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Effects of Taxotere and taxol on *in vitro* colony formation of freshly explanted human tumor cells

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Taxotere (RP 56976, NSC 628503) is a new semisynthetic analog of taxol (NSC 125973) with promising antitumor activity in a variety of preclinical screening systems. Clinical responses after treatment with taxol have been observed in ovarian cancer, breast, lung cancer and melanoma. Both agents act through induction of microtubule polymerization. We have studied and compared the antiproliferative action of Taxotere and taxol against a variety of freshly explanted human tumor specimens using an *in vitro* soft agar cloning system. Final concentrations of 0.025–10 μg/ml were used for both agents in short-term (1 h) or continuous (14 days) incubations. Taxotere was studied using a 1 h incubation in a total of 167 tumor specimens of which 85 (51%) were evaluable. At 10 μg/ml, Taxotere inhibited 32 out of 78 (41%) specimens (colony formation $\leq 0.5 \times$ control). Cytotoxicity of Taxotere was observed against breast, lung, ovarian, colorectal cancer and melanoma tumor colony forming units. For comparison, 227 specimens were exposed to taxol for 1 h. At 10 μg/ml, 32 out of 97 evaluable specimens (33%) were significantly inhibited. Cytotoxicity was observed against breast, lung, ovarian, colorectal cancer and melanoma tumor colony forming units. In head-to-head comparisons, 29 specimens were found more sensitive to Taxotere than taxol, while only 13 were more sensitive to taxol than to Taxotere. These data indicate that cross-resistance between the two agents is incomplete and that on a concentration basis Taxotere is more cytotoxic than taxol in the majority of human primary tumor specimens evaluated.

Key words: Colony formation, taxol, Taxotere, tumor cells.

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Introduction

Taxotere (RP 56976, NSC 628503) is a semisynthetic analog of the diterpene taxol (NSC 125973), a natural product isolated from various species of yew.[1–5] Both agents are mitotic spindle poisons inducing uncontrolled tubulin polymerization and are active in preclinical murine screening models including P388, L1210 and B16 melanoma.[2,6–9] Taxol is also active against tumor xenografts such as LX-1 lung, CX-1 colon and MX-1 breast tumors.[10] Taxol has clinical activity in refractory ovarian cancer, breast and lung cancer as well as in melanoma, adenocarcinoma of unknown primary, and possibly gastric and colon cancer.[11–13] Taxotere is currently undergoing clinical phase I trials. It has been reported to be 2.5- to 5-fold more active against cancer cell lines *in vitro* compared with taxol.[2,14] *In vivo* sensitive mouse tumors include pancreatic and colon adenocarcinomas, melanoma, and lung cancer.[15] We report here the evaluation and the comparison of the *in vitro* cytotoxicity of Taxotere and taxol using a soft agar cloning system of freshly explanted human tumors.

Material and methods

Compounds

Taxol was kindly provided by the National Cancer Institute (Bethesda, MD). Taxotere was a gift from Rhône-Poulenc Inc. (Vitry-Sur-Seine, France).

Taxotere was dissolved in ethanol. Taxol was dissolved in a mixture of polyethoxylated castor oil and ethanol to an initial concentration of 6 mg/ml. Final concentrations of both compounds ranged from 0.025 to 10 μg/ml.

Human tumor cloning system

Tumor specimens (biopsies, pleural effusions or ascites) were obtained by sterile standard techniques as part of routine clinical procedures. Biopsies of solid tumors were stored in McCoy's 5A medium containing 10% newborn calf serum, 10 mM HEPES, 90 U/ml penicillin and 90 μg/ml streptomycin (all Gibco, Grand Island, NY) for transport to the laboratory. Preservative-free heparin (10 U/ml, O'Neill, Johns and Feldman, St Louis, MO) was added immediately after collection of fluids to prevent coagulation. Solid specimens were minced and repeatedly passed through metal sieves with 40 μm mesh (EC Apparatus, St Petersburg, FL) to obtain a single cell suspension. Effusions were centrifuged at 150 *g* for 5–7 min and passed through 25 g needles to obtain single cell suspensions when necessary. All specimens were suspended in McCoy's 5A medium (Gibco) containing 5% horse serum (HS), 10% fetal calf serum (FCS) (both Hyclone, Logan, UT), 2 mM sodium pyruvate, 2 mM glutamine, 90 U/ml penicillin, 90 μg/ml streptomycin and 35 μg/ml L-serine (all Gibco).

The human tumor cloning assay (HTCA) was performed using the two-layer system described by Hamburger and Salmon with several modifications.[16] Base layers contained 0.5% agar (Difco, Detroit, MI) in a mixture of McCoy's 5A medium as described above, 0.6% soy broth (Difco) and 100 μg/ml asparagine (Gibco). Cells were plated at a density of 5×10^5/dish in a 35 mm petri dishes (Corning) in a mixture of 0.3% agar in CMRL 1066 medium (Irvine Scientific) containing 15% HS, 2% FCS, 5 mg% vitamin C (Gibco), 90 U/ml penicillin, 90 μg/ml streptomycin, 0.1 mM non-essential amino acids, 2 mM glutamine (all Gibco), 2 U/ml insulin (Iletin I®, Eli Lilly), 2 μg/ml transferrin, 4 ng/ml hydrocortisone (both Sigma). Immediately prior to plating, HEPES (Gibco, 10 mM final concentration), asparagine (100 μg/ml final concentration) and sodium pyruvate (2 mM final concentration) were added. All determinations were done in triplicates. Each experiment included a control with orthosodiumvanadate (10^{-3} M, Sigma) to assure the presence of a good single-cell suspension (positive control).[17] Plates were incubated at 37°C, 5% CO_2, 100% humidity. After 14 days, colonies were counted with an inverted microscope. An experiment was considered evaluable when the water control had ≥ 20 colonies/plate and the positive control showed $\leq 30\%$ colony formation compared to the solvent control. An increase in tumor colony formation was considered significant if survival of colonies was ≥ 1.5-fold compared to the control. A decrease in tumor 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 triplicate determinations. Percent survival was calculated by expressing the average number of tumor colony forming units from taxol- or Taxotere-treated cells in percent of the average number of tumor colony forming units from untreated controls. A significant inhibition was defined as colony formation of $\leq 0.5 \times$ control.[18] Statistical analyses were performed using the χ^2 test for linear trend.

Results

The effects of Taxotere on colony formation were studied in a total of 176 tumor specimens (167 specimens with 1 h short-term incubation and 15 specimens with 14 days continuous incubation). The most common tumor types were lung, breast and ovarian cancer. Final concentrations ranged from 0.025 to 10 μg/ml. As shown in Table 1, Taxotere had a significant concentration-dependent effect on the frequency of growth inhibition. After short-term incubation, 11 out of 85 specimens were inhibited at 0.25 μg/ml as compared with 32 out of 78 specimens at 10 μg/ml ($p = 0.0005$). Although the sample size was more limited with continuous incubation, a similar trend was noted (data not shown). The majority of tumor specimens showed concentration-dependent response curves.

A total of 234 tumor specimens were studied for the effects of taxol on clonogenic proliferation *in vitro*. Of these, 227 specimens were exposed to the drug using a short-term incubation while 14 specimens were studied using a continuous incubation. Ten specimens were confirmed benign and were excluded from analysis. The major tumor

Table 1. Concentration-dependent inhibition of colony formation by Taxotere after short-term (1 h) exposure

Tumor type	No. of specimens with inhibition[a]/no. of specimens evaluable			
	0.025	0.25	2.5	10.0
	(μg/ml)			
Breast	0/1	0/20	3/20	6/19
Lung, non-small cell	0/3	0/11	1/11	3/8
Melanoma	0/0	4/9	5/9	6/9
Ovary	0/3	3/26	4/26	8/23
Colorectal	0/0	1/8	2/8	5/8
Other	0/0	3/11	2/11	4/11
Total	0/7 (0%)	11/85 (13%)	17/85 (20%)	32/78[b] (41%)

[a] Colony survival $\leq 0.5 \times$ control.
[b] $p = 0.0005$, χ^2-test for linear trend.

Table 2. Concentration-dependent inhibition of colony formation by taxol after short term (1 h) incubation

Tumor type	No. of specimens with inhibition[a]/ no. of specimens evaluable				
	0.1	0.25	1	2.5	10
			(μg/ml)		
Breast	1/5	0/19	1/5	1/19	7/23
Lung, non-small cell	0/2	1/10	1/3	1/10	4/10
Melanoma	0/1	1/9	1/3	4/9	8/10
Ovary	0/6	0/19	0/6	3/19	4/24
Colorectal	0/1	1/7	0/1	2/7	2/8
Other	0/10	2/12	0/10	3/12	7/22
Total	1/25 (4%)	5/76 (7%)	3/28 (11%)	14/76 (18%)	32/97[b] (33%)

[a] Colony survival $\leq 0.5 \times$ control.
[b] $p = 0.0005$, χ^2-test for linear trend.

types accrued were breast cancer, ovarian cancer, and lung cancer. Final concentrations of taxol ranged from 0.1 to 10 μg/ml. A clear, concentration-dependent inhibition of colony formation was observed (Table 2). Using a short-term incubation, one out of 25 tumor specimens was inhibited at 0.1 μg/ml as compared with 32 out of 97 tumors at 10 μg/ml ($p = 0.0005$). A similar effect was observed using the continuous incubation with none out of five specimens sensitive at 0.025 μg/ml as compared with five out of five sensitive tumors at 25 μg/ml (data not shown). The majority of tumors studied showed a concentration-dependent decrease in colony formation.

In 78 head-to-head comparisons, 29 specimens were more sensitive to Taxotere than to taxol (eight breast, seven ovarian, four colorectal, three non-small cell lung, three melanoma, two pancreas, one renal cell and one cervix) while only 13 tumors were more sensitive to taxol than to Taxotere (three non-small cell lung, three breast, three melanoma, two ovarian, one brain and one renal cell). No difference in activity was observed in 36 tumor specimens (10 ovarian, eight breast, six non-small cell lung, four melanoma, three colorectal, two pancreas and one each small-cell lung, renal cell and unknown primary site). These data indicate that cross-resistance of taxol and Taxotere is incomplete, and that on a concentration basis, Taxotere is more active than taxol in a subgroup of primary tumor specimens.

Discussion

Taxol is a polycyclic natural compound isolated from the bark of the Pacific yew *Taxus brevifolia*.[9,19] It has shown antitumor activity in preclinical murine tumor systems, as well as in human tumor xenografts. Clinical phase I studies have been recently completed and have shown antitumor activity in ovarian, lung, gastric, colon and breast cancers as well as in melanoma.[11–13] Currently, this agent is undergoing clinical phase II studies in a variety of tumor types.

Considerable efforts are directed towards synthesis of taxol or taxol-like compounds since the parent compound can presently be made available in only small amounts. Taxotere is a semisynthetic analog of taxol and is synthesized from its precurser 10-deacetyl-baccatin III which can be isolated from the leaves of *Taxus baccata*, a renewable source. It has antitumor activity in preclinical murine systems and is currently undergoing clinical phase I evaluation.[15,20]

In the present study, we have demonstrated that both taxol and Taxotere possess antiproliferative activity against freshly explanted human tumor specimens in a soft agar cloning system. Taxol is highly active at clinically achievable concentrations both with short-term or continuous incubations. Pharmacokinetic studies revealed peak plasma concentrations of taxol between 1 and 10 μM (0.9 and 9 μg/ml).[12,13,21] When tested at equimolar concentrations, Taxotere also showed considerable antitumor activity and was even more active than taxol on a molar basis in the majority of tumors studied. The most sensitive tumor types to Taxotere

were breast, lung and ovarian cancers as well as melanomas. Pharmacokinetic studies with Taxotere are ongoing and should help to put the concentrations we have tested *in vitro* into perspective.

The *in vitro* activity of taxol corresponds to the reported clinical activity of this agent. Wiernik *et al.*[12] have reported on antitumor effects of taxol in patients with colorectal and gastric cancer as well as in a patient with adenocarcinoma of unknown primary origin. In another clinical phase I study, Donehower *et al.*[11] have observed antitumor effects of taxol in a patient with non-small cell lung cancer and a patient with ovarian cancer. Most recently, our group has reported on a clinical phase I trial of taxol using a 6 h infusion schedule. In this study, partial responses were observed in four patients (two adenocarcinoma of the lung, one squamous cancer of the lung and one adenocarcinoma of unknown primary). One heavily pretreated patient is in partial remission for more than 24 months.[13]

Our data indicate that Taxotere and taxol have an overlapping spectrum of activity. As demonstrated in head-to-head comparisons in 78 specimens, Taxotere has superior activity in a subgroup of tumors and cross-resistance between these two agents was incomplete. Our data are in accordance with cell line studies showing that Taxotere is 2.5-fold more potent than taxol and 5-fold more potent in taxol resistant cells.[2] This may offer an additional clinical benefit for Taxotere. A comparison of our results with published clinical data indicates that soft agar cloning of freshly explanted human tumors can be of value for the prediction of clinical activity of taxol-related compounds and may be a helpful tool for the identification of potentially sensitive tumor types suitable for clinical phase II studies with Taxotere.

References

1. Wani MC, Taylor HL, Wall ME, *et al.* Plant antitumor agents: VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia. J Am Chem Soc* 1971; **93**: 2325–7.
2. Ringel I, Horwitz SB. Studies with RP 56976 (Taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 1991; **83**: 288–91.
3. Kaldor JM, Day NE, Hemminki K. Quantifying the carcinogenicity of antineoplastic drugs. *Eur J Cancer Clin Oncol* 1988; **24**: 703–11.
4. Mangatal L, Adeline M-T, Guéard D, *et al.* Application of the vicinal oxyamination reaction with asymmetric induction to the hemisynthesis of taxol and analogues. *Tetrahedron* 1989; **13**: 4177–90.
5. Denis J-N, Correa A, Greene AE. An improved synthesis of the taxol side chain and of RP 56976. *J Org Chem* 1989; **55**: 1957–9.
6. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly *in vitro* by taxol. *Nature* 1979; **277**: 665–7.
7. Parness J, Horwitz SB. Taxol binds to polymerized tubulins *in vitro. J Biol Chem* 1981; **91**: 479–87.
8. Schiff PB, Horwitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA* 1980; **77**: 1561–5.
9. Bender RA, Hamel E, Hande KR. Plant Alkaloids. In: Chabner BA, Collins JM, eds. *Cancer chemotherapy. principles and practice.* New York: JB Lippincott 1990; 253–75.
10. Douros J, Suffness M. New natural products under development at the National Cancer Institute. *Recent Results Cancer Res* 1981; **76**: 153–75.
11. Donehower RC, Rowinsky EK, Grochow LB, *et al.* Phase I trial of taxol in patients with advanced cancer. *Cancer Treat Rep* 1987; **71**: 1171–7.
12. Wiernik PH, Schwartz EL, Strauman JJ, *et al.* Phase I clinical and pharmacokinetic study of taxol. *Cancer Res* 1987; **47**: 2846–93.
13. Brown T, Havlin K, Weiss G, *et al.* A phase I trial of taxol given by a 6-hour intravenous infusion. *J Clin Oncol* 1991; **9**: 1261–7.
14. Lavelle F, Fizames C, Gueritte-Voegelein F, Guenard D, Polier P. Experimental properties of RP 56976, a taxol derivative. *Proc Am Assoc Cancer Res* 1989; **30**: 566. (Abstract).
15. Bissery M-C, Guénard D, Guéritte-Voegelein F, *et al.* Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. *Cancer Res* 1991; **51**: 4845–2.
16. Hanauske A-R, Hanauske U, Von Hoff DD. The human tumor cloning assay in cancer research and therapy. *Curr Prob Cancer* 1985; **9**: 1–50.
17. Hanauske U, Hanauske A-R, Marshall MH, *et al.* Biphasic effects of vanadium salts on *in vitro* tumor colony growth. *Int J Cell Cloning* 1987; **5**: 170–178.
18. Clark GM, Von Hoff DD. Statistical considerations for *in vitro/in vivo* correlations using a cloning system. In: Dendy P, Hill BT, eds. *Human tumor drug sensitivity testing in vitro.* New York: Academic Press 1983; 225–33.
19. Slichenmyer WJ, Von Hoff DD. New natural products in cancer chemotherapy. *J Clin Pharmacol* 1990; **30**: 770–88.
20. Extra JM, Rousseau F, Bourhis J, *et al.* Phase I trial of Taxotere (RP 56976, NSC 628503). *Proc Am Ass Cancer Res* 1991; **32**: 205 (Abstract).
21. Longnecker SM, Donehower RC, Cates AE, *et al.* High-performance liquid chromatographic assay for taxol in human plasma and urine and pharmacokinetics in a phase I trial. *Cancer Treat Rep* 1987; **71**: 53–9.

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