Anti-Cancer Drugs, 4, pp. 487–490

A saturation threshold for taxol cytotoxicity in human glial and neuroblastoma cells

L Helson, C Helson, S Malik, S Ainsworth[1] and J Mangiardi[2]

New York Medical College, Valhalla, NY 10595, USA. Tel: (+1) 914 285 8374; Fax: (+1) 914 993 4420.
[1]NaPro Biotherapeutics Inc., Boulder, CO 80301, USA.
[2]Lenox Hill Hospital, New York, NY 10021, USA

The cytotoxic effects of taxol at concentrations of 0.001–1.0 μg/ml were determined in two human glioblastoma multiforme, two neuroblastoma and two primitive neuroectodermal tumor cell lines. The neuroectodermal cell lines were established from previously treated patients, while the glioblastomas were from untreated patients. At exposure durations of 1, 4 and 24 h there was an inverse taxol concentration–survival relationship for all six cell lines as measured by the MTT method. Significant differences in sensitivity to taxol among these cell lines were observed; the most resistant cell line SK-N-FI is characterized by very high levels of MDR1 expression and the most sensitive SK-N-AS by very low levels. An additional level of complexity concerns a saturation threshold for taxol-induced cytotoxic effects which when reached precludes additional effects of prolonged or additional exposure. Tumors of the brain and peripheral nervous system appear to be sensitive to taxol. However, dosage necessary to maximize cytocidal effects in tumors requires knowledge of at least the range of each tumors constitutive sensitivity to taxol and a way to optimize drug delivery.

Key words: Glioblastoma multiforme, MTT assay, neuroblastoma, primitive neuroectodermal tumor, taxol.

Introduction

Taxol, isolated from the bark of the Western yew, *Taxus brevifolia*, is a complex diterpine which stabilizes tubulin and promotes assembly of unusually stable microtubules, microtubule bundling and abnormal spindle aster formation. This distinguishes it from other agents such as vincristine and estramustine which have microtubular destabilizing activity.[1,2] Once microtubules are stabilized, the cells lose their capacity to undergo normal dynamic reorganization of their microtubular network. This network is necessary for mitogenic signaling, intracellular transport of metabolites and movement of chromosomes associated with mitosis.

Neurotoxic effects of taxol after 24 h infusion have been described as an acute onset of tingling of the fingertips and toes.[3,4] These symptoms subsequently merged into a myalgia syndrome. Chronic effects included glove and stocking sensory neuropathy associated with loss of deep tendon reflexes, paresthesias, hyperalgesia and even fine motor skill diminution. Since replicating tumor cells of nervous system origin may exhibit even greater sensitivity to taxol than non-replicating neurons, we investigated the effects of taxol on human glial and neural tumor cell lines. We specifically addressed concentration and exposure durations necessary to maximize cytotoxicity.

Materials and methods

Cell culture

Taxol was added to glioblastoma multiforme cell lines VA-MG-SL and U-373-MG, neuroblastoma cell lines SK-N-AS, SK-N-FI and VA-N-BR, and two primitive neuroectodermal tumor cell lines SK-N-LO and SK-PN-DW at different concentrations.

All cell lines except U-373-MG (obtained from the American Type Culture Collection, Rockville, MD, USA), were established by C.H. They were all obtained from patients and cultured as previously described.[5] The cell lines SK-N-AS, SK-N-FI, SK-N-LO and SK-PN-DW have been reported in several publications[6–9] and are believed to be representative of their respective lineages.

Cytotoxicity assays

The cytotoxic effects of taxol were determined in tumor cells growing as attached monolayers. Stock

Correspondence to L Helson

cells in 25 cm^2 flasks were incubated in Dubelco's modified Eagle's medium (Sigma, St Louis, MO) containing 10% fetal bovine serum at 37°C and 5% CO_2 in air for 5 days. Media was renewed on the sixth day and on the seventh day the cells were detached into a single cell suspension with trypsin–EDTA, counted and aliquots plated at 3000–5000 cells in 0.1 ml fresh media in separate wells of a 96-well microtiter plate (Becton-Dickinson Labware, Lincoln Park, NJ). By 24 h the cells formed attached monolayers. Six replicate wells were used in every experiment as controls and for each test concentration. Each experiment was repeated three separate times. Viability of the cell lines 5 days after exposure to drugs was determined by the MTT assay following published procedures.[7] Taxol, obtained as a 98.2% purified product (NaPro BioTherapeutics Inc., Boulder, CO) was solubilized in 95% alcohol to 1000 μg/ml and further diluted with media to 0.1–20 μg/ml. Various concentrations of freshly diluted taxol were added to replicate wells in 0.1 ml volumes and removed after 1, 4 or 24 h. In a separate experiment we compared 0.3 μg/ml taxol added at 30 min intervals twice versus 0.6 μg/ml; both lines were washed free of taxol after a total of 1 h exposure.

To distinguish between taxol-induced growth inhibition and cytocidal activity, we compared MTT readings and direct viable cell enumerations at baseline and at 5 days after drug exposure of control and treated cells. Cytocidal activity was defined when the number of cells or the MTT surrogate optical density value was less than the initial inoculum. This is usually the case when ≥80% toxicity is recorded under these *in vitro* conditions.

Statistical analysis

The significance of the differences between the mean values of control and treated cells on the fifth day after exposure to taxol were determined using Students *t*-test. Differences were considered to be statistically significant for a *p*-value of 0.05 or less.

Results

Concentrations of 0.001–1.0 μg/ml taxol for 1–24 h caused a range of graded responses which remained consistent over three separate experiments (Table 1). SK-N-AS was the least resilient of the cell lines to taxol and SK-N-FI the most. The order of sensitivity to taxol exhibited by these cell lines remained consistent within the concentration range 0.01–1.0 μg/ml. The extent of toxicity following incubation for 1, 4 or 24 h with 0.01, 0.1 and 1.0 μg/ml taxol was similar at each concentration for the two glioblastoma, and the VA-N-BR neuroblastoma cell line. At 0.01 μg/ml taxol for 24 h the neuroblastoma SK-N-AS and the primitive neuroectodermal tumor cell line SK-N-LO exhibited enhanced cytotoxicity when compared with a 1 h exposure.

Table 1. Taxol toxicity

Cell line	Concentration	× 1 h	× 4 h	× 24 h
VA-MG-SL	0.1	58	56	58[b]
	1.0	87	90	86[b]
U-373-MG	0.1	46	56	57[b]
	1.0	74	79	79[b]
SK-N-AS	0.01	15	25	50[a]
	0.1	65	67	81[a]
	1.0	87	82	89[b]
SK-N-FI	0.1	0	0	14[a]
	1.0	0	58	76[a]
VA-N-BR	0.1	61	66	68[b]
	1.0	87	84	83[b]
SK-N-LO	0.01	0	0	17[a]
	0.1	43	56	47[b]
	1.0	54	58	57[b]

All values presented are the means of sextuplicate wells compared to untreated controls 5 days after exposure to taxol. Concentrations of taxol, 0.01 μg/ml were toxic in SK-N-AS and SK-N-LO at ≥1 and 24 h exposure, respectively. The remaining cell lines were affected only at concentrations ≥0.1 μg/ml.

[a] Significant differences ($p < 0.05$) comparing 1 and 24 h incubation.

[b] Toxicity ≤80% was growth inhibition relative to untreated controls after 5 days incubation. When ≥80% cytotoxicity was recorded, it was cytocidal in nature.

After exposure of SK-N-AS to 1 μg/ml taxol for 1 h, cytopathologic changes were characterized by apoptosis at 6–24 h, abnormal mitosis and polyploidy at 36–72 h, and death of cells between 72 and 144 h. Approximately 30% of the cells appeared to be altered by taxol at any of the time points (24, 48 and 72 h) of observation. Apoptosis was recognized as acute change in the cells. These include chromatin clumping at the periphery of the nucleus, loss of cytoplasmic detail and finally apoptotic bodies, either readily detached or floating with several being phagocytosed by surrounding cells.

The cytotoxic effects of a single exposure to taxol at 0.6 μg/ml/h in SK-N-AS were greater than when taxol was scheduled as 0.3 μg/ml given once and once again after 30 min (Table 2).

Table 2. Effects of spacing exposure to taxol on toxicity in SK-N-AS cells

Concentration	Survival (%)
6.0 μg/ml for 60 min	24 ± 4
0.3 μg/ml for 30 min × 2	61 ± 6

Discussion

While taxol-induced alteration of microtubules in murine dorsal root ganglia, fibroblasts, satellite cells, Schwann cells, oligodendroglia and human glial tumors in nude mice have been reported,[11,12] this is the first evidence that taxol also causes cytopathic effects and death of human neuroblastoma and primitive neuroectodermal tumor cells. These data are not surprising in view of taxol-induced neuropathy[3,4] and requirements for microtubule assembly and reorganization in replicating neural cells. The clinical use of taxol in primary highly malignant glial brain tumors lacking blood–brain barrier protection should be more effective than where individual glioblastoma cells have migrated to sites in the brain where drug penetration is limited.[13,14]

Of these six tumor cell lines, the neuroblastoma SK-N-FI exhibited the lowest sensitivity to taxol. Both SK-N-FI and the most sensitive SK-N-AS cell line were obtained from bone marrow metastases of heavily pre-treated patients. Both patients were treated with radiation cyclophosphamide, vincristine, cisplatin, etoposide, phenylalanine mustard and ditriazinoimidocarboamide. While SK-N-AS exhibits a minimal degree of multidrug resistance, SK-N-FI resistance expression is 14-fold higher by rhodamine expulsion and polymerase chain reaction quantitative determination.

Since determination of the growth rates of untreated and treated cells is dependent upon the size of their initial inocula and the degree of confluency in each well during the time of treatment, we standardized cell inocula and the incubation times in order to minimize these variables. We also compared Trypan blue viable cell enumeration daily with MTT values in replicate wells and found them to be sufficiently consistent to use either technique.

The similarity in quantitative cytopathic effects following taxol exposures of 1, 4 or 24 h at concentrations within the range 0.01–1.0 μg ml in three cell lines suggests that an upper limit of drug cytotoxic effect exists. When reached within a short time frame, this precludes additional drug-induced effects, suggesting for taxol, the notion of a saturable microtubular target. Assuming taxol-induced cytopathic effects are maximal at a specific level of microtubule alteration, then intracellular taxol concentrations exceeding these critical levels may not be effective. This hypothesis appears to be corroborated when comparing a single 1 h exposure of SK-N-AS cells to 0.6 μg/ml taxol with 0.3 μg/ml taxol at two 30 min intervals within 1 h. In cell lines such as SK-N-LO which is relatively insensitive to taxol saturation a 'threshold' would require the cells to be exposed for prolonged intervals in order to achieve the same effect. There is a body of evidence which suggests that within minutes of taxol entry into cells, changes in microtubule assembly and topological organization occur. In using the MTT assay we are in fact identifying a discrete subpopulation of surviving tumor cells which obviously exhibit lesser sensitivity to taxol than cells which died. This lack of homogeneity in taxol sensitivity of serially cultured tumor cells reflects differences in replicative activity as well as cytotoxic susceptibility and could explain why the 0.6 μg/ml impacts upon a broader cohort of the 'taxol-susceptible' cells than 0.3 μg/ml.

In conclusion, taxol may exert considerable cytostatic and cytocidal effects on neuroblastoma and glial cells. There appears to be a limited microtubule target which when fully occupied by taxol may not necessarily lead to cell death or even allow further cytotoxicity because additional factors controlling susceptibility to taxol appear to exist and are heterogeneously distributed among tumor cells.

References

1. Wall ME, Wani MC. Antineoplastic agents from plants. *Ann Rev Pharmacol Toxicol* 1977; **17**: 117–32.
2. Rowinsky EK, Cazenave LA, Donehower RC. Taxol: a novel investigational antimicrotubule agent. *J Natl Cancer Inst* 1990; **82**: 1247–59.
3. Wiernik PH, Schwartz EL, Winzig A, *et al.* Phase I trial of taxol given as a 24 hour infusion every 21 days: responses observed in metastatic melanoma. *J Clin Oncol* 1987; **5**: 1232–9.
4. Lipton RB, Apfel SC, Dutcher JP, *et al.* Taxol produces a predominantly sensory neuropathy. *Neurology* 1989; **39**: 368–73.
5. Helson L, Helson C. Establishment of a new cell line, VA-N-BR, from a primitive neuroblastoma tumor of the abdomen. *Anticancer Res* 1992; **12**: 467–72.
6. Sugimoto T, Tatsumi E, Kemshead JT, *et al.* Determination of cell surface membrane antigens common to both

neuroblastoma and leukemia lymphoma cell lines by a panel of 38 monoclonal antibodies. *J Natl Cancer Inst* 1984; **73**: 51–8.

7. Helson L, Helson C. Human neuroblastoma cells and 13-*cis* retinoic acid. *J Neuro-Oncol* 1985; **3**: 39–42.
8. Buck J, Brachelt G, Girgert R, *et al.* Specific uptake of iodine-125M iodobenzyl guanidine in the human neuroblastoma cell line SK-N-SH. *Cancer Res* 1985; **45**: 6366–70.
9. Helson L, Weinberger C, Helson C, *et al.* Neuroectodermal tumor and monoclomal antibodies. *Am J Pediatr Hematol Oncol* 1984; **6**: 227–31.
10. Carmichael J, DeGraff WG, Gazdor AF, *et al.* Evaluation of tetrazolium-based semi-automated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987; **47**: 936–42.
11. Masurovsky EB, Peterson ER, Crain SM, *et al.* Taxol effects on glia in organotypic mouse spinal cord-DRG cultures. *Cell Biol Int Rep* 1985; **9**: 539.
12. Riondel J, Jacrot M, Fessi H, *et al.* Effects of free and liposome-encapsulated taxol on two brain tumors xenografted into nude mice. *In Vivo* 1992; **6**: 23–8.
13. Helson L. Cephalomannine and 10-deacetyltaxol cytotoxicity in human glial and neuroblastoma cell lines. *Int J Oncol* 1993; **2**: 297–9.
14. Walter KA, Cahan M, Brem H. Taxol's effectiveness against malignant brain tumor cell lines. *Proc Am Ass Cancer Res* 1993; **34**: 383 (abstr).

(Received 10 May 1993; accepted 10 June 1993)