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Preclinical Activity of Taxotere (RP 56976, NSC 628503) Against Freshly Explanted Clonogenic Human Tumour Cells: Comparison with Taxol and Conventional Antineoplastic Agents

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Taxotere (TER) and taxol (TA) are new antitumour agents currently undergoing clinical evaluation. We studied the antineoplastic effects of these agents (final concentrations: 4.0, 0.4, 0.04 μmol/l) on the *in vitro* proliferation of clonogenic cells from freshly explanted human tumours using a capillary soft agar cloning system. We also compared the activity of these new compounds to conventional antineoplastic agents (bleomycin, cisplatin, dacarbazine, doxorubicin, etoposide, 5-fluorouracil, vinblastine, interferon-α_2). Using a 21–28-day continuous drug exposure, 54/81 specimens (67%) were evaluable for comparisons, and using a 1-h drug exposure followed by 21–28 days incubation, 50/80 specimens (63%) were similarly evaluable. With both schedules, TA and TER showed concentration-related antitumour activity. At 0.4 μmol/l, median colony survival was 0.61 × control (range 0.09–0.96) for TA and 0.51 × control (0.15–0.81) for TER in the 1-h incubation ($P = 0.0002$). Median colony formation was also reduced significantly more by TER as compared to TA in the long-term incubation schedule. Statistical analysis indicated that TER but not TA was significantly more active than cisplatin ($P = 0.02$), doxorubicin ($P = 0.01$), 5-fluorouracil ($P = 0.01$) and interferon-α_2 ($P = 0.01$). We conclude that TER and TA are more active against *in vitro* tumour colony formation from freshly explanted human tumours. TER appears to be slightly more active than taxol and promises to be active against tumours resistant to conventional antineoplastics.
Eur J Cancer, Vol. 29A, No. 14, pp. 2009–2014, 1993.

INTRODUCTION

TAXOL AND taxotere are new antitumour agents obtained either by extraction (taxol) from the bark of *Taxus brevifolia* or by semisynthesis (taxotere) from 10-deacetyl baccatin III, a non-cytotoxic precursor extracted from the needles of the European yew, *Taxus baccata* L. [1–3]. Both agents induce the formation of stable microtubule polymers and thus disturb the architecture of the cytoskeleton as well as the orderly progression through mitosis [4, 5]. Taxol has shown activity against cisplatin-refractory ovarian cancer, breast cancer, lung cancer and possibly

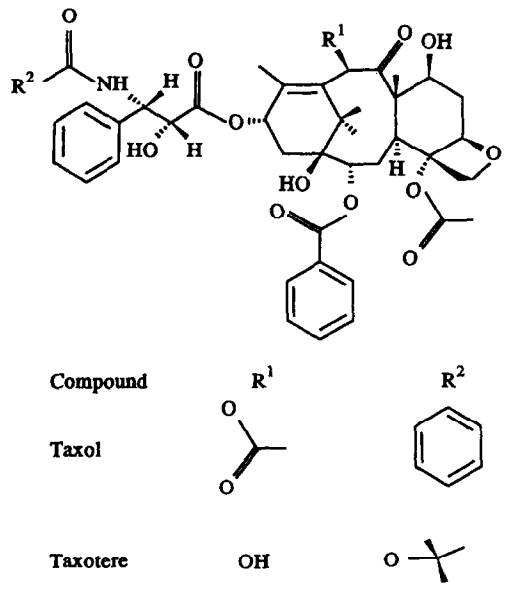

Fig. 1. Structural formulae of taxol and taxotere.

melanoma in clinical phase II studies [6, 7]. Similarly, taxotere has induced tumour responses in animals and in clinical phase I studies while phase II studies with taxotere are still ongoing [8–12]. In addition to their direct antitumour effects, *in vitro* studies indicate that both agents are potentially important radiation sensitisers [13, 14]. Taxotere appears slightly more active in the induction of tubulin polymerisation and in preclinical models [4, 15]. Using a soft agar proliferation system for freshly explanted clonogenic tumour cells and two different treatment schedules, we have compared the antiproliferative activity of taxotere with taxol and the activity of each of these compounds with a variety of clinically used antineoplastic agents.

MATERIALS AND METHODS

Compounds

Taxotere was provided as powder by the EORTC/PTMG (Preclinical Therapeutic Models Group) and Rhône-Poulenc Rorer S.A. (Antony Cedex, France). Taxol was provided by the NCI-EORTC liaison office through the EORTC/PTMG. The structures of taxol and taxotere are provided in Fig. 1. Stock solutions of 10 mg/ml were prepared in 96% ethanol, aliquoted and stored at −20°C prior to use. All further dilutions were made in sterile water in a stepwise fashion to give concentrations of 40, 4 and 0.4 μmol/l. Final concentrations were 4.0, 0.4 and 0.04 μmol/l. These concentrations represent approximately 1.0, 0.1 and 0.01 of the observed peak plasma concentrations in humans.

Other antitumour agents used for this project were clinical preparations and were used at final concentrations corresponding to 0.1 of their clinically observed peak plasma level. Stock solutions of 10 × final concentration for these agents were stored at −80°C prior to use. Dacarbazine was activated prior to use as previously described [16].

Human tumour cloning system

Tumour specimens were obtained by sterile standard procedures as part of routine clinical measures. Biopsies of solid tumours were stored in McCoy's 5A medium containing 10% newborn calf serum, 10 mmol/l HEPES, 90 U/ml penicillin, and 90 μg/ml streptomycin (all supplied by Gibco, Eggenstein, F.R.G.) for transport to the laboratory. Preservative-free heparin (10 U/ml, Novo, Mainz, F.R.G.) was added immediately after collection of fluids to prevent coagulation. Solid specimens were minced and repeatedly passed through metal sieves with mesh widths of 100 μm (Linker, Kassel, F.R.G.) to obtain single cell suspensions. Effusions were centrifuged at 150 *g* for 5–7 min. All specimens were suspended in McCoy's 5A medium containing 5% horse serum, 10% fetal calf serum, 2 mmol/l sodium pyruvate, 2 mmol/l glutamine, 90 U/ml penicillin, 90 μg/ml streptomycin and 35 μg/ml L-serine (all supplied by Gibco). If clumps of cells were detected by microscopic inspection the suspension was aspirated through 27 G needles and passed through metal sieves with mesh widths of 50 μm (Linker).

Soft agar tumour cloning experiments were performed in glass capillary tubes as described earlier [17–19]. Cells were plated at a median density of 5.6×10^4 cells/capillary (range 3.4×10^4–7.2×10^4) after a 1-h drug exposure for further incubation and similarly at 5.5×10^4 cells/capillary (range 3.2×10^4–6.4×10^4) for the 21–28 days drug exposure. Plating was performed using glass capillaries and a mixture of 0.3% agar in CMRL medium 1066 containing 90 U/ml penicillin, 90 μg/ml streptomycin, 0.1 mmol/l non-essential amino acids (all supplied by Gibco), 10^{-10} mol/l epidermal growth factor and 4 ng/ml hydrocortisone (Sigma, Deisenhofen, F.R.G.). Immediately prior to plating, HEPES (10 mmol/l final concentration; Gibco), glutamine (2 mmol/l final concentration; Gibco), asparagine (100 μg/ml final concentration) and sodium pyruvate (2 mmol/l final concentration) were added. Each experiment included a positive control with orthosodiumvanadate (10^{-3} mol/l final concentration; Sigma) to assure the presence of a good single-cell suspension [20]. Capillaries were incubated at 37°C, 5% CO_2 and 100% humidity. After 21–28 days, colonies were counted with an inverted microscope. An experiment was considered evaluable if the water control had ≥ three colonies/capillary and the positive control showed ≤ 30% colony formation compared to the negative (water) control. A decrease in tumour colony formation was considered significant if survival of colonies was ≤ 0.5-fold compared to the control.

Statistical analysis

Data were expressed as means and standard deviations of 6-fold determinations for each tested concentration and all controls. A significant inhibition was defined as colony formation ≤ 0.5 × negative control. For direct comparisons, an agent was considered more active than the other if colony formation differed by more than 0.1 × control. Statistical analyses were

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Table 1. Tumour types studied

Tumour type	No. evaluable/no. attempted (%) Continuous incubation	1-hour incubation
Renal	16/22 (73)	15/21 (71)
Colorectal	7/8 (88)	5/8 (63)
Gastric	8/14 (57)	8/14 (57)
Melanoma	7/8 (88)	8/9 (89)
Breast	3/4 (75)	3/4 (75)
Ovary	3/4 (75)	2/4 (50)
Urinary tract	3/3 (100)	2/2 (100)
Prostate	2/3 (67)	2/3 (67)
Lung, non-small cell	1/4 (25)	1/4 (25)
Lung, small cell	1/2 (50)	1/2 (50)
Oesophagus	1/2 (50)	1/2 (50)
Hodgkin's lymphoma	1/2 (50)	1/2 (50)
Gall bladder	1/1 (100)	1/1 (100)
Endometrium	0/1 (0)	0/1 (0)
Leiomyosarcoma (uterus)	0/1 (0)	0/1 (0)
Ewing sarcoma	0/1 (0)	0/1 (0)
Unknown primary site	0/1 (0)	0/1 (0)
Total	54/81 (67)	50/80 (63)

performed using the Wilcoxon signed rank test and the McNemar's test.

RESULTS

The effects of taxotere, taxol and clinically used antitumour agents were studied in a total of 93 tumour specimens.

Of the specimens tested in a 21–28-day (long-term) incubation, 12 had to be excluded from further analysis due to benign histology (2), bacterial or fungal contamination (8) and technical reasons (2). Thirteen specimens tested in a 1-h (short-term) incubation had to be excluded from further analysis because of benign histology (2), contamination (9) and technical failures (2). As summarised in Table 1, a total of 54/81 specimens (67%) were evaluable in the continuous incubation group. In the short-term incubation group, 50/80 specimens (63%) were evaluable.

After long-term incubation, median colony formation in water controls was 20.5 colonies/capillary. At 0.4 μmol/l, median colony survival for taxol was 0.57 × control (range 0.04–0.96) and for taxotere 0.43 × control (range 0.04–0.77; $P = 0.00001$, Wilcoxon signed rank test) (Fig. 2a). After short-term (1-h) incubation, median colony formation in water controls was 15.9 colonies/capillary. Median colony survival for taxol (0.4 μmol/l) was 0.61 × control (range 0.09–0.90) as compared to 0.51 × control (range 0.15–0.83) for taxotere (0.4 μmol/l; $P = 0.0002$, Wilcoxon signed rank test) (Fig. 2b). The antitumour activity of taxotere and taxol appeared to be largely independent of the schedule used. This was also observed in individual concentration–response curves (data not shown).

Tables 2 and 3 summarise the antitumour activity of taxotere and taxol on *in vitro* tumour colony formation after long-term and short-term incubation. Both agents had a profound concentration-dependent effect on the frequency of growth inhibition in long-term as well as in short-term incubation. In either case, taxotere was more active than taxol.

Table 4 shows the comparison between taxol, taxotere and clinically used antineoplastic agents. Taxotere was significantly more active than cisplatin, doxorubicin, 5-fluorouracil (1-h exposures) and interferon-α (21–28 days exposure) with a trend to superiority when compared with bleomycin and vinblastine (1-h exposure). Interestingly, taxol was not statistically more active than conventional antineoplastic agents.

In renal cell cancer, taxotere was significantly more active than interferon-α and slightly more active than bleomycin, but no difference could be detected when compared with vinblastine (Table 5).

Although the sample size for melanoma was more limited, none of the specimens was sensitive to any clinically used agent and at the same time resistant to taxotere (Fig. 3a). However, some specimens were sensitive to taxotere while resistant to conventional agents. Taxol, while having some activity, appeared to be slightly less active than taxotere. Similar results

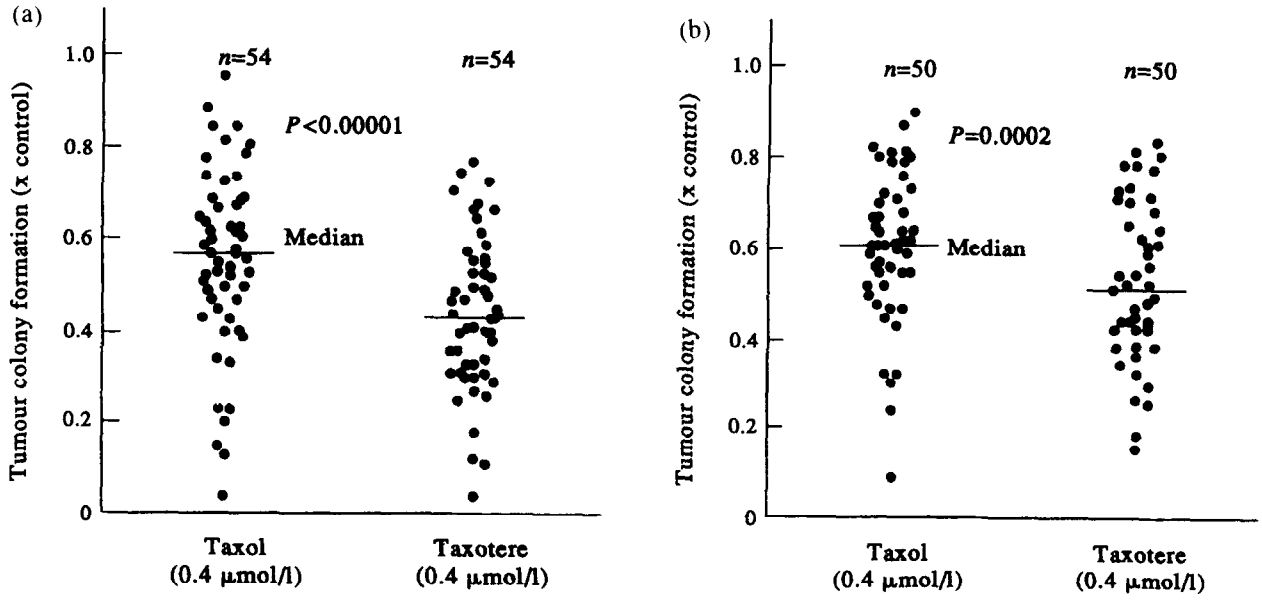

Fig. 2. Tumour colony formation after treatment with taxotere or taxol at 0.4 μmol/l. Taxotere was significantly more active than taxol. (a) Long-term incubation (21–28 days). (b) Short-term incubation (1-h).

Table 2. Concentration-dependent inhibition of tumour colony formation with long-term (21–28 days) incubation

Tumour type	No. of specimens with inhibition*/no. of specimens evaluable					
	Taxotere (μmol/l)			Taxol (μmol/l)		
	0.04	0.4	4.0	0.04	0.4	4.0
Renal	2/14	13/16	14/16	2/16	5/16	13/16
Colorectal	1/ 7	4/ 7	6/ 7	0/ 7	2/ 7	5/ 7
Gastric	1/ 8	5/ 8	7/ 8	1/ 8	2/ 8	5/ 8
Melanoma	1/ 7	4/ 7	6/ 7	1/ 7	3/ 7	5/ 7
Breast	1/ 3	3/ 3	3/ 3	1/ 3	2/ 3	3/ 3
Ovary	0/ 3	3/ 3	3/ 3	0/ 3	2/ 3	3/ 3
Urinary tract	1/ 3	2/ 3	3/ 3	0/ 3	1/ 3	3/ 3
Prostate	0/ 2	0/ 2	1/ 2	0/ 2	0/ 2	1/ 2
Lung, non-small cell	0/ 1	1/ 1	1/ 1	0/ 1	1/ 1	1/ 1
Lung, small cell	0/ 1	1/ 1	1/ 1	0/ 1	0/ 1	1/ 1
Oesophagus	0/ 1	1/ 1	1/ 1	0/ 1	0/ 1	0/ 1
Hodgkin's lymphoma	0/ 1	0/ 1	1/ 1	0/ 1	0/ 1	0/ 1
Gall bladder	0/ 1	0/ 1	0/ 1	0/ 1	0/ 1	0/ 1
Total	7/52 (13%)	37/54 (69%)	47/54 (87%)	5/54 (9%)	18/54 (33%)	40/54 (74%)

* Colony survival ≤ 0.5 × control.

Table 3. Concentration-dependent inhibition of tumour colony formation with short-term (1-h) incubation

Tumour type	No. of specimens with inhibition*/no. of specimens evaluable					
	Taxotere (μmol/l)			Taxol (μmol/l)		
	0.04	0.4	4.0	0.04	0.4	4.0
Renal	0/15	4/15	10/15	0/15	2/15	9/15
Colorectal	0/ 5	3/ 5	4/ 5	0/ 5	0/ 5	2/ 5
Gastric	0/ 8	5/ 8	7/ 8	1/ 7	2/ 8	5/ 8
Melanoma	1/ 8	4/ 8	7/ 8	0/ 8	3/ 8	5/ 8
Breast	1/ 3	3/ 3	3/ 3	1/ 3	2/ 3	3/ 3
Ovary	0/ 2	2/ 2	2/ 2	0/ 2	1/ 2	2/ 2
Urinary tract	0/ 2	2/ 2	2/ 2	0/ 2	0/ 2	2/ 2
Prostate	1/ 2	1/ 2	2/ 2	1/ 2	1/ 2	1/ 2
Lung, non-small cell	0/ 1	0/ 1	1/ 1	0/ 1	0/ 1	0/ 1
Lung, small cell	0/ 1	0/ 1	1/ 1	0/ 1	0/ 1	1/ 1
Oesophagus	0/ 1	0/ 1	1/ 1	0/ 1	0/ 1	0/ 1
Hodgkin's lymphoma	0/ 1	0/ 1	1/ 1	0/ 1	0/ 1	0/ 1
Gall bladder	0/ 0	0/ 1	1/ 1	0/ 1	0/ 1	1/ 1
Total	3/49 (6%)	24/50 (48%)	42/50 (84%)	3/49 (6%)	11/50 (22%)	31/50 (62%)

* Colony survival ≤ 0.5 × control.

Table 4. Direct comparison of the effects of taxanes with clinically used agents

Agent	Taxol (0.4 μmol/l)		Taxotere (0.4 μmol/l)	
	Sensitive*	Resistant†	Sensitive*	Resistant†
Bleomycin (0.2 μg/ml)	$P = 0.62$		$P = 0.07$	
Sensitive	2	1	3	0
Resistant	3	18	5	16
Cisplatin (0.2 μg/ml)	$P = 0.62$		$P = 0.016$	
Sensitive	4	1	4	1
Resistant	3	17	10	10
Doxorubicin (0.04 μg/ml)	$P = 0.62$		$P = 0.008$	
Sensitive	5	1	6	0
Resistant	3	15	9	9
5-Fluorouracil (6.0 μg/ml)	$P = 1.00$		$P = 0.008$	
Sensitive	2	1	2	1
Resistant	2	8	7	3
Vinblastine (0.05 μg/ml)	$P = 1.00$		$P = 0.114$	
Sensitive	2	3	3	2
Resistant	4	19	8	15
Interferon-α_2‡ (100 IE/ml)	$P = 0.18$		$P = 0.009$	
Sensitive	0	2	1	1
Resistant	7	13	11	9

* Number of tumours with colony formation ≤ 0.5 × control. † Number of tumours with colony formation > 0.5 × control. ‡ Long-term incubation. The two-by-two tables provide the sample sizes for tumours tested with taxol/taxotere and the specified clinically used agent. Statistical significance was determined for each comparison separately using the McNemar's test. Taxol was not significantly more active than any clinically used agent. In contrast, taxotere was significantly more active than cisplatin, doxorubicin, 5-fluorouracil and interferon-α_2. It was borderline more active than bleomycin.

Table 5. Antitumour activity of taxotere and taxol in renal cell cancer

Agent	Taxol (0.4 μmol/l) Sensitive*	Taxol (0.4 μmol/l) Resistant†	Taxotere (0.4 μmol/l) Sensitive*	Taxotere (0.4 μmol/l) Resistant†
Bleomycin (0.2 μg/ml)	P‡ = 0.48		P = 0.134	
Sensitive	0	0	0	0
Resistant	2	13	4	11
Vinblastine (0.05 μg/ml)	P = 0.48		P = 1.00	
Sensitive	2	2	2	2
Resistant	0	11	2	9
Interferon-α_2§ (100 IE/ml)	P = 0.45		P = 0.004	
Sensitive	0	2	2	0
Resistant	5	8	10	3

* Number of tumours with colony formation ≤ 0.5 × control. † Number of tumours with colony formation > 0.5 × control. ‡ McNemar's test. § Long-term incubation.

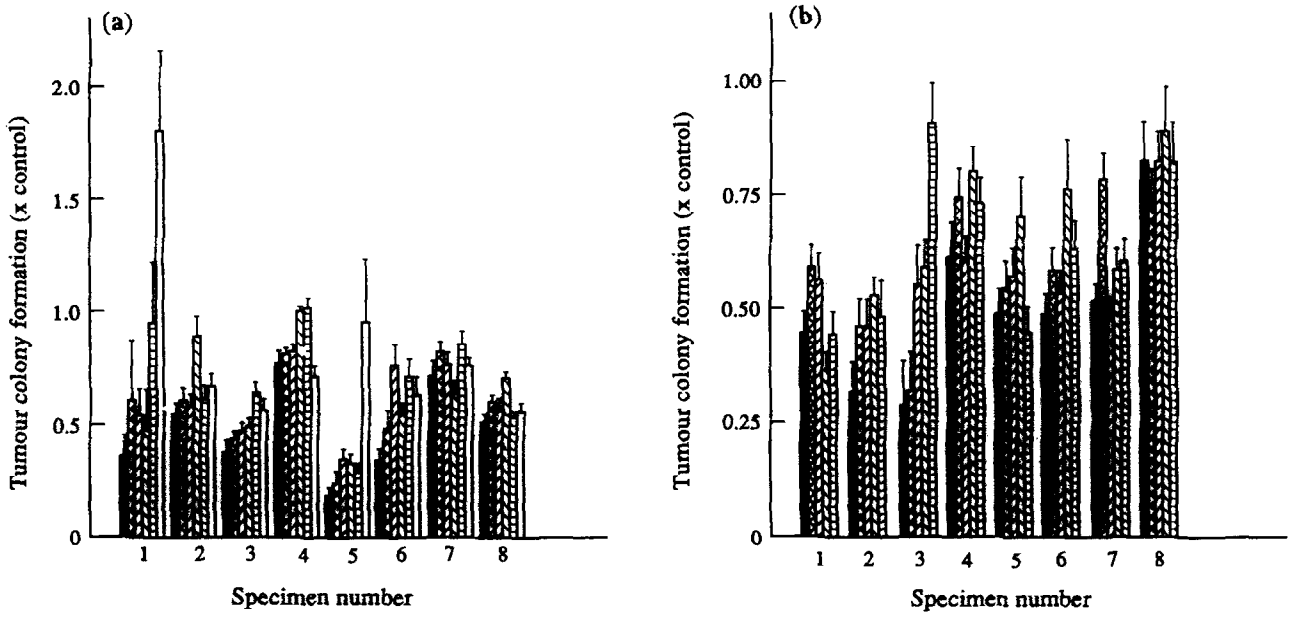

Fig. 3. Comparison of antitumour activity of taxol and taxotere with clinically used antineoplastic agents. Taxol was consistently slightly less active than taxotere. (a) Melanoma (n = 8). (b) Gastric cancer (n = 8). Columns from left to right—melanoma: taxotere (0.4 μmol/l), taxol (0.4 μmol/l), cisplatin (0.2 μg/ml), bleomycin (0.2 μg/ml), dacarbazine (2.0 μg/ml), vinblastine (0.05 μg/ml). Gastric cancer: taxotere (0.4 μmol/l), taxol (0.4 μmol/l), cisplatin (0.2 μg/ml), doxorubicin (0.04 μg/ml), etoposide (3.0 μg/ml).

were observed in eight evaluable gastric cancer specimens (Fig. 3b).

In direct comparisons using long-term incubation at a concentration of 0.4 μmol/l, 36/54 specimens (67%) were more sensitive to taxotere than to taxol (11 renal cell, six gastric, five colorectal, four melanoma, three breast, two ovarian and two ureteral, and one of each of prostate, oesophagus and small cell lung), while only two tumours (4%) were more sensitive to taxol than to taxotere (one each of renal cell and non-small cell lung). No difference in activity was observed in 16 tumour specimens (30%; four renal cell, three melanoma, two gastric, two colorectal, one of each of ureteral, prostate, ovarian, gall bladder and Hodgkin's lymphoma).

In direct comparisons using 1-h short-term incubation, 28/50 tumour specimens (56%) were more sensitive to taxotere than to taxol at a concentration of 0.4 μmol/l (seven melanoma, five renal cell, five gastric, three colorectal, two ureteral, two breast, one of each of ovarian, prostate, gall bladder and oesophagus), while four tumour specimens (8%) were more sensitive to taxol than to taxotere (two renal cell, one gastric, one breast). In 18 specimens (36%) no difference in antitumour activity was noted (eight renal cell, two gastric, two colorectal, one of each of ovarian, prostate, melanoma, Hodgkin's lymphoma, non-small cell and small cell lung cancer).

DISCUSSION

Our study has demonstrated that taxotere and taxol inhibit clonogenic growth of tumour cells from freshly explanted human tumours at clinically achievable concentrations. This activity appears to be largely independent of the schedule used, indicating that prolonged exposure is not required for sufficient antitumour activity. Schedule independency has also been reported by other investigators [12].

In both schedules, taxotere was statistically significantly more

active than taxol. This finding is in agreement with observations by other investigators. Barasoian *et al.* and the EORTC-Cologenic Assay Screening Study Group observed similar differences *in vitro* [21]. Ringel and Horwitz have reported a 2.5-fold higher potency in inhibition of cell replication of taxotere as compared to taxol [4]. Accordingly, taxotere has been reported to be twice as potent in inhibiting the cold or calcium-induced depolymerisation of microtubules [22]. In addition, taxotere is approximately 2-fold more potent than taxol at inducing tubulin polymerisation *in vitro* [23]. Recent evidence indicates that taxotere is not only more active than taxol *in vitro* but also that the optimal non-toxic dose for taxotere is slightly lower than for taxol *in vivo* [24]. Since there is a wide overlap of tumour responses to taxotere and taxol the clinical importance of this difference may be small.

The *in vitro* activity of taxol and taxotere reflects results obtained in early clinical studies. Brown *et al.* have observed partial responses in lung cancer patients treated with taxol [25]. Responses in non-small cell lung cancer and ovarian cancer were noted by Donehower *et al.*, and Wiernik *et al.* have reported responses in colon cancer and gastric cancer after treatment with taxol [26, 27]. Partial responses in pretreated breast cancer patients have been observed in a phase I study with taxotere [28]. However, our findings also indicate that taxotere, like taxol, does not have significant activity in notoriously drug-resistant tumours like renal cell cancer. After short-term incubation, the majority of tumour colony forming units of this tumour type was sensitive to taxotere only at concentrations that cannot easily be achieved in the clinical setting. However, at very high concentrations or after long-term incubation, more responses were observed.

When studied in direct comparisons, taxotere was significantly more active than a variety of potent antitumour agents. This suggests that taxotere may be a valuable agent for tumours refractory to conventional antineoplastics. Also, combinations of taxotere with other cytotoxic agents, preferably with non-overlapping toxicities, may hold clinical promise [29].

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