Cancer Chemother Pharmacol (1992) 30: 444–450

Cancer Chemotherapy and Pharmacology

Comparative in vitro cytotoxicity of taxol and Taxotere against cisplatin-sensitive and -resistant human ovarian carcinoma cell lines*

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Received 20 December 1991/Accepted 16 April 1992

Summary. Using the sulforhodamine B assay, we compared the cytotoxic properties of the novel microtubule agent taxol and the semi-synthetic related compound Taxotere in nine human ovarian-carcinoma cell lines, including three pairs of cell lines rendered resistant to cisplatin or carboplatin. In addition, the cytotoxicity of the commonly used anticancer drugs cisplatin and adriamycin and the topoisomerase II inhibitor etoposide was determined. The results of continuous drug exposure showed that taxol [mean concentration producing 50% growth inhibition (IC_{50}), 1.1×10^{-9} M; range, $2.8 \times 10^{-9} - 5 \times 10^{-10}$ M and Taxotere (mean IC_{50}, 5.1×10^{-10} M; range, $7.2 - 3.3 \times 10^{-10}$ M) were >1,000 times more cytotoxic than either cisplatin (mean IC_{50}, 3.1×10^{-6} M; $P < 0.05$) or etoposide (mean IC_{50}, 2.3×10^{-6} M; $P < 0.05$) and >100 times more cytotoxic than Adriamycin (mean IC_{50}, 6.9×10^{-8} M; $P < 0.05$). Taxotere was more cytotoxic than taxol; following continuous exposure, the mean difference across the cell lines was 2 orders of magnitude (range, 1.1–3.9 orders of magnitude for individual lines). Although this difference did not reach statistical significance for any individual cell line (P values ranged from 0.17 for HX/62 to 0.9 for OVCAR-3), when all IC_{50} values for the 96-h experiments were pooled, Taxotere was found to be significantly more potent than taxol ($P = 0.05$). Following 2 h exposure, the mean cytotoxicity of Taxotere was 3.9-fold > that of taxol across the nine lines (range, 0.75- to 10-fold; $P < 0.05$ for the CH1 cell line; overall pooled IC_{50} data, $P = 0.05$). Although a 71-fold range of sensitivity to cisplatin was observed across the six parent cell lines (IC_{50} most resistant line/IC_{50} most sensitive line), this was largely abolished by treatment with taxol (5.6-fold range) and Taxotere (2.2-fold range). Following continuous exposure of the three pairs of lines exhibiting acquired resistance to platinum, no cross-resistance with either Taxotere or taxol was found (resistance factors, <1.5). In the 41M and 41McisR pair of lines, in which previous studies have shown resistance to be due to reduced platinum accumulation, taxol and Taxotere exhibited some collateral sensitivity (resistance factors, 0.69 and 0.66, respectively). Taxotere and, particularly, taxol showed a pronounced concentration times exposure duration (C × T) dependence as compared with cisplatin ($P < 0.05$). The mean loss in potency across the nine lines for 2 vs 96 h exposure was 97 for taxol, 35 for Taxotere, 30 for Adriamycin and only 9.9 for cisplatin. However, these differences in potency loss observed between taxol and Taxotere did not reach statistical significance ($P = 0.18$). These data indicate that Taxotere is approximately 2 times more cytotoxic than taxol and shows an encouraging lack of cross-resistance in three cell lines exhibiting acquired resistance to cisplatin and carboplatin.

Introduction

Ovarian carcinoma is the seventh most common cancer in women, with approximately 140,000 new cases being annually diagnosed worldwide [29]. The standard chemotherapy for this disease generally consists of a platinum-based drug (cisplatin or carboplatin) combined with a classic alkylating agent (commonly cyclophosphamide [25, 28]. Despite the high initial response rates, most patients ultimately die of their disease, primarily due to the emergence of tumour resistance to the platinum drugs [25, 28]. A recent analysis of some 45 different clinical trials has concluded that cisplatin and carboplatin are equally effective against advanced ovarian cancer [1]. Moreover, the

* This study was supported by grants to the Institute of Cancer Research, Royal Cancer Hospital, from the Cancer Research Campaign and, through the European Organisation for Research and Treatment of Cancer (EORTC) Clonogenic Assay Screening Group, by grant 90031 from Rhone-Poulenc Rorer.

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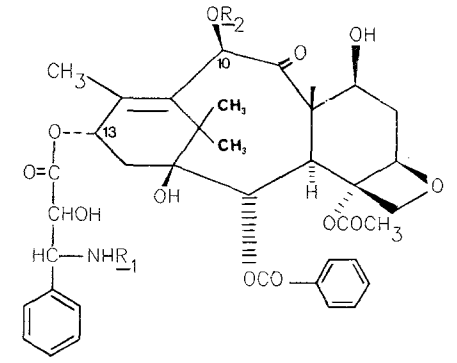
results of cross-over trials suggest that cisplatin and carboplatin are effective against essentially the same population of tumours [10, 15]. Therefore, it is apparent that new clinical strategies are urgently required to improve the survival of patients with this disease.

Over the past few years, a novel microtubule-stabilising agent, taxol, has been shown to exhibit promising activity in a variety of human cancers [26]. Some 20 years ago, taxol was shown to be the major active species of extracts derived from the bark of the Pacific yew, *Taxus brevifolia* [41]. Its mechanism of action was later demonstrated to be unique, constituting tubulin polymerisation and stabilisation of microtubules rather than the depolymerisation reported for the classic antimicrotubule agents vincristine and colchicine [36]. In the clinical trials thus far performed, taxol has shown particularly encouraging activity against advanced ovarian cancer; in the first phase II trial an unusually high response rate of 30% (partial plus complete response) was observed [25]. Furthermore, a notable proportion of the responses occurred in patients considered to be resistant to cisplatin. Subsequently, this exciting level of activity has been demonstrated in further trials [12, 39]. Unfortunately, the structural complexity of taxol has precluded its chemical synthesis. In addition, its extreme scarcity has thus far limited a more extensive clinical evaluation. Pacific yew is extremely slow-growing, and stripping of the bark to produce taxol results in the death of the tree as well as a low yield of drug.

One approach to alleviating the problems of taxol supply may involve use of the semi-synthetic compound Taxotere (RP 56976; NSC 628503; *N*-debenzoyl-*N*-tert-butoxycarbonyl-10-deacetyl taxol) [3, 16, 23]. This analogue is prepared from a non-cytotoxic precursor (10-deacetyl baccatin III) extracted from the renewable needles of the English yew, *Taxus baccata*, by esterification with a synthetic side chain [9, 24]. The aim of the present study was to compare the cytotoxic properties of Taxotere versus taxol against a panel of human ovarian-carcinoma cell lines representative of both intrinsic and acquired platinum drug resistance. Nine cell lines were used, including three pairs of lines exhibiting acquired platinum resistance. In addition, data on the cytotoxicity of cisplatin, Adriamycin and etoposide were obtained for comparative purposes.



	R_1	R_2
Taxol	CO–	$COCH_3$
Taxotere	$COOC(CH_3)_3$	H

Fig. 1. Structures of taxol and Taxotere

Materials and methods

Cell lines. Six parent human ovarian-carcinoma cell lines (SKOV-3, HX/62, PXN/94, OVCAR-3, 41M and CH1) were used in this study. Their biological properties and sensitivity to the clinically used platinum drugs cisplatin and carboplatin have been described elsewhere [18]. Two of these lines, SKOV-3 and HX/62, may be considered as being intrinsically resistant to platinum agents [18, 21]. In the two most cisplatin-sensitive cell lines, 41M and CH1, acquired resistance to cisplatin was generated by exposing cells to increasing concentrations of cisplatin [starting at those producing 10% growth inhibition (IC_{10})] over a 12- to 18-month period. Typically, cells were exposed three times to each concentration, whereafter the concentration was doubled. Exposure was continuous over 3 days; the drug was then removed, and the cells were again exposed when normal growth had resumed. In addition, resistance to carboplatin was generated in the OVCAR-3 cell line as described above.

All lines grew as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (Imperial Laboratories, Andover, UK), 2 mM glutamine, 10 μg insulin/ml, 0.5 μg hydrocortisone/ml plus 50 μg gentamicin/ml and 2.5 μg amphotericin B/ml in an atmosphere comprising 10% CO_2/90% air. Cells were routinely checked for the presence of mycoplasma and were found to be negative throughout the course of this study. Lines were used within a defined range of 20 passages; no difference in tumour-doubling time or tumour morphology became apparent during the study.

Drugs. Cisplatin was synthesised by and obtained from the Johnson Matthey Technology Centre (Reading, UK), Adriamycin was obtained from Farmitalia Carlo Erba (Herts, UK) and etoposide (VP16, vepesid) was supplied by Bristol Myers Squibb (Hounslow, UK). Taxotere was provided by Dr. M. C. Bissery (Rhone-Poulenc Rorer, Vitry sur seine, France) and taxol, by Dr. O. Yoder (National Cancer Institute, Brussels, Belgium). A structural comparison of Taxotere and taxol is shown in Fig. 1.

Cytotoxicity assay. Cytotoxicity was assessed using the sulforhodamine B (SRB) assay as described previously [20–22, 27, 35, 37]. SRB is a bright pink aminoxanthene dye with two sulfonic groups, which binds to protein basic amino acid residues in trichloroacetic acid (TCA)-fixed cells under mildly acidic conditions. Dead cells either lyse or are removed during the staining procedure and thus do not contribute to the colorimetric endpoint [35]. In other studies, IC_{50} values obtained in the SRB assay have been shown (using a diverse range of chemotherapeutic agents across multiple panels of tumour cell lines) to correlate closely with those obtained in the tetrazolium (MTT) semi-automated assay [20, 35]. Moreover, good correlation has been demonstrated for IC_{50} values between the MTT and clonogenic assays [7], and our studies using cisplatin reveal the same cell-line ranking and similar IC_{50} values for the SRB versus clonogenic assays (unpublished observations).

Briefly, $5 \times 10^3 - 1 \times 10^4$ cells/well were seeded into 96-well microtitre plates in 200 μl growth medium. After overnight incubation, agents were then added following appropriate serial dilution in 50 μl medium to quadruplicate wells. Immediately before their use, cisplatin and Adriamycin were dissolved in saline and water, respectively. Taxol and Taxotere were made up in absolute ethanol at 5 mM and were kept as stock solutions at –20°C. Stock solutions were initially diluted at least 1 : 100 (v/v) in growth medium and were re-diluted thereafter as required. As taxol is soluble in medium at concentrations of up to 40–50 μM [30],

Table 1. Cytotoxicity of various agents in six parent human ovarian-carcinoma cell lines as determined following continuous drug exposure

Cell line	IC_{50} (M)[a]				
	Cisplatin	Taxol	Taxotere	Adriamycin	Etoposide
HX/62	1.1×10^{-5} $\pm4\times10^{-7}$	2.8×10^{-9} $\pm1.3\times10^{-9}$	7.2×10^{-10} $\pm2\times10^{-10}$	1.2×10^{-7} $\pm3.5\times10^{-9}$	5.5×10^{-6} $\pm2\times10^{-6}$
SKOV-3	3.9×10^{-6} $\pm7\times10^{-8}$	1.2×10^{-9} $\pm4\times10^{-10}$	4.2×10^{-10} $\pm2\times10^{-10}$	8×10^{-8} $\pm3\times10^{-9}$	1.4×10^{-6} $\pm3.2\times10^{-7}$
PXN/94	2.7×10^{-6} $\pm1.5\times10^{-6}$	7.4×10^{-10} $\pm1\times10^{-10}$	5×10^{-10} $\pm2\times10^{-10}$	3.1×10^{-8} $\pm1\times10^{-10}$	5.2×10^{-7} $\pm9\times10^{-8}$
41M	2.3×10^{-7} $\pm5\times10^{-8}$	8.1×10^{-10} $\pm2\times10^{-10}$	6.2×10^{-10} $\pm2\times10^{-10}$	4.9×10^{-8} $\pm5\times10^{-9}$	5.5×10^{-7} $\pm4\times10^{-8}$
CH1	1.6×10^{-7} $\pm3\times10^{-8}$	5×10^{-10} $\pm9\times10^{-11}$	3.3×10^{-10} $\pm7\times10^{-11}$	4.5×10^{-9} $\pm7\times10^{-10}$	6.2×10^{-8} $\pm7\times10^{-9}$
OVCAR-3	5.1×10^{-7} $\pm1\times10^{-8}$	5.1×10^{-10} $\pm1\times10^{-10}$	4.8×10^{-10} $\pm2\times10^{-10}$	1.3×10^{-7} $\pm2\times10^{-9}$	5.6×10^{-6} $\pm1\times10^{-7}$

[a] Data represent mean values $\pm$SD ($n = 3-5$)

no precipitation of drug was observed. The final concentration of ethanol in these stock solutions was <0.1% and was non-toxic to the cell lines as previously reported [21]. Etoposide, obtained as a liquid formulation, was diluted as required in growth medium immediately prior to its use.

Cells were exposed to agents either for 2 h or for the total 96-h assay period. In the 2-h experiments, drug was removed at the completion of the exposure period by washing the wells first with phosphate-buffered saline and then with growth medium, after which fresh growth medium was used for the remainder of the 96-h assay period. Cytotoxicity was then assessed by analysis of the basic amino acid content using 0.4% SRB in 1% acetic acid (Sigma Chemicals) as described elsewhere [20–22, 27, 35, 37]. The drug concentrations required to inhibit 50% of cell growth (IC_{50}) relative to unexposed control values were then determined.

Statistical analysis. All results represent mean values for 3–5 independent experiments. Results were analysed in terms of IC_{50} values, and differences noted across the cell-line panel and within individual cell lines were tested for statistical significance using the Newman-Keuls test or Student's paired *t*-test.

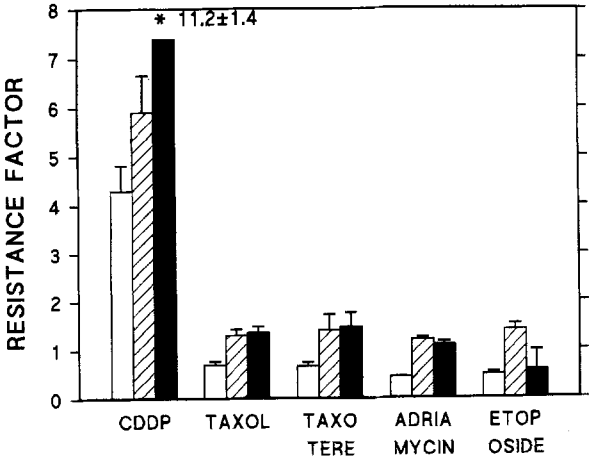


Fig. 2. Cross-resistance profile (96 h exposure) of 41M versus 41McisR (*open boxes*), CH1 and CH1cisR (*hatched boxes*) and OVCAR-3 and OVCAR-3carboR (*filled boxes*) to cisplatin (*CDDP*), taxol, Taxotere, Adriamycin and etoposide. Resistance factors = IC_{50} resistant line/IC_{50} parent line. Error bars = $\pm$SD ($n = 3-5$)

Results

The cytotoxicity of cisplatin, taxol, Taxotere, Adriamycin and etoposide as determined in the six parent human ovarian-carcinoma cell lines following continuous drug exposure (96 h) is shown in Table 1. Taxol (mean IC_{50}, 1.1×10^{-9} M) and Taxotere (mean IC_{50}, 5.1×10^{-10} M) were markedly more cytotoxic than either cisplatin (mean IC_{50}, 3.1×10^{-6} M), etoposide (mean IC_{50}, 2.3×10^{-6} M) or Adriamycin (mean IC_{50}, 6.9×10^{-8} M). In the individual cell lines, taxol, Taxotere and Adriamycin were significantly more cytotoxic than were cisplatin and etoposide ($P<0.05$). In addition, taxol and Taxotere were significantly more cytotoxic than was Adriamycin in all six lines ($P<0.01$). Although the panel of cell lines exhibited a wide range of sensitivity to cisplatin (71-fold; IC_{50} most resistant line/IC_{50} most sensitive line), etoposide (90-fold) and Adriamycin (29-fold), this was not apparent for taxol and Taxotere. The latter two agents produced a very narrow range of IC_{50} values across the panel of lines (5.6-fold range for taxol; 2.2-fold range for Taxotere). Overall, the cytotoxicity of Taxotere averaged 2- $\pm$1.1-fold > that of taxol across the six cell lines tested (range, from 3.9 orders of magnitude for HX/62 to 1.1 orders of magnitude for OVCAR-3). Although this difference did not reach statistical significance for any individual cell line (P values ranged from 0.17 for HX/62 to 0.9 for OVCAR-3), when all IC_{50} values for the 96-h experiments were pooled, Taxotere was found to be significantly more potent than taxol ($P = 0.05$).

Effects on cell lines exhibiting acquired resistance to platinum

The cross- resistance profiles obtained for the three pairs of platinum-resistant lines 41M and 41McisR, CH1 and CH1cisR and OVCAR-3 and OVCAR-3carboR using cisplatin, taxol, Taxotere, Adriamycin and etoposide are shown in Fig. 2. Resistance factors (RF; IC_{50} resistant

Table 2. Cytotoxicity of various agents in six parent human ovarian-carcinoma cell lines as determined following 2 h drug exposure

Cell line	IC_{50} (M)[a]			
	Cisplatin	Taxol	Taxotere	Adriamycin
HX/62	7.3×10^{-5} $\pm1.7\times10^{-5}$	2.1×10^{-8} $\pm1\times10^{-10}$	2.8×10^{-8} $\pm7\times10^{-9}$	2.7×10^{-6} $\pm1.5\times10^{-6}$
SKOV-3	3.3×10^{-5} $\pm1.5\times10^{-6}$	5.1×10^{-8} $\pm3\times10^{-8}$	7.7×10^{-9} $\pm4\times10^{-10}$	1×10^{-6} $\pm1\times10^{-7}$
PXN/94	2.1×10^{-5} $\pm3.2\times10^{-6}$	3.4×10^{-8} $\pm8\times10^{-9}$	1.2×10^{-8} $\pm4\times10^{-9}$	5.8×10^{-7} $\pm1.2\times10^{-7}$
41M	2.2×10^{-6} $\pm5\times10^{-7}$	5.9×10^{-8} $\pm1.8\times10^{-8}$	3.4×10^{-8} $\pm1.2\times10^{-8}$	1×10^{-6} $\pm1.9\times10^{-7}$
CH1	2.3×10^{-6} $\pm5.5\times10^{-7}$	1.1×10^{-7} $\pm7\times10^{-8}$	1.1×10^{-8} $\pm3.7\times10^{-9}$	1.9×10^{-7} $\pm2.5\times10^{-8}$
OVCAR-3	3.1×10^{-6} $\pm5\times10^{-7}$	1.3×10^{-8} $\pm4.3\times10^{-9}$	1.1×10^{-8} $\pm1.8\times10^{-9}$	2.6×10^{-6} $\pm2.3\times10^{-6}$

[a] Data represent mean values $\pm$SD ($n = 3-5$)

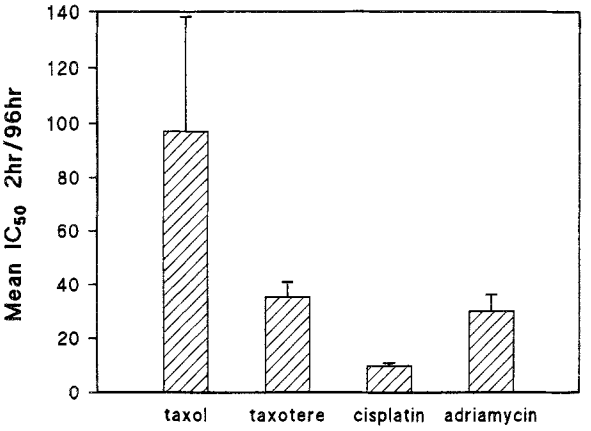


Fig. 3. Effect of concentration $\times$ time of exposure ($C\times T$) on the cytotoxicity of taxol, Taxotere, cisplatin and Adriamycin. Data represent the mean IC_{50} values (across the nine human ovarian-carcinoma cell lines used in this study) obtained using a 2-h period of drug exposure versus 96-h (continuous) drug exposure. Error bars = SD ($n = 3$ experiments)

line/IC_{50} parent line) found for cisplatin were 4.3 in 41McisR, 5.9 in CH1cisR and 11.2 in OVCAR-3carboR. Resistance in the OVCAR-3 line was generated using carboplatin (RF = 8). Both taxol and Taxotere (as well as Adriamycin and etoposide) failed to exhibit cross-resistance with acquired platinum resistance (RF values, <1.5) in all three lines. In the 41M pair of lines, a degree of collateral sensitivity (RF values, <1) was observed for taxol, Taxotere, Adriamycin and etoposide.

Effect of duration of exposure on cytotoxicity

The cytotoxicity values found for cisplatin, taxol, Taxotere and Adriamycin following a 2-h period of drug exposure are shown in Table 2 for the six parent lines. The table shows a pattern of response similar to that obtained following continuous exposure, but the IC_{50} values were higher for each drug. Taxol (mean IC_{50}, 4.8×10^{-8} M) and Taxotere (mean IC_{50}, 1.7×10^{-8} M) were significantly more cytotoxic than either cisplatin (mean IC_{50}, 2.2×10^{-5} M) or Adriamycin (mean IC_{50}, 1.3×10^{-6} M; $P<0.01$). Adriamycin was significantly more cytotoxic than cisplatin ($P<0.05$). In addition, the range of sensitivity observed across the six lines was wider for cisplatin (33-fold) and Adriamycin (14-fold) than for taxol (8.5-fold) or Taxotere (4.4-fold). Again, Taxotere was generally more cytotoxic than taxol (mean, 3.9 orders of magnitude; range, from 10 orders of magnitude for CH1 to 0.75 orders of magnitude for HX/62). Although Taxotere was found to be significantly more cytotoxic than taxol when all IC_{50} values obtained for the six cell lines were pooled ($P = 0.05$), among the individual lines, statistical significance was apparent only for the CH1 cell line.

The effect of the duration of drug exposure on the cytotoxicity of taxol, Taxotere, cisplatin and Adriamycin is shown in Fig. 3. For each drug, the ratio of IC_{50} values for 2-h/96-h exposures was calculated for each cell line, and an overall mean value was then determined. All four drugs exhibited statistically higher IC_{50} values across the six cell lines for 2- vs 96-h exposure periods ($P<0.01$ for taxol, Taxotere and Adriamycin; $P<0.05$ for cisplatin). Taxol showed the greatest $C\times T$ dependence, being an average of 97 times less cytotoxic after 2 h as compared with 96 h. However, there was a large degree of variability across the cell lines; individual cell-line ratios ranged from only 7.5 for HX/62 to 220 for CH1. Taxotere, Adriamycin and, especially, cisplatin showed a less pronounced $C\times T$ dependence than did taxol and exhibited markedly lower individual cell-line variability. Although these differences were statistically significant ($P<0.05$) for taxol versus cisplatin, Taxotere versus cisplatin and Ariamycin versus cisplatin, they did not reach significance for taxol versus Taxotere ($P = 0.18$), taxol versus Adriamycin ($P = 0.09$) or Taxotere versus Adriamycin ($P = 0.5$).

In addition, 2-h experiments using taxol and Taxotere were performed in the three lines exhibiting acquired resistance to platinum. RF values found for cisplatin were 5.9 in the 41M/41McisR pair, 5.5 in the CH1/CH1cisR pair and 20 in the OVCAR-3/OVCAR-3carboR pair. Following continuous exposure, collateral sensitivity to taxol and Taxotere was observed for the 41M pair of lines (RF values, 0.27 and 0.46, respectively). However, the agents were less effective (as compared with continuous exposure) in overcoming resistance in CH1cisR (RF values, 4.1 for taxol and 2 for Taxotere) and OVCAR-3carboR (RF values, 2.3 for taxol and 5.6 for Taxotere).

Discussion

The present study compared the cytotoxic properties of the semi-synthetic analogue of taxol, Taxotere, with those of taxol itself as well as the commonly used anticancer drugs cisplatin, Adriamycin and etoposide. Taxol has shown significant clinical activity in advanced ovarian cancer [12, 26, 39]. In six human ovarian-carcinoma cell lines, Taxotere was statistically significantly more cytotoxic than

taxol following both continuous (96 h) and brief (2 h) exposure (average, 2 and 3.9 orders of magnitude respectively). Moreover, following continuous exposure, Taxotere was significantly more cytotoxic than either cisplatin (average, 6,000-fold difference), etoposide (average, 4,500-fold difference) or Adriamycin (average, 135-fold difference).

The cytotoxicity of taxol in human leukaemia cell lines has been shown to be directly related to the taxol-induced formation of irreversible microtubule bundles [31]. In sensitive cell lines, these arrays of disorganised microtubules (often aligned in parallel bundles) were formed during all phases of the cell cycle. However, in taxol-resistant cells, microtubule bundling appeared to be reversible and cells remained unaffected during traverse through the G0/G1 and S phases of the cell cycle [31]. Thus, sensitive cells were critically affected during interphase, whereas resistant cells acumulated in the G2/M phase and formed multiple abnormal spindle asters [31]. The 2- to 4-fold difference in potency between Taxotere and taxol correlates closely with the respective ability of these agents to promote polymerisation of tubulin; in two studies, Taxotere was 2- to 5-fold more potent than taxol [2, 30].

The relative IC_{50} values obtained for taxol and Taxotere in the present study using 6 human ovarian cell lines are similar to those obtained in a recent study, in which IC_{50} values ranging from 1.6×10^{-10} to 3.1×10^{-9} M were determined for Taxotere in a panel of 12 mammalian cell lines using either clonogenic or MTT assays following a 24-h period of drug exposure [17]. In common with our findings, the cytotoxicity of Taxotere averaged 3-fold that of taxol and showed pronounced schedule dependency. In another in vitro study, Taxotere was 2.5 times more potent than taxol in the two murine cell lines J774.2 and P388 [30]; another noteworthy finding in this study was that Taxotere was >5 times more active then the parent drug in a taxol-resistant variant of the J774.2 cell line. In this study it was postulated that at least part of the increased potency of Taxotere over taxol might have been attributable to the greater water solubility of Taxotere as compared with taxol (47 vs 35 μM).

The comparative in vivo data available to date also indicate that Taxotere appears to be more active than taxol; using B16 melanoma, Bissery and co-workers [3, 4] obtained tumour log-cell-kill values of 3 for Taxotere and only 1.1 for taxol on equitoxic intravenous schedules [3, 4]. In another study using two human ovarian-carcinoma xenografts, both taxol and Taxotere exhibited higher antitumour activity than did cisplatin [14].

In three pairs of cell lines made resistant to platinum in the present study, both Taxotere and taxol failed to exhibit cross-resistance, producing RF values of <1.5. Previous studies have shown that the resistance of the 41M/41McisR and CH1/CH1cisR pairs of lines to cisplatin is due to contrasting mechanisms [22]. The resistance of 41McisR is predominantly attributable to a reduced accumulation of platinum through the plasma membrane, whereas that of CH1cisR occurs via a mechanism involving enhanced DNA repair of platinum-DNA adducts or through an increased tolerance to platinum-DNA adducts. The mechanism of platinum resistance in OVCAR-3carboR has not yet been determined. It is noteworthy that some collateral sensitivity (RF values, <1) to taxol and Taxotere (and to Adriamycin and etoposide) was observed in 41McisR. In addition, Taxotere (and taxol) virtually abolished the large (71-fold) range of cisplatin sensitivity observed across the six parent lines. These data therefore add some support to the clinical observations of taxol's activity in cisplatin-refractory ovarian cancer [26]. However, in another in vivo study using a cisplatin-resistant variant of murine L1210 leukaemia, Taxotere was inactive [4].

Although mechanisms of resistance to Taxotere have been little studied to date, investigations using taxol have shown two major mechanisms to be responsible for drug resistance, namely, transport-related and tubulin-related processes [8, 19, 32, 34]. In common with etoposide and Adriamycin, resistance to taxol has been associated with amplification of the 170-kDa membrane P-glycoprotein, which is responsible for the efflux of a broad range of anticancer drugs and is encoded by the multidrug resistance (*mdr*) gene in man [11]. In the murine macrophage-like cell line J774.2, selected for resistance to taxol, resistance was reported to be associated with increased levels of a 130-kDa membrane phosphoglycoprotein [19, 34]. A second major mechanism of resistance to taxol appears to involve alterations in α- and β-tubulin, resulting in impaired microtubule assembly [6].

A comparison of continuous and 2-h drug exposure revealed that the cytotoxicity of taxol and Taxotere were highly C × T-dependent as compared with that of cisplatin. Whereas taxol was an average of 97 times less cytotoxic and Taxotere, 35 times less cytotoxic after a 2- vs 96-h exposure period, the difference found for cisplatin amounted to only 9.9 orders of magnitude. Although the differences observed between the former two agents and cisplatin were highly statistically significant, the differences noted between taxol and Taxotere did not reach statistical significance (most of the difference being due to the CH1 cell line, which was >200 times less sensitive to taxol following a 2-h exposure period). In addition, CH1cisR and OVCAR-3carboR exhibited a small degree of cross-resistance to taxol and Taxotere along with their acquired platinum resistance following brief exposure periods.

These observations suggest that the in vitro activity of both taxol and Taxotere is highly schedule-dependent. Therefore, cytotoxic plasma concentrations may have to be maintained for several hours to ensure that maximal clinical antitumour activity is obtained. In addition, taxol has been shown to exhibit some schedule dependency in vivo [8]. In experiments using the P388 murine lymphoblastic leukaemia model, intraperitoneal doses of taxol given every 3 h for 1 day produced antitumour activity superior to that obtained using a single dose given on day 1 [increase in life span (ILS), 78% vs 0]; thus, these experiments also allude to the possibility of gaining an antitumour benefit using continuous exposure schedules. Interestingly, in in vivo experiments using colon adenocarcinoma 38, Taxotere did not show any marked schedule dependency [4]. The pharmacokinetic profile of taxol appears to be best characterised by a bi-exponential model,

with alpha and beta (elimination) half-lives of approximately 18 min and 5 h, respectively, being obtained [42, 43]. Peak plasma levels of around of 5 and 0.8 μM appear to be achievable following 6- and 24-h infusion schedules, respectively [8, 32, 42, 43]. It should be noted that these plasma concentrations of taxol (in patients) are substantially higher than the IC_{50} values obtained in the present study. Full details of the pharmacokinetics of Taxotere are not yet available.

In summary, these in vitro data suggest that Taxotere is approximately 2 times more cytotoxic than taxol and shows a lack of cross-resistance in cell lines exhibiting both acquired and intrinsic platinum resistance. To date, attempts to achieve the complete chemical synthesis of this important novel class of antimicrotubule agent have not been successful; structure-activity studies indicate that the taxane ring system plus the substitution of carbon 13 (whereby taxol contains 11 chiral carbons; see Fig. 1) are essential for cytotoxicity (see [32] for a review; [38]). Therefore, at present, Taxotere would appear to represent the most ecologically appropriate compound of this class of agent. Phase I clinical trials in Europe and the United States are currently testing Taxotere given as a 1-h infusion every 2 weeks [13], as a 6-h infusion every 3 weeks [40] and as a 24-h infusion every 3 weeks [5]. These studies should shed light on the possible schedule-dependent effects of Taxotere. Furthermore, the use of a combination of Taxotere and cisplatin, which is now being investigated in a phase I trial with taxol [33], might be a rational step in the future.

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