buffered saline. Cells were then harvested and centrifuged. The cell pellet was suspended in 0.4 ml of medium and 50 μl of cell suspension was counted in the Coulter counter. An aliquot of the cell suspension was dissolved in an equal volume of 4% SDS –0.1N NaOH and radioactivity counted in a scintillation counter. A [H^3]-taxol standard was counted simultaneously to determine dpm/mg of taxol.

ELISA assay. After incubation of CHO and A2780 cell monolayer with taxol, the medium was removed and the cells washed with cold phosphate-buffered saline, harvested and centrifuged. The cell pellet was resuspended in cold phosphate-buffered saline and an aliquot was counted in the Coulter counter. Cells were then diluted with phosphate-buffered saline to a concentration of 10^6/ml and homogenized on ice (Polytron homogenizer, Brinkman) for 1 min and assayed soon after. ELISA assay kit was purchased from Hawaii Biotechnology Group. The ELISA assay was conducted in 96-well plates coated with taxol-coating antigen in phosphate-buffered saline, which acts by hydrophobic binding in 1 h incubation, followed by washing. Excess binding sites on wells were blocked with 1% BSA (1 h) and then washed with TRIS-buffered saline (TBS) containing 0.05% Tween 20. Taxol-treated cell lysates/standards were added to the wells and incubated for 1 h. Taxol in the sample and taxol bound to wells compete for a limited number of sites on antibody so that increasing taxol in the sample decreases the amount of antibody bound to the plate. After the wells were washed with TBS, they were treated with alkaline phosphatase labelled goat anti-rabbit serum and incubated for 1 h and washed. Enzyme substrate pNPP (*p*-nitrophenylphosphate) was added and color read on an ELISA plate reader. A standard curve was plotted with a negative slope as expected of a competitive binding immunoassay. Sample values were extrapolated from the standard curve.

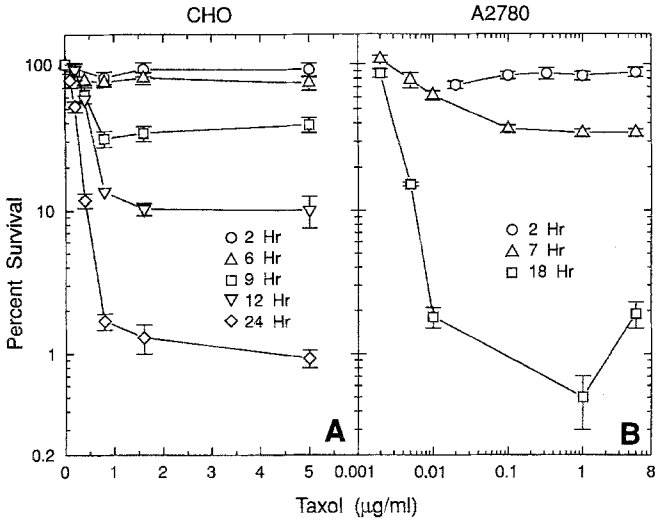

Fig. 1 A, B. Survival of *CHO* and *A2780* cells exposed to different taxol concentrations for various times. Survival was determined by a colony-formation assay. **A** CHO cells; **B** A2780 cells

Results

Growth inhibition

When CHO and A2780 cells were exposed to taxol for 72 h, dose-dependent growth inhibition was observed. Growth of CHO cells was completely inhibited at 0.5 μg/ml and A2780 at 0.02 μg/ml. Thus, A2780 cells were at least 25 × more sensitive to taxol.

Cell survival

The survival of cells after different periods of exposure to several concentrations of taxol are shown in Fig. 1. For each exposure time, cell kill in an asynchronous culture increased with taxol concentration, but reached a plateau at about 0.8–1.6 μg/ml for CHO cells compared to a plateau at about 0.01 μg/ml for A2780 (Fig. 1). Increasing the dose beyond this point did not increase cell kill for all exposure times tested, which suggests that the asynchronous cell population consists of a taxol-sensitive and taxol-resistant fraction. The survival of cells after different periods of exposure to several concentrations of taxol are shown in Fig. 1. This dose survival pattern, in which cell kill seems to be related more to increased exposure time than to increased drug concentration, supports the concept of a phase-specific agent (see discussion). The cells were also more responsive to increased exposure time than to increased taxol dose above the plateau concentration. Thus, the percent of CHO cells killed after exposure to 1.6 μg/ml of taxol increased with increasing exposure times from 13% at 4 h to 99% at 24 h.

Taxol is selectively toxic to mitotic CHO cells

Two different approaches were followed in order to determine taxol sensitivity of mitotic as compared to interphase cells.

Table 1. Sensitivity of mitotic and interphase cells to taxol[a]

	Mitotic		Interphase	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Taxol (μg/ml)	1.5	5.0	1.5	5.0
Mitotic cells (%)	89	93.6	4.6	3.6
Survival (%)	42.8±4	42.9±5	84.8±2	95.2±5

[a] An asynchronous culture was exposed to 1.5 or 5 μg/ml of taxol for 4 h, following which mitotic cells were selectively harvested. Survival of harvested mitotic cells and attached interphase cells were then determined. The percent survival is normalized to that of an untreated asynchronous population

1. Mitotic cells (77% were mitotic) were selectively harvested, without any pretreatment, from an asynchronous culture and planted at 0 h to initiate a synchronized culture. The cells progressed through the cell cycle such that after 2 h the cells were in G_1 and by 6 h 50% of the cells had entered S. When cells in mitosis (0 h) or in G_1 (2 h) were exposed to taxol for 1 h, 36.7% and 2.2% of the cells, respectively, were killed. When mitotic cells were exposed to taxol for 4 h (i.e., 0–4 h exposure) 71.8% were killed as compared to 6.8% of cells killed when exposure (2.5–6.5 h) started in G_1. Although the degree of synchrony of this population (mitotic index = 77%) was lower than desirable, the results show that mitotic cells were much more sensitive than interphase cells.

2. It is difficult to obtain a pure population of mitotic cells without using any mitotic-blocking agent. Therefore, we used a second approach in which mitotic cells were selectively harvested after an asynchronous culture was exposed to taxol for 4 h. In two experiments (Table 1), the mitotic population (89–94% mitotic) and the interphase (95–96% non-mitotic) population were quite pure. Survival results clearly show that taxol was more toxic to mitotic cells (57% killed) and marginally toxic to interphase cells (5–15% killed). However, we were surprised that taxol did not kill all the mitotic cells even after 4 h exposure to 5 μg/ml (Table 1). This could be due to the fact that mitotic cells harvested after 4 h taxol exposure constituted a heterogeneous population. Some mitotic cells had entered M shortly before harvest and thus received a very short taxol exposure, whereas others entered M at the beginning of taxol exposure and had received a complete 4 h exposure.

In order to determine the time course of the killing of mitotic cells by taxol, selectively harvested mitotic cells were exposed to the drug for varying periods. The results (Table 2) show that the majority (≅86%) of the mitotic cells harvested after 2 h exposure to taxol were viable. It required a further 2–4 h exposure to kill most of these mitotic cells.

Mitotic block

Since the mitotic phase is most sensitive to taxol, we expected a close relationship between the dose required to block cells in mitosis and the plateau cell-killing dose (see Fig. 1).

Table 2. Sensitivity of mitotic and interphase cells to taxol[a]

Exposure to taxol (h)	Survival (%)
0	86.2
2	23 ±6
4	15.7±3
6	13.2±1
8	12.3±0.2

[a] An asynchronous culture was treated with 1.6 μg/ml of taxol for 2 h, following which mitotic cells (89% mitotic) were selectively harvested. These mitotic cells were planted and further exposed to 1.6 μg/ml of taxol and cell survival determined. The percent survival is normalized to that of an asynchronous population. The percent survival of the 0 h sample represents the survival of mitotic cells harvested after 2 h taxol exposure

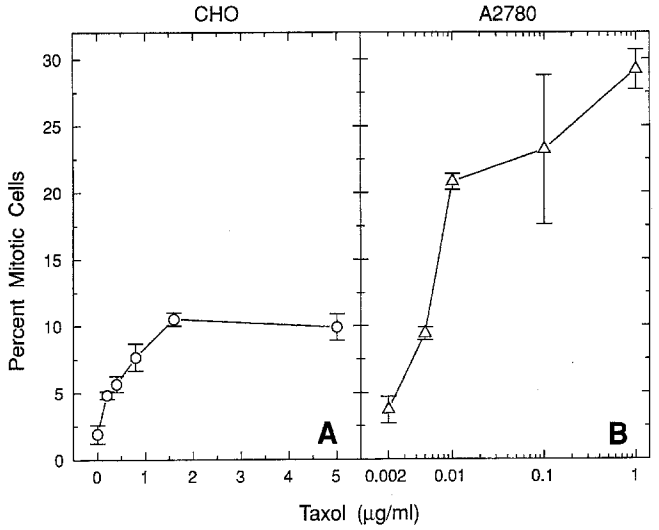

Fig. 2 A, B. Dose-response for taxol induced mitotic block in CHO and A2780 cells. Cells were exposed to taxol for 4 h following which the percent of mitotic cells was determined. Error bars represent mean ± SD

Dose response curves (Fig. 2) show that CHO and A2780 cells were blocked in mitosis by 1.6 and 0.01 μg/ml of taxol, respectively. These doses approximately correspond to the doses required for maximim cell kill at a particular exposure period. Also A2780 was again 10- to 20-fold more sensitive than CHO. The time course of accumulation of CHO cells in mitosis during taxol exposure was then determined (Fig. 3). The percent of CHO cells blocked in mitosis increased with increasing taxol exposure times and reached a maximum at 9 h after which the mitotic index decreased.

Cell progression

The DNA histograms of CHO cells exposed continuously to a growth-inhibitory concentration (1.6 μg/ml) of taxol are shown in Fig. 4 (top). The actual percentage of cells in G_1, S, and G_2-M were calculated from the DNA histograms and are shown (Fig. 4, lower half) as the time course

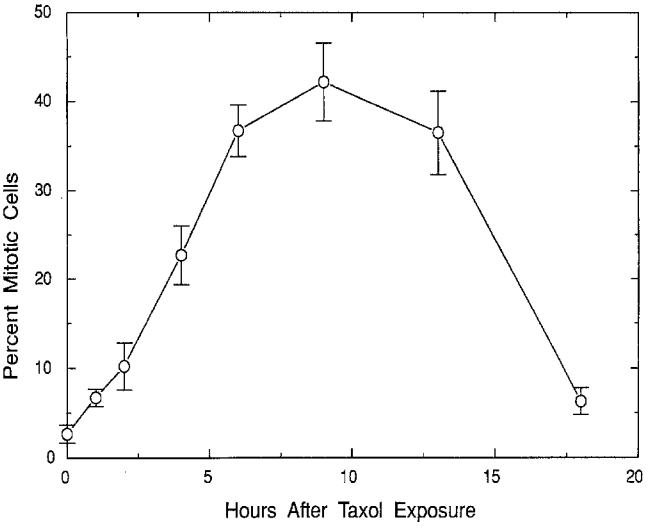

Fig. 3. Time course of blocking CHO cells in mitosis. The percent of mitotic cells was determined after different periods of exposure to 1.6 μg/ml taxol. Error bars represent mean ± SD

of the change in cell distribution during drug exposure. The percentage of cells in G_1 declined from an initial value of 25% to 3% at 8 h. The percentage of cells in S-phase increased slightly from 60% initially to 77% at 4 h as cells entered S from an unreplenished G_1 pool. This transient increase in percent of cells in S, at about 4 h, was seen in several experiments. S-Phase then declined to 39% at 8 h as cells continued to exit S and enter G_2. As a result of the mitotic block exerted by taxol, about 67% of the cells had accumulated in G_2M (4n DNA content) by 8 h. By 12 h few diploid G_1 or S-phase cells remained and cells entered a second round of DNA synthesis without completing cytokinesis. At this time 48% of cells were in tetraploid G_1 (4n) and 52% in tetraploid S-phase. By 24 h, the majority of cells progressed to tetraploid G_2M (8n) whereupon a third round of DNA synthesis began, resulting in an octaploid population at 48 h. These results show that cells escape the mitotic block and begin new rounds of DNA synthesis without prior cytokinesis. Also the progression of cells through several rounds of DNA synthesis in the presence of taxol suggests that the drug does not significantly inhibit DNA synthesis.

The appearance of multinucleated (polyploid) cells depended on taxol concentration. For example, after 24 h exposure of CHO cells to a lower dose (0.2 μg/ml) of taxol, a larger proportion of cells which had accumulated in G_2-M divided and entered diploid G_1, whereas a smaller proportion became tetraploid. At 0.8 and 1.6 μg/ml, these proportions were reversed and a major proportion of cells became tetraploid S.

The response of A2780 cells (Fig. 5) were similar to that of CHO cells but required a much lower concentration (0.02–0.1 μg/ml) of taxol. Cells exposed to taxol accumulated in G_2-M by 8 h and escaped the G_2-M block by 24 h to become polyploid. At the lower dose of taxol (0.02 μg/ml, 24 h) almost equal proportions of cells either divide to become diploid G_1 or became tetraploid. At 0.1

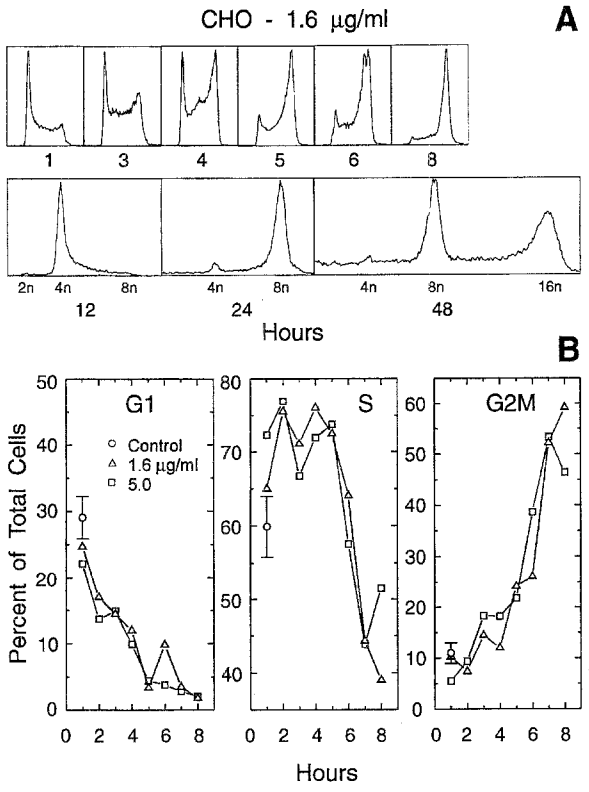

Fig. 4 A, B. Progression of CHO cells in the presence of 1.6 µg/ml of taxol. **A** DNA histograms. **B** Analysis of the histograms to determine percent G_1, S, and G_2-M cells at different times

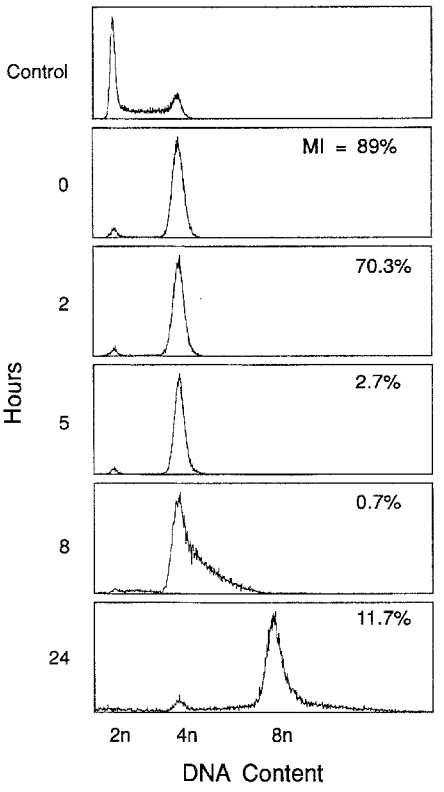

Fig. 6. Progression of mitotic CHO cells in the presence of taxol. DNA histograms at 0 h shows the mitotic population (89% mitotic). At 8 h the histogram shows polyploid S-phase and at 24 h most cells are in polyploid G_2-M

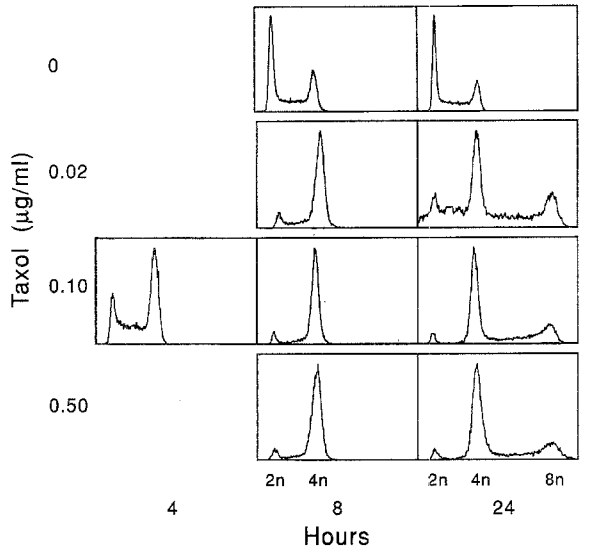

Fig. 5. Progression of A2780 cells in the presence of taxol. The DNA histogram of the control (0 ng/ml taxol) shows the diploid G_1 (2 n) and G_2-M (4 n) peaks. DNA histograms of taxol-treated cells at 4 and 8 h show accumulation in G_2-M. At 24 h, polyploid (>4 n DNA) cells are seen

and 0.5 µg/ml, cell division did not occur and most of the cells became tetraploid.

We then determined the progression of mitotic CHO cells incubated with taxol (Fig. 6). During the first 5 h, the percent mitotic cells decreased from 89% to 2.7% without any significant change in the DNA histograms. This is due to cells progressing from diploid M (4n DNA) to tetraploid G_1 (4n DNA) without cytokinesis. By 8 h, cells had progressed into tetraploid S and by 24 h the population contained tetraploid G_2-M (8n DNA) and small percent of octaploid S (>8n DNA). These results show that mitotic cells escape the mitotic-block even in the presence of taxol.

Since both taxol and colcemid block cells in mitosis but by different mechanisms, we compared the effects of taxol and colcemid on progression of CHO cells (results not shown). Both taxol (1.6 µg/ml) and colcemid (0.05 µg/ml) caused a transient accumulation of cells in mitosis at about 4 h and induced the formation of tetraploid cells by 24 h.

Cellular uptake of taxol

In order to explain the greater toxicity of taxol for A2780 as compared to CHO cells, we measured the intracellular accumulation of taxol both by [H^3]-taxol uptake and by an ELISA assay (Table 3). Both assays showed that total uptake by CHO cells was much lower than by A2780 cells. For example, total uptake by CHO cells exposed to

Table 3. Taxol binding measured by H^3-taxol uptake and by ELISA assay

[H^3]-Taxol uptake		Binding (ng/10^6 cells)		
Cell line	[H^3]-taxol (μg/ml)	Total	Non-specific[a]	Specific[a]
CHO	1.0	1.45 ±0.21	0.35	1.1
	0.5	0.98 ±0.05	0.19	0.79
	0.2	0.59 ±0.06	0.22	0.37
A2780	0.5	24.4 ±3.7		
	0.2	17.2 ±1.6	3.8	13.4
	0.05	14.7 ±1.3	1.53	13.2
Taxol uptake by ELISA[b]				
CHO	1.0	2.9 ±0.43		
	0.5	0.95 ±0.05		
	0.2	0.57 ±0.25		
A2780	0.2	24.0 ±1.1		

[a] Nonspecific binding (in the presence of 0.3 μg/ml colcemid) was subtracted from total binding to give specific binding values. CHO and A2780 cells were exposed to [H^3]-taxol for 1 h

[b] Cells were exposed to taxol for 3 h when the total uptake of taxol was measured by ELISA assay

0.2 μg/ml of taxol for 1 h was 0.59 ng/10^6 cells compared to 17.2 ng/10^6 A2780 cells. Specific binding of taxol to cellular microtubules was determined by subtracting non-specifically bound taxol (i.e., [H^3]-taxol bound in the presence of colcemid) from the total taxol bound to cells. Specifically bound taxol, after 1 h exposure to 0.2 μg/ml of drug, was also higher in A2780 (13.4 ng/10^6 cells) than in CHO (0.37 ng/10^6 cells). These results clearly show that the greater sensitivity of A2780 is due to greater taxol uptake by A2780 cells.

Discussion

Bruce et al. [7] have classified anti-cancer agents into three groups. Agents belonging to the cycle non-specific group, such as nitrogen mustard and gamma radiation, kill cycling and non-cycling cells in all phases of the cell cycle and give exponential dose survival curves. Agents such as 5-fluorouracil and cyclophosphamide belong to the cycle-specific group and kill only cycling cells and also give exponential dose-survival curves. In contrast, agents that are phase specific (i.e., kill cells only in certain phases of the cell cycle) give dose response curves that decrease to a constant saturation value (plateau survival) at high doses. Increasing drug concentration beyond the breakpoint does not result in increased cell kill. Bhuyan et al. [2] have extended the above studies to identify several S-phase-specific agents. For example, with asynchronous cultures cytosine arabinoside and camptothecin (S-phase-specific agents) give dose survival curves that reach a plateau at a certain dose and in synchronized cultures they kill only S-phase cells. In contrast actinomycin D, nogalamycin, and alkylating agents give exponential dose survival curves with asynchronous cultures and kill cells in several phases of the cell cycle in synchronous cultures.

Several lines of evidence clearly show that taxol is a phase-specific agent, which is most cytotoxic to mitotic cells:

1. In asynchronous cultures of both CHO and A2780 cells, survival decreased to a plateau at a certain taxol concentration. Increasing the taxol concentration beyond this break-point did not increase cell kill.

2. Taxol concentration needed to attain maximum cell kill (approximately 0.8–1.6 μg/ml for CHO and 0.01 μg/ml for A2780) corresponded to the concentrations needed to block cells in mitosis. The plateau survival value obtained at different exposure times approximately corresponded to the percent of cells expected to enter M (taxol-sensitive phase) during that period.

3. In synchronous cultures, mitotic cells were taxol sensitive, whereas interphase (G_1, S, G_2) cells were resistant to taxol.

This dose-survival pattern (i.e., plateau survival at certain doses) has also been seen with other cell lines exposed to taxol [23]. It must be realized that this dose-survival pattern can only be observed when an asynchronous culture is exposed to a phase-specific agent for periods less than the cell cycle time for that cell line. Exposure periods longer than the cell cycle time will allow all the cells to enter M and be killed. To our knowledge the only pertinent study is that of Rowinsky et al. [2] who have reported survival values for short exposures to taxol in human leukemic cell lines. We reanalyzed their data and observed that survival reached a plateau at a certain dose for Daudi (2 and 4 h exposure), K562 (2, 4 or 22 h), and LC (2 and 22 h) cell lines. However, HL-60 gave exponential dose survival curve at all exposure times. Therefore, we can conclude that taxol gives a dose-survival pattern characteristic of a phase-specific agent in several cell lines (CHO, A2780, Daudi, K562 and LC), but not necessarily in all cell lines (e.g., HL60).

Microtubules, the target of taxol, are critical for many cellular functions besides mitosis, such as cellular motility and intracellular transport. They also modulate the interaction of growth factors with their receptors. In view of the many cellular functions in which microtubules are involved, it is surprising to find that taxol is phase-specific in several cell lines. This may suggest that the interaction of taxol with spindle microtubules may be much more traumatic to these cell lines than the other effects of taxol.

Our results suggest that a long period of exposure to a lethal taxol dose is necessary to kill a high percent of cells in an asynchronous population. It should be noted that long-term exposure to a low concentration of taxol can be more cytotoxic than short-term exposure to a high concentration, even though both treatments have a similar concentration × time product. For example, Fig. 1 shows that exposure of CHO cells to 0.8 μg/ml for 24 h (concentration × time = 19.2) killed 99% of the cells as compared to 30% of cells killed when exposed to 5 μg/ml for 6 h (cxt = 30). Thus to kill a high percent of cells, the exposure has to be long enough (≅1 cell cycle time) to allow most of the cells to enter mitosis. Also, even cells in mitosis require 4 to 6 h exposure in order to be killed. For example, although 90% of the CHO cells ultimately die as a result of 12 h exposure to 1.6 μg/ml of taxol (Fig. 2), these cells

escape the mitotic block and subsequently proceed through several cycles of DNA synthesis for at least 48 h. These results suggest that taxol-treated cells progress through a sequence of events, which may consist of unbalanced growth or apoptosis that results ultimately in cell death.

We observed that both human (A2780) and hamster (CHO) cells lines responded similarly to taxol-induced mitotic blockade. Cells progressed into the next round of DNA synthesis, without undergoing cytokinesis, and became multinucleated (polyploid). Our observations are similar to those reported by Roberts et al. [22] in an elegant study of the progression of human leukemic cells exposed to taxol. They showed that taxol apparently slowed DNA synthesis to a greater extent in drug-sensitive HL-60 cells than in relatively resistant K-562 cells. This correlates with our observation of a transient accumulation of taxol-treated CHO cells in S-phase. Taxol accumulated both HL-60 and K-562 cells in G_2 and in mitosis. Our flow cytometric studies do not enable us to differentiate between G_2 and M cells. Finally, they differentiated between the taxol-induced polyploidy in relatively drug-resistant K-562 cells and absence of polyploidy induction in HL-60 cells. In our study, both the drug-resistant CHO and the more sensitive A2780 cell lines became polyploid.

Our observations with hamster and human cell lines differ from those of Kung et al. [15] who have reported a species-specific difference in the response of human and rodent cell lines to mitotic blockade by microtubule-depolymerizing agents such as colchicine and nocodazole. Human cell lines remain blocked in M whereas rodent cells escape M without cytokinesis and become polyploid. They related this difference in behavior to their cyclin B and cdc-2 kinase metabolism. Human cell lines treated with colchicine maintain their mitotic level of cyclin B and cdc-2 kinase and therefore the cells cannot exit M. In rodent cells, cyclin B degrades shortly after mitosis, thus allowing the cells to exit M without cytokinesis and become polyploid. The difference between our results and Kung's suggest that the species-specific response may not be universal but may depend on the cell lines used.

These studies identifying mitosis as the most sensitive phase can have a significant impact on the use of taxol in the clinic. Since the transit time for mitosis is usually short, it suggests that taxol will be ineffective when exposure times are short irrespective of the dose used. Only long exposure times will allow a large percent of the cells to enter M and be killed by the drug. In phase II clinical trials, taxol has been administered as 6 h or 24 h infusion. At the maximum tolerated dose of a 6 h infusion, taxol plasma levels have been maintained at about 10 μM for 2–4 h and exceeded 1 μM for 24 h [6]. Therefore, the effectiveness of taxol after 6 h infusion may be accounted for by the long period of exposure of cells to a possibly cytotoxic level of the drug. Our results may also be useful in preparing rational combinations of taxol. It is likely that agents which prevent cells from entering M (e.g., hydroxyurea, which blocks cells in S, or cisplatin, which blocks cells in G_2) would be antagonistic if they precede taxol exposure. We are presently attempting combination studies to prove the above speculation. Our speculation is corroborated by the observation of Citardi et al. [8], who have reported that cisplatin-taxol combination is most cytotoxic when cisplatin follows taxol as compared to the reverse schedule or simultaneous exposure to cisplatin and taxol.

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