Cancer Chemother Pharmacol (1994) 34: 335–343

Cancer Chemotherapy and Pharmacology

Comparative effects of taxol and Taxotere on two different human carcinoma cell lines

Patrick Garcia[1], Diane Braguer[1], Gérard Carles[1], Said El Khyari[2], Yves Barra[2], Concepcion de Ines[3], Isabel Barasoain[3], Claudette Briand[1]

[1] Groupe de Recherche sur les Interactions des Protéines en Pharmacologie, Faculty of Pharmacy, 27 Bd Jean Moulin, F-13385 Marseille Cedex 5, France
[2] Laboratory of Molecular Biology, Faculty of Pharmacy, 27 Bd Jean Moulin, F-13385 Marseille Cedex 5, France
[3] Centro de Investigaciones Biologicas, C.S.I.C., Velazquez 144, 28006 Madrid, Spain

Received: 5 November 1993/Accepted: 10 February 1994

Abstract. The effects of taxoids (taxol and Taxotere) were followed on two human cancerous cell lines (bladder carcinoma J82 cells and epidermoid carcinoma KB 3-1 cells). Three cellular parameters were studied, viz., the qualitative effect on cellular microtubules, the quantitation of tubulin, and the antimitotic action, using two-parametric flow-cytometric analyses in treated cells. In both of the cell lines the tubulin content increased after taxoid treatment before the accumulation of cells in the G_2/M phase. The effects of taxoids on tubulin appeared at about a 10-fold lower concentration on KB cells than on J82 cells. After drug exposure, the microtubule network showed a striking difference between the two cell lines: microtubule bundles were predominant in the J82 cell line, whereas multiple asters were prevalent in the KB cell line. The formation of these structures was dose- and time-dependent. Asters were observed in mitotic cells and bundles were seen in interphase cells. The reversibility of these structures in both cell lines varied with the duration of exposure to drug. Some differences were shown between taxol and Taxotere: the effects of Taxotere as compared with taxol appeared at a 2-fold lower concentration and their reversibility was slower.

Key words: Taxol – Taxotere – Tubulin – Microtubule

Introduction

The taxoids currently used in patients are two diterpenoid compounds: taxol (paclitaxel), isolated from *Taxus brevifolia*, and Taxotere (docetaxel), obtained by hemisynthesis from 10-deacetylbaccatin III, a precursor isolated from *Taxus baccata*. These two recent antineoplastic agents have demonstrated substantial clinical activity in patients with ovarian cancer, breast cancer, non-small-cell cancer, and leukemia [2, 18].

The mechanism of action of taxol has been largely elucidated by Manfredi and Horwitz [9]. In vitro, taxol promotes microtubule assembly and binds preferentially to microtubules rather than to tubulin dimers, unlike the antimicrotubule agents that induce microtubular disassembly (vinca alkaloids, colchicine). Taxol reduces the critical concentration of tubulin required for polymerization. Taxol-treated microtubules are stable and resist disassembly even after treatment with calcium or low temperatures, both of which usually promote disassembly. Recently it has been shown that [^{3}H]-taxol binds covalently to the β-subunit of tubulin [13]. Finally, taxol and Taxotere do not inhibit cellular DNA, RNA, or protein synthesis [9, 15].

Intact cells incubated with taxol accumulate in the G_2 and M phases of the cell cycle. Cells exposed to taxol produce two distinct patterns of cellular microtubules: either abundant arrays of disorganized microtubules arranged in parallel "bundles" or abnormal mitotic asters that do not require centrioles to form. The significance of these two morphological changes in cytotoxicity has not been fully established. Moreover, results obtained on the reversibility of taxol's action vary largely according to the cell line involved [3, 15]. The effect of Taxotere on different leukemias and PtK_2 epithelial-like cells is being studied [1].

The present work is a follow-up of the effects of taxoids on two human carcinoma cell lines. Our aim was to study the effect of the drugs on three cellular parameters: the quantitation of tubulin, the qualitative effect on cellular microtubules, and the antimitotic action. The study was performed using taxol and Taxotere at different doses over time, i.e. from the beginning of contact between drug and cell to either cellular death or reversal of the drug effect. This approach made it possible, first, to compare the action of the two drugs and, second, to evidence the differences between the two cell lines, such as the formation of bundles or asters.

This study was supported by grants from the ARC and the FNCLCC and by grant PB 87-0230 from the DGICYT

Correspondence to: C. Briand

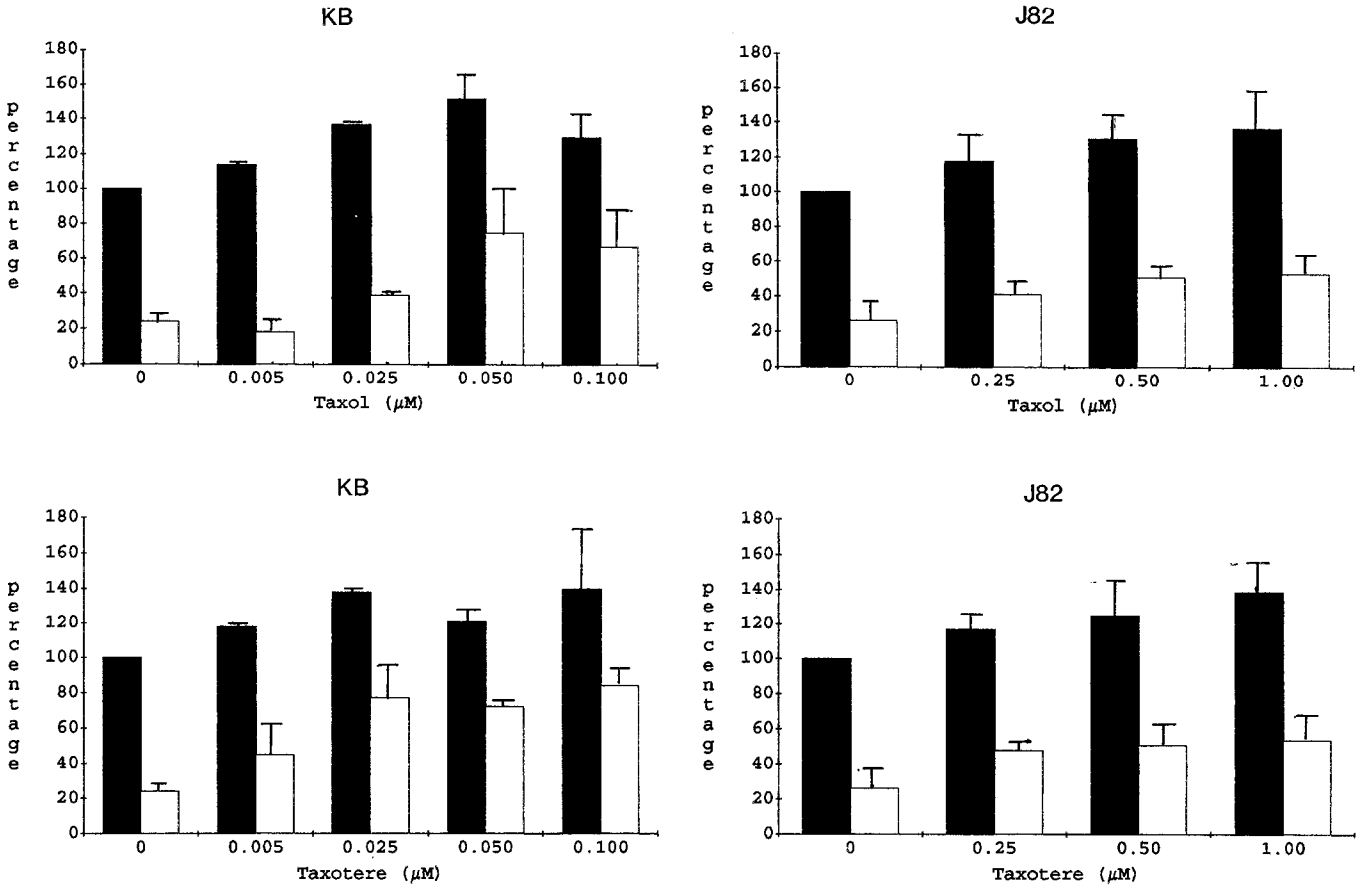

Fig. 1. Effects of taxoids on tubulin content and on the percentage of cells in the G_2/M phase as measured by two-parametric flow cytometry. After overnight incubation of KB or J82 cells with different doses of taxol or Taxotere, tubulin and DNA were measured by flow cytometry. The tubulin content (*black bars*) of the treated cells was quantified using anti-α-tubulin antibody. It was expressed as a percentage of that of untreated control cells. The percentage of cells in the G_2/M phase (*white bars*) was determined by cell-cycle analysis after staining of DNA with propidium iodide. Data represent mean values ± SE for at least three experiments

Materials and methods

Chemicals. Taxol was obtained from Sigma (St. Louis, Mo.) and Taxotere, from Rhône Poulenc Rorer (Paris, France). Stock solutions (0.01 *M*) of taxol and Taxotere were made in dimethylsulfoxide (DMSO) such that the highest concentration of DMSO used was 0.2%. [^{14}C]-Taxotere was supplied by Rhone Poulenc Rorer. The specific activity was 50 mCi/mmol. It was dissolved in DMSO (1.2 m*M*) and stored at –80° C. Mouse monoclonal anti-α-tubulin antibody and anti-mouse immunoglobulin, fluorescein isothiocyanate (FITC)-linked whole antibody were obtained from Amersham (Buckinghamshire, England).

Cell culture. KB 3-1 human epidermoid carcinoma cells were obtained from M. Gottesman (National Cancer Institute, Bethesda, Md.) and were maintained in Dulbecco's modified Eagle's medium supplemented with 4.5 g glucose/l, 0.1 *M* sodium pyruvate (Sigma) plus 10% fetal bovine serum, L-glutamine, penicillin, and streptomycin. J82 human bladder carcinoma cells were provided by M. Manfait (Laboratoire de Spectroscopie Biomoléculaire, Reims, France) and were grown in RPMI medium (Sigma) supplemented with 2 m*M* glutamine, 10% fetal bovine serum, and antibiotics.

Drug incubation. Log-phase cultures were plated in 25-cm^2 Costar tissue-culture flasks for two-parametric flow-cytometric analyses or were plated on coverslips for immunofluorescence study. For the evaluation of dose- and time-related effects, exponentially growing cells were treated with taxol or Taxotere at various concentrations for the below-mentioned times, washed, and stained. For reversibility studies, exponentially growing cells were incubated with drug for 3 h or overnight, quickly washed with PBS three times, and resuspended in the culture medium during the recovery period before staining.

Flow-cytometric measurements. Cellular tubulin and DNA contents were measured by flow cytometry. Cells (70%–80% confluence) from the KB or J82 cell lines were trypsinized and fixed in cold methanol. Cells were then washed, centrifuged, and resuspended for 30 min in PBS containing 60 μg RNAse/ml (Type I-A, Sigma). Monoclonal anti-α-tubulin antibody [200 μl (KB cells) and 300 μl (J82 cells) of a 1:400 dilution] was then added to 10^5 cells and incubated for 1 h at 37° C. FITC-conjugated anti-mouse antibody in PBS (dilution, 1:20) containing 1% bovine serum albumin was then added for 1 h incubation at 37° C. Before analysis, the cells were stained with 20 μg propidium iodide/ml (Sigma). Measurements wee performed using an ATC 3000 flow cytometer. Glass spheres (Fluresbrite-Fluorescent; 1.97-μm diameter) were used for calibration and normalization of the flow cytometer from experiment to experiment. The percentages of cells in the G_0/G_1, S, and G_2/M cell-cycle phases were calculated using the mathematical technique of Fried [4]. The relative fluorescence intensity of cells stained with anti-α-tubulin antibody was measured and compared with that of cells stained with the secondary antibody alone. Measurements were performed at least in triplicate. To confirm the validity of the method, we treated cells with taxol and colchicine overnight before performing flow-cytometric measurements as de-

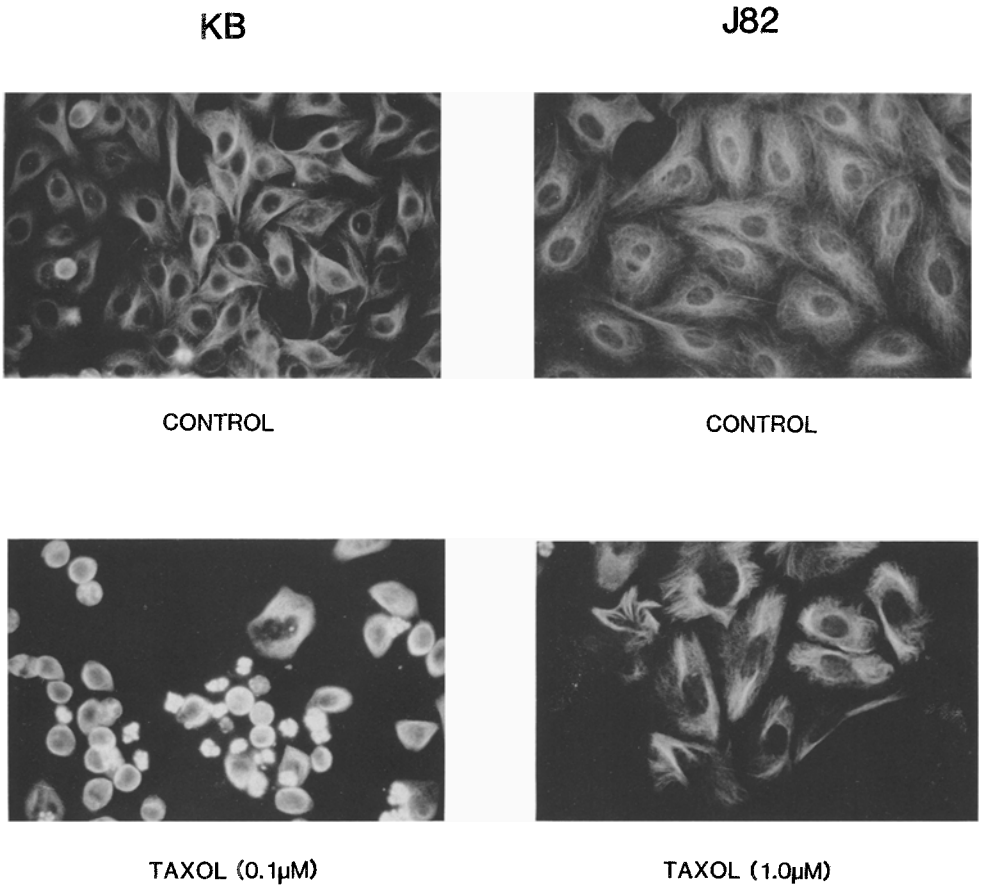

Fig. 2. Indirect immunofluorescence staining of tubulin in the KB and J82 cell lines, showing multiple mitotic asters and bundles in KB cells treated overnight with 0.10 μ*M* taxol and microtubule bundles in J82 cells treated overnight with 1.0 μ*M* taxol

scribed elsewhere [5, 7]. Exposure to taxol increased the cellular tubulin content as compared with that of untreated cells. In contrast, disruption of microtubules after treatment with colchicine was accompanied by a decrease in tubulin content.

Immunofluorescence microscopy. For immunofluorescence microscopy, cells were treated according to a technique similar to that previously described by Peyrot et al. [12]. Coverslips were then stained with 4,6 Diamidino-2-phenylindole (DAPI; 0.1–1 μg/ml, 2 min), rinsed, and mounted on glass slides. They were viewed with a Nikon Diaphot Photomicroscope (objective, ×40). To quantify the changes in cellular microtubules, we scored a minimum of 200 consecutive cells on each coverslip. The percentages of normal cells and cells with bundles or abnormal asters were determined for each concentration of taxol and Taxotere and for each incubation period. Experiments were performed in duplicate.

Cellular drug accumulation. Total binding of [^{14}C]-Taxotere to KB and J82 cells was determined according to the technique of Manfredi et al. [10]. Protein determinations were performed using the method of Lowry et al. [8], and radioactivity was determined in a Beckman LS 1701 counter.

Cytotoxicity assays. The cytotoxicity of taxol to both cell lines was assessed using the tetrazolium assay [11]. After 24 h taxol incubation, cytotoxicity was assessed by analysis of the optical density of wells using a Dynatech MR 7000 apparatus. The taxol concentrations required to inhibit 50% of cell growth (IC_{50}) relative to unexposed control values were then determined.

P-glycoprotein determination. P-glycoprotein levels were measured in these cell lines by flow-cytometric analysis using P-glycochek C_{219} FITC (Centocor).

Results

Dose effect

Flow-cytometric measurements. The amount of tubulin quantified by flow-cytometric measurements (Fig. 1) increased in taxoid-treated cells relative to untreated cells after overnight treatment with different drug concentrations, whatever the cell line. KB cells seemed more sensitive than J82 cells to taxoids, since we observed a 50% increase in tubulin content and cell-cycle blockage in KB cells treated with 0.050 μ*M* taxol, whereas no effect was detected on J82 cells at this concentration. The tubulin content in J82 cells incubated with 0.250 μ*M* taxol increased similarly to that of KB cells incubated with 0.025 μ*M* taxol. Moreover, the tubulin content was higher in nontreated J82 cells relative to nontreated KB cells because of the greater size of the J82 cells (relative fluorescence intensities were 102 and 55 for J82 and KB cells, respectively). The differences in sensitivity and size explain the different ranges of drug concentrations used in subsequent experiments (0.025–0.100 μ*M* and 0.25–1.00 μ*M* for KB and J82 cells, respectively).

For taxoid-treated cells, the tubulin-content increase was accompanied by a dose-dependent accumulation of cells in the G_2/M phase (Fig. 1). The two-parametric flow-cytometric method used to measure DNA versus tubulin content allowed us to determine the amount of tubulin in cells in the G_0/G_1, S, or G_2/M phases. For both of the cell lines we

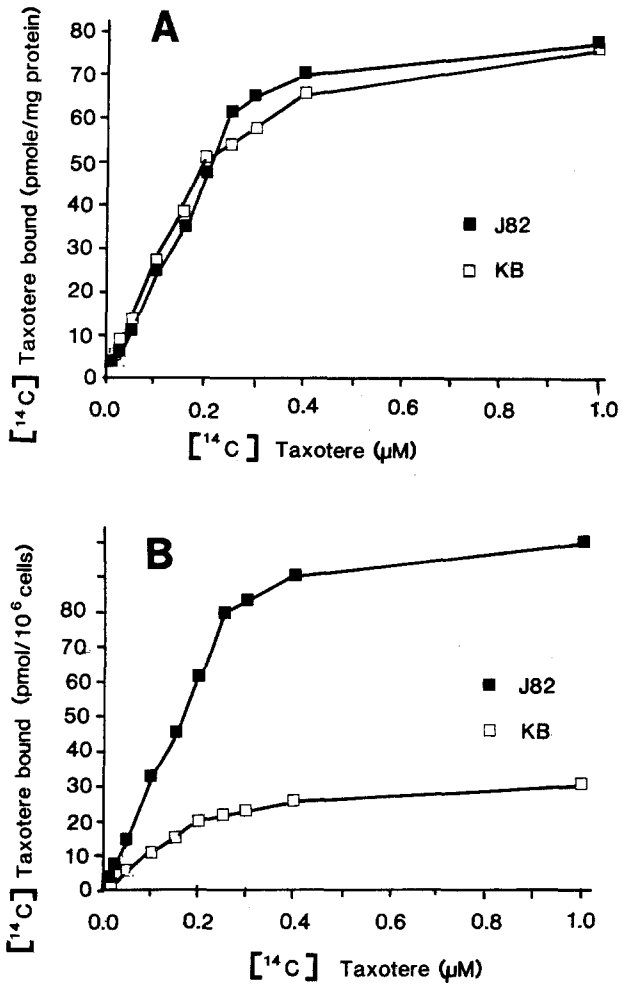

Fig. 3A, B. Intracellular [^{14}C]-Taxotere concentration in J82 and KB cells as expressed per milligram of protein (**A**) or per 10^6 cells (**B**). Confluent KB or J82 cells were incubated overnight with different concentrations of [^{14}C]-Taxotere in 35-mm dishes. Cells were washed and lyzed in 0.1 *M* NaOH. Radioactivity, protein concentrations, and cell counts were determined as described in Materials and methods

observed a similar increase in cellular tubulin content, whatever their DNA content (data not shown).

A difference was observed between the two drugs: 80% of KB cells were blocked in the G_2/M phase after exposure to 0.025 μ*M* Taxotere, whereas 0.050 μ*M* taxol was needed to induce a similar blockage. The J82 cell line behaved similarly at higher concentrations; 0.25 μ*M* Taxotere induced the same accumulation of cells (50%) in the G_2/M phase that was caused by 0.50 μ*M* taxol.

Immunofluorescence microscopy. After overnight exposure to taxoids, the microtubule networks of the two cell lines differed markedly (Fig. 2). Microtubule bundles were predominant in J82 cells, whereas abnormal mitotic asters were observed in only a small percentage of J82 cells (< 10%). In contrast, multiple asters were prevalent in KB cells (> 50% at taxol or Taxotere concentrations of ≥0.050 μ*M*). Taxol-induced microtubule bundles appeared as fluorescent cytoplasmic bands disrupting the cellular shape and radiating from one or two common sites in the peripheral cytoplasm. After overnight treatment of J82 cells, the quantity of microtubule bundles was relatively independent of the taxol concentration (0.250–1 μ*M*). Multiple fluorescent asters (usually four to eight) of short microtubules radiating from the center were scattered throughout the cytoplasm of KB cells. The proportion of KB cells containing these asters was dose-dependent (30% of cells treated with 0.025 μ*M* taxol contained asters versus 55% of those treated with 0.050 μ*M* taxol). Asters were present in mitotic cells and bundles appeared in interphase cells as observed on DAPI staining.

Determination of intracellular drug concentration. To check whether the difference in the sensitivity of the two cell lines was correlated with the intracellular drug concentration, we measured the latter using [^{14}C]-Taxotere (0.01–1 μ*M*). Figure 3 shows that when intracellular drug concentrations were expressed in picomoles per milligram of protein, the results were highly similar for both cell lines. In contrast, when expressed per 10^6 cells, intracellular concentrations of Taxotere differed because of the greater size of the J82 cells.

Cytotoxicity assay. The assay was performed to evidence differences in sensitivity to taxol between the two cell lines. After 24 h exposure, the mean IC_{50} in the KB 3-1 cell line was 0.02 ± 0.002 μ*M* and 0.01 ± 0.002 μ*M* for taxol and Taxotere, respectively, and it was about 1000-fold higher in the J82 cell line. Similar differences were observed after 48 h exposure.

Time effect

Flow-cytometric measurements. In both the KB and the J82 cell lines, the tubulin content increased earlier than the DNA content. For example, for KB cells treated with 0.025 μ*M* taxol or Taxotere, the increase in tubulin content relative to untreated cells was observed at 3 h drug exposure, whereas the increase in the number of cells blocked in the G_2/M phase began only at 6 h (Fig. 4). During this time course (3–24 h), the tubulin content did not appear to vary. Similar results were obtained in J82 cells treated with 0.25–1 μ*M* taxol or Taxotere (data not shown).

Immunofluorescence analysis. Table 1 shows the distribution over time (30 min–24 h) of bundles and asters in KB and J82 cells treated with different concentrations of taxol and Taxotere. For both cell lines, at 30 min contact time we observed a large decrease in the number of normal cells as compared to controls: 77% and 63% of the cells were normal in the J82 cell line following treatment with 1 μ*M* taxol and Taxotere, respectively, as opposed to < 50% in the KB cell line, whatever the drug concentrations. The decrease was clearly time-dependent for the two cell lines, as was the formation of bundles in J82 cells. The number of asters appearing during 24 h contact time remained low (< 10%). In contrast, the formation of asters in KB cells was time-dependent (taxol at 0.050 μ*M*: 35% and 55% proportions of asters at 6 and 24 h contact time, respectively). Moreover, as can be seen in Table 1, Taxotere in-

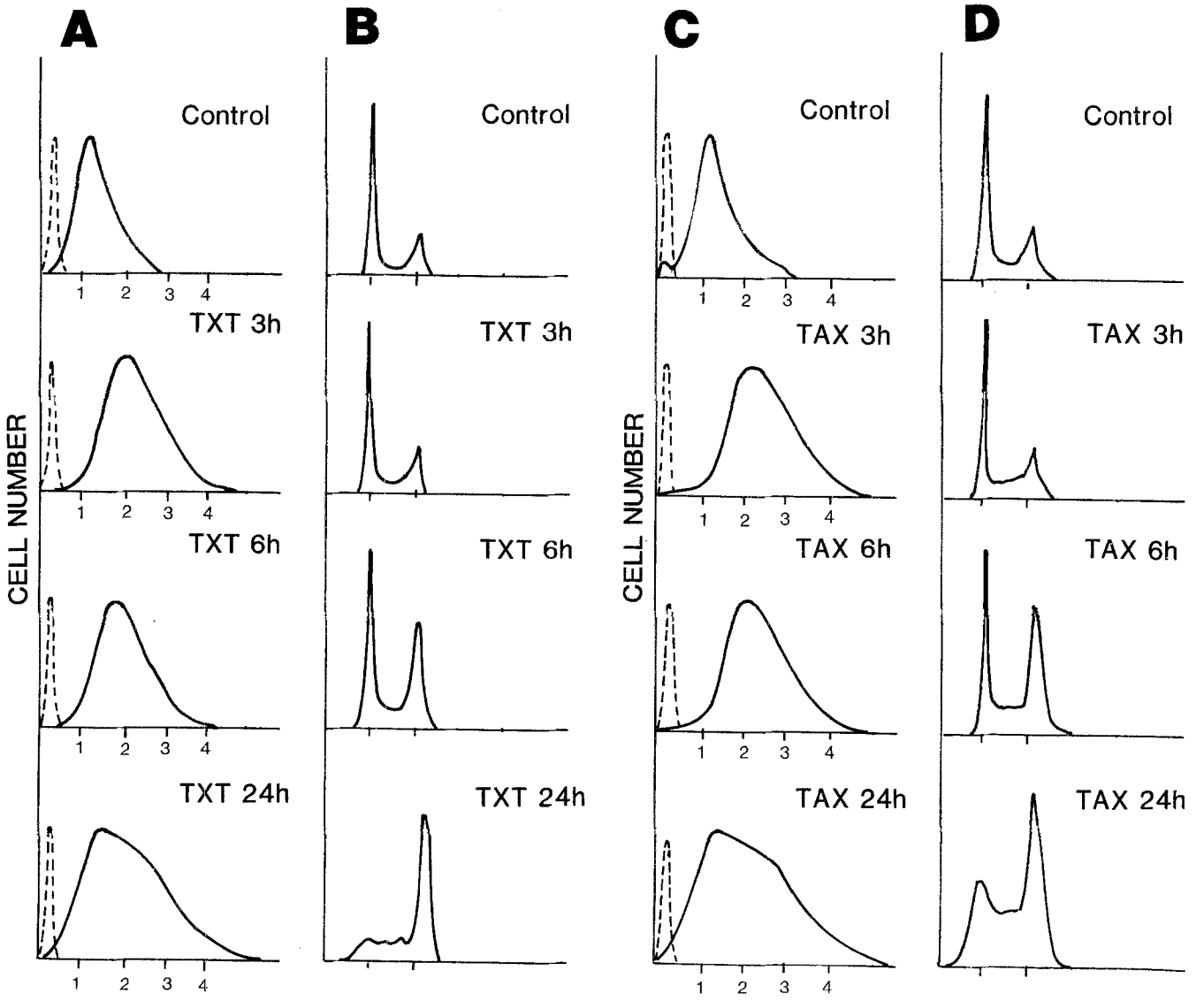

Fig. 4A–D. Time course of the immunofluorescent distribution of KB cells treated with Taxotere (*TXT*) at 0.025 μ*M* (**A, B**) and with taxol (TAX) at 0.025 μ*M* (**C, D**). Cells were stained with anti-α-tubulin antibody and then with FITC-linked secondary antibody (**A, C**), and the DNA was stained with propidium iodide (**B, D**). *Dashed line* (**A, C**), cells steined with FITC-linked secondary antibody alone; *solid line* (**A, C**), cells stained with specific anti-α-tubulin antibody

duced a lower proportion of normal cells than did taxol, whatever the contact time (J82 cells: 35% and 22% proportions of normal cells after 6 h contact with 0.5 μ*M* taxol and Taxotere, respectively; KB cells: 20% and 13% contents of normal cells after 6 h contact with 0.025 μ*M* taxol and Taxotere, respectively).

Reversibility of drug effects

The reversibility of taxoid effects was studied both after a short contact time (3 h) and after overnight incubation.

Reversibility after 3 h contact: Immunofluorescence analysis. As observed by immunofluorescence analysis, the microtubule network differed slightly according to the cell line examined (Fig. 5). In KB cells, the number of normal cells continued to decrease for at least 30 min after the end of the contact time; at 6 h the proportion of normal cells and asters increased simultaneously. In J82 cells (Fig. 5), the number of bundles increased during the 1st h after drug elimination and then steadily decreased.

Reversibility after 3 h of contact: flow-cytometric measurements. For KB cells, the number of cells in the G_2/M phase increased during the recovery period (32.3% and 44% at 3 and 6 h, respectively), whereas the proportion was similar to that observed in control cells at the end of drug incubation (13.7%). At 24 h after drug elimination the DNA content of treated KB cells was nearly the same as that of control cells. In contrast, we noted a low variation in the percentage of cells in the G_2/M phase in treated J82 cells (47.1%) as compared with control cells (41.4%).

Table 1. Time effect of taxoid-induced microtubule effects as assessed by indirect immunofluorescence on the KB and J82 cell lines

Cell line	Drug	Contact time (h)	Normal (%)	Bundle (%)	Aster (%)
KB	Taxol 0.025 μ*M*	0.5	47	52	1
		3	41	54	5
		6	20	60	20
		24	10	61	29
	Taxol 0.050 μ*M*	0.5	35	59	6
		3	33	59	8
		6	11	54	35
		24	5	40	55
	Taxol 0.100 μ*M*	0.5	31	62	7
		3	28	66	6
		6	2	71	27
		24	1	48	51
	Taxotere 0.025 μ*M*	0.5	33	67	0
		3	33	64	3
		6	13	57	30
		24	3	70	27
	Taxotere 0.050 μ*M*	0.5	32	68	0
		3	27	68	5
		6	12	61	27
		24	1	54	45
	Taxotere 0.100 μ*M*	0.5	21	77	2
		3	20	70	10
		6	5	61	34
		24	0	54	46
J82	Taxol 0.25 μ*M*	0.5	87	13	0
		3	64	28	8
		6	56	41	3
		24	26	65	9
	Taxol 0.50 μ*M*	0.5	83	15	2
		3	39	58	3
		6	35	63	2
		24	18	78	4
	Taxol 1.00 μ*M*	0.5	77	21	2
		3	34	65	1
		6	25	72	3
		24	15	85	0
	Taxotere 0.25 μ*M*	0.5	63	36	1
		3	43	54	3
		6	36	56	8
		24	18	80	2
	Taxotere 0.50 μ*M*	0.5	67	33	0
		3	30	70	0
		6	22	73	5
		24	8	84	8
	Taxotere 1.00 μ*M*	0.5	63	37	0
		3	27	73	0
		6	13	81	6
		24	5	91	4

Percentages of normal cells, microtubule bundles, and asters are expressed as a function of taxol and Taxotere concentration and contact time. Standard errors of the means are less than 5% of the mean values. Control cells without drug correspond to 100% of the normal cells

Reversibility after overnight incubation: immunofluorescence analysis. In J82 cells, treatment with 0.25–1 μ*M* taxol and Taxotere led to a regular increase in the proportion of normal cells during the recovery period (Fig. 5). Only normal cells were visualized at 48 h after the end of the contact time, except for some bundles observed at the highest doses. In KB cells, treatment with 0.025 and 0.050 μ*M* taxol led to a regular increase in the proportion of normal cells because of a decrease in the numbers of bundles and asters. At 0.1 μ*M* taxol and all tested doses of Taxotere, the reversibility of the drug effect was slower since the number of asters increased at the beginning of the recovery period. By 48 h after drug elimination, almost all asters had disappeared, and bundles or normal cells were observed; the proportion of normal cells was dose-dependent (0.025 μ*M* Taxotere, 85%; 0.1 μ*M* Taxotere, 27%).

Reversibility after overnight incubation: flow-cytometric measurements. Tubulin and DNA contents were higher at the end of overnight incubation as described above. Moreover, for both cell lines, neither the tubulin content nor the percentage of cells in the G_2/M phase showed significant variation at 24 h after the end of the contact time (data not shown).

Discussion

Flow cytometric measurements

An increase in tubulin content was observed in the J82 and KB cell lines after exposure to taxol or Taxotere; it was accompanied by a higher proportion of cells blocked in the G_2/M phase as compared with the control cell lines. Cellular tubulin content, measured by flow cytometry, reflected either the whole-cell tubulin content or the microtubule content alone if soluble tubulin was removed by the cell-fixation technique. The rise in tubulin content may have been due to an increase in the proportion of polymerized tubulin, with a lower loss of tubulin subunits, and/or to an increase in total tubulin synthesis. Indeed, cells contain an autoregulatory control mechanism that monitors the pool of depolymerized tubulin subunits and adjusts the rate of tubulin synthesis [19]. The variation in tubulin content measured by flow cytometry did not appear dose- or time-dependent, in contrast to the DNA content. However, a weakly dose-dependent action and a progressive effect of the drugs were observed on immunofluorescence (Table 1). Thus, the apparent absence of variation in tubulin content may have been due to the lack of sensitivity of the technique.

The antimitotic effect classically observed with antitubulin agents was manifested by the time-dependent accumulation of cells in the G_2/M phase. As the tubulin content increased much sooner than the DNA content, it can be concluded that the mechanism for increased tubulin expression after taxoid treatment does not depend on DNA synthesis.

The taxoid concentrations necessary to produce identical increases in cellular tubulin content were lower for the KB cell line than for the J82 cell line. The higher intrinsic sensitivity of the KB 3-1 cell line to taxoids was confirmed by the identical intracellular concentration of drug, expressed per milligram of protein, and by the cytotoxicity results. Indeed, taxol is more cytotoxic to the KB 3-1 cell line than to the J82 cell line. Moreover, the P-glycoprotein level was insignificant in both cell lines, indicating that multidrug resistance does not contribute to their sensitivity

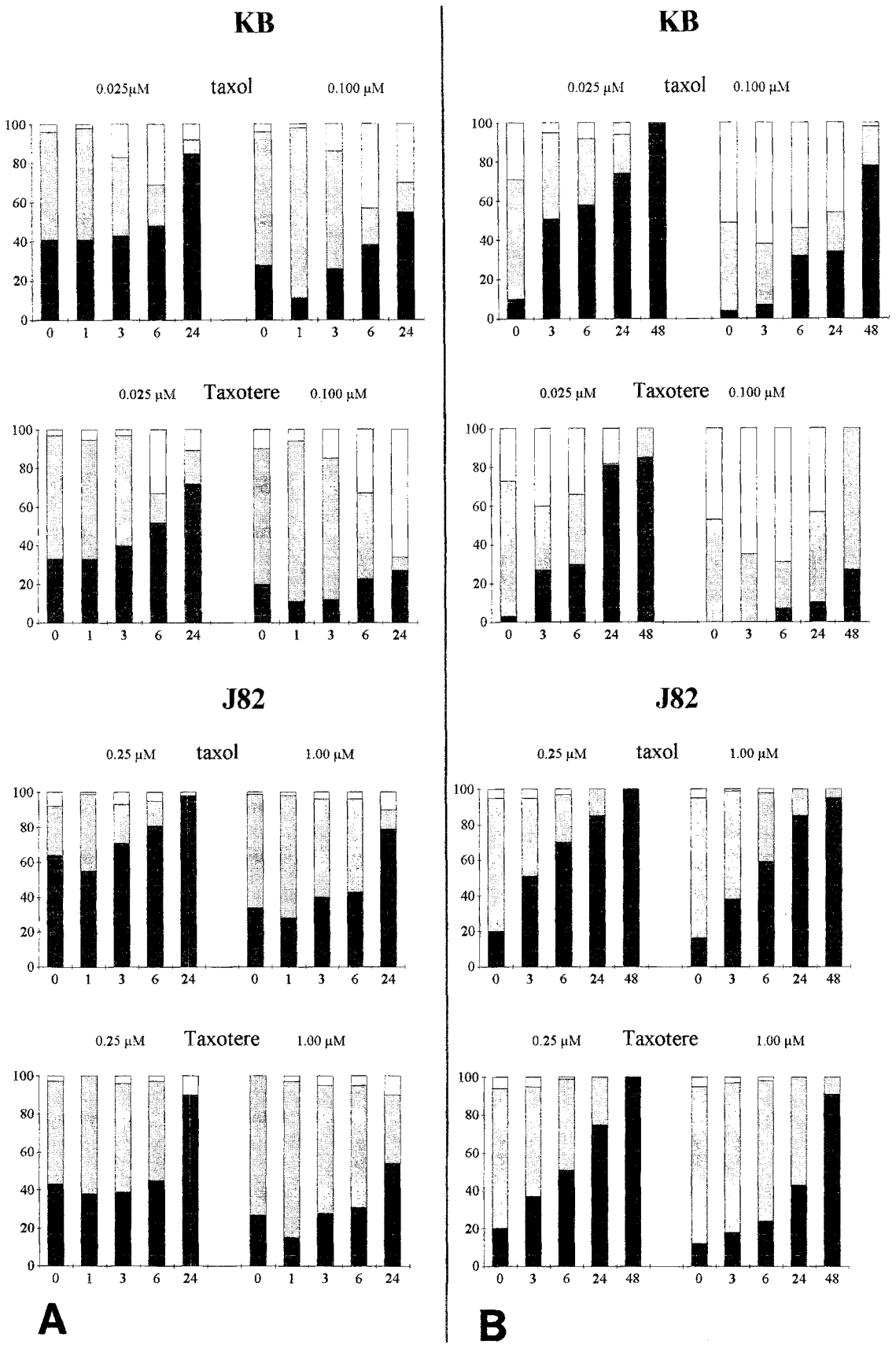

Fig. 5 A, B. Quantitation of taxoid-induced microtubule effects on the KB and J82 cell lines during the recovery period: **A** after 3 h contact time and **B** after overnight incubation. Percentages of normal cells (*black bars*), microtubule bundles (*gray bars*), and abnormal asters (*white bars*) are expressed as a function of taxol and Taxotere concentration. Standard errors of the means were less than 5% of the mean values. Untreated cells corresponded to 100% of the normal cells. Time *0* indicates the end of the 3-h contact time in **A** and the end of overnight incubation in **B**

to taxoids. Contrary to leukemia cell lines, the cell line most sensitive to taxoids is the one that shows asters in mitosis.

Immunofluorescence analysis

A striking difference between the two cell lines was observed in the microtubule network: the predominant formation of bundles (J82 cells) or asters (KB cells). Taxol has previously been reported to induce these two microtubule structures in cells blocked in the G_2/M phase [9]. DAPI staining evidenced that the presence of bundles in J82 cells corresponded to interphase cells, whereas the presence of asters in KB 3-1 cells treated with taxoids was due to cell blockage in mitosis; this explains why the formation of asters was clearly time-dependent in the KB cell line (Table 1). The relative proportions of bundles, asters, and normal cells appearing in KB cells treated with in 0.050 μM taxol after 6 and 24 h contact time suggested that bundles were transformed into asters as the cells entered mitosis. Moreover, the presence of asters in taxoid-treated cells could lead to polyploidization as observed on DAPI staining and previously described by Roberts et al. [16].

Reversibility

First, the reversibility of the effect of taxoids on cytoplasmic microtubules was studied after a short contact time allowing no accumulation of cells in mitosis. A biphasic effect was observed in KB cells: the drug effect initially persisted after the end of incubation (increase in bundles, asters, and G_2/M-blocked cells), then reversibility was evidenced by a decrease in the proportion of abnormal structures and the number of cells in the G_2/M phase. In J82 cells, the same biphasic effect was observed, albeit to a slighter extent. During the recovery period, the DNA-content increase was higher in KB 3-1 cells than in J82 cells after 3 h drug exposure, because the former remained blocked in mitosis longer than the latter, which progress through the mitotic block into interphase. This interpretation is consistent with the presence of asters in KB cells and their relative absence in J82 cells. Finally, in the cell lines tested, both the bundles and the asters induced by short-term incubation of cells with drug showed reversibility at 24 h after the end of exposure. The reversibility of these two structures has previously been described: that of asters, in leukemic cells [17] and that of bundles, in PtK_2 cells [3]. However, taxol-induced bundles have been reported to be irreversible in leukemic cells, albeit after a longer contact time (22 h) [17]. We tested the reversibility after overnight incubation (sufficient time required for accumulation of cells in the G_2/M phase). Reversibility was difficult to evaluate because flow-cytometric measurements of tubulin and DNA contents did not vary significantly during the recovery period. Nevertheless, the results obtained by immunofluorescence analysis indicated that the drug effect was reversible. This apparent discrepancy may be explained by the difference in the techniques used. Indeed, on immunofluorescence, only the living cells attached on coverslips were observed; the dead ones were excluded during the washes. In contrast, with flow-cytometric measurements, large numbers of cells (5000), including dead ones and debris, were analyzed, reflecting the total cell population. Thus, the apparent reversibility of the drug effect might have been attributable to a few surviving cells that grew during the recovery period.

Comparison of the effects of taxol and Taxotere

A difference between the actions of the two taxoids was evidenced both by flow-cytometric measurements and by immunofluorescence analysis. A 2-fold lower concentration of Taxotere relative to taxol induced the same accumulation of cells in the G_2/M phase, whatever the cell line. A lower proportion of normal cells was quantified by immunofluorescence in Taxotere-treated cells as compared with cells treated with the same taxol dose, whatever the contact time. Thus, under our experimental conditions, Taxotere appeared to induce 2 times more potent alterations in cellular tubulin than did taxol. We previously obtained a similar result, i.e., a 2-fold lower concentration of Taxotere relative to taxol was required to produce the same effect on pure tubulin polymerization in vitro [1] and as described in cytotoxicity studies on various cell lines [6, 14]. However, a comparative study of the two drugs in KB 3-1 cells treated for 1 h showed that the cytotoxicity of Taxotere was 10-fold that of taxol [15]. Moreover, the reversibility of the drug effect after a short contact time was faster for taxol than for Taxotere in both KB and J82 cells, whatever the tested doses. The greatest difference in reversibility was observed after overnight incubation of KB cells: 78% and 27% of the cells were normal following treatment with 0.1 μM taxol and Taxotere, respectively, after a 48-h recovery period. The effects of Taxotere as compared with taxol occurred at lower doses and their reversibility was slower in the two cell lines investigated.

In conclusion, the present findings evidenced differences between the two carcinoma cell lines, i.e., the appearance of either bundles or asters. The formation of these structures raises two important questions. First, what is the role of bundles and, especially, of asters in the fate of the cell? Second, is there a correlation between their presence in the cell and its malignancy?

Acknowledgements. We wish to thank Dr. Guiraud-Dauriac and Dr. Capo for their assistance with the flow-cytometric measurements.

References

1. Barasoain I, Ines C de, Diaz F, Andreu JM, Peyrot V, Leynadier D, Garcia P, Briand C (1991) Interaction of tubulin and cellular microtubules with Taxotere (RP 56976), a new semisynthetic analog of taxol. Proc Am Assoc Cancer Res 32: 1952
2. Bissery MC, Guenard D, Gueritte-Voegelein F, Lavelle F (1991) Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res 51: 4845
3. Brabander M de, Geuens G, Nuydens R, Willebrords R, Mey J de (1982) Microtubule stability and assembly in living cells: the in-

fluence of metabolic inhibitors, taxol and pH. Cold Spring Harbor Symp Quant Biol 46: 227
4. Fried J (1976) Method for the quantitative evaluation of data from flow microfluorometry. Comp Biomed Res 9: 263
5. Garcia P, Carles G, Braguer D, El Khyari S, Barra Y, Briand C (1992) Effects of mitotic spindle poisons on tubulin synthesis quantified by flow cytometric measurements. Proceedings, Second Interface of Clinical and Laboratory Responses to Anticancer Drugs. Marseille, France, April 2–4
6. Kelland L, 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
7. Leung MF, Sokoloski JA, Sartorelli AC (1992) Changes in microtubules, microtubule-associated proteins, and intermediate filaments during the differentiation of HL-60 leukemia cells. Cancer Res 52: 949
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265
9. Manfredi JJ, Horwitz SB (1984) Taxol: an antimitotic agent with a new mechanism of action. Pharmacol Ther 25: 83
10. Manfredi JJ, Parness J, Horwitz SB (1982) Taxol binds to cellular microtubules. J Cell Biol 94: 688
11. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55
12. Peyrot V, Leynadier D, Sarrazin M, Briand C, Rodriguez A, Nieto JM, Andreu JM (1989) Interaction of tubulin and cellular microtubules with the new antitumor drug MDL 27048. J Biol Chem 264: 21296
13. Rao S, Horwitz SB, Ringel I (1992) Direct photoaffinity labeling of tubulin with Taxol. J Natl Cancer Inst 84: 785
14. Ringel I, Horwitz SB (1992) Studies with RP 56976 (Taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83: 288
15. Riou JF, Naudin A, Lavelle F (1992) Effects of Taxotere on murine and human cell lines. Biochem Biophys Res Commun 187: 164
16. Roberts JR, Allison DC, Donehower RL, Rowinsky EK (1990) Development of polyploidization in taxol-resistant human leukemia cells in vitro. Cancer Res 50: 710
17. Rowinsky EK, Donehower RC, Jones RJ, Tucker RW (1988) Microtubule changes and cytotoxicity in leukemic cell lines treated with Taxol. Cancer Res 48: 4093
18. Rowinsky EK, Cazenave LA, Donehower RC (1990) Taxol: a novel investigational antimicrotubule agent. J Natl Cancer Inst 82: 1247
19. Theodorakis NG, Cleveland DW (1992) Physical evidence for cotranslational regulation of β-tubulin mRNA degradation. Mol Cell Biol 12: 791