Characterization of paclitaxel (Taxol®) sensitivity in human glioma- and medulloblastoma-derived cell lines[1]

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Paclitaxel (Taxol®), a cytotoxic natural product that disrupts microtubule integrity, is being clinically evaluated for use against gliomas. We examined paclitaxel-induced killing in seven cell lines derived from human malignant astrocytic gliomas and medulloblastomas with the goal of characterizing range of sensitivity, contribution of P-glycoprotein 170–mediated drug efflux to resistance, and cross-resistance with alkylating agents. Exposure to paclitaxel for 8 h or less produced biphasic survival curves for all lines, with 40–75% of cells comprising a subpopulation that was 9–26 times more resistant to paclitaxel than the more sensitive fraction. Increasing exposure to 24 h eliminated the resistant subpopulation, increasing sensitivity 50- to 400-fold. The dose producing one log of kill (LD_{10}) after a 24-h exposure ranged from 4 to 18 nM, comparable to concentrations in the cerebrospinal fluid of brain tumor patients given a 3-h infusion of paclitaxel. Concurrent exposure to paclitaxel and either nimodipine or verapamil, inhibitors of P-glycoprotein activity, did not increase sensitivity, demonstrating that the fivefold range in sensitivity was not due to P-glycoprotein–mediated drug efflux. Importantly, there was no correlation between LD_{10} for paclitaxel and LD_{10} for 1,3-bis(2-chloroethyl)-1-nitrosourea, streptozotocin, and temozolomide, indicating no expression of cross-resistance to these different classes of tumoricidal agents. Our results suggest that greater clinical efficacy of paclitaxel against malignant brain tumors may be obtained by infusion for 24 h or longer and support the use of paclitaxel in combination with alkylating agents.
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The continuing poor prognosis for malignant brain tumors is, in part, a consequence of the lack of effective chemotherapy for newly diagnosed and recurrent disease. During the last three decades, the methylating agent procarbazine, and the chloroethylating agents BCNU[3] (carmustine) and CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; lomustine) have been the most commonly used tumoricidal drugs for malignant gliomas and medulloblastomas (Prados and Russo, 1998). The cytotoxicity of these agents is mediated by the formation of approximately 15 alkyl base adducts in DNA, including the lethal lesions O^6-alkylguanine and N^3-alkyladenine (Beranek, 1990; Ludlum, 1997). Unfortunately, intrinsic or acquired resistance limits clinical response to alkylators in most malignant brain tumors. In vitro evidence implicates DNA repair as one mechanism of resistance to alkylating agents. For example, numerous studies have shown that the DNA

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[3]Abbreviations used are as follows: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; D_{37}, dose required to reduce survival on linear portion(s) of survival curves by 63%; LD_{10}, dose that produces one log kill and thus reduces survival to 10%; P-pg, P-glycoprotein 170.

repair protein O^6-methylguanine-DNA methyltransferase limits alkylating agent–induced killing in human brain tumor–derived cell lines and xenografts (Bobola et al., 1995a, 1995b, 1996; Friedman et al., 1995; Schold et al., 1996) by removing lethal alkyl adducts from the O-6 atom of guanine (Pegg et al., 1995). Moreover, results from our laboratory (Bobola et al., 1995a, 1995b, 1996) indicate that brain tumor alkylating agent resistance is multifactorial, which will complicate the development of antiresistance therapies. In light of the limited efficacy of alkylators, new chemotherapeutic agents need to be evaluated for malignant brain tumors. Agents, the cytotoxicity of which is not mediated by DNA damage, are particularly attractive since their lethality will not be limited by DNA repair activities that restrict alkylating agent–induced killing.

Paclitaxel (Taxol®), one of a number of tumoricidal agents that target microtubules, has produced clinical responses against a number of solid tumors (Hajek et al., 1996), including tumors refractory to DNA-damaging agents (Ezcurdia et al., 1997; Gore et al., 1995; Ravdin, 1995; Woo et al., 1996). At stoichiometric concentrations, paclitaxel binds to αβ–tubulin heterodimers and suppresses normal microtubule remodeling necessary for cell cycle progression (Jordan and Wilson, 1998). The resulting kinetic stabilization of spindle microtubules promotes mitotic arrest in G_2-M (Rudner and Murray, 1996) with only subtle disorganization of the spindle. Blocked cells ultimately exit mitosis and undergo apoptosis. Paclitaxel-induced apoptosis, which is p53 independent (Vasey et al., 1996; Woods et al., 1995), is preceded by the activation of Raf-1, a protein kinase that functions in cellular proliferation and survival (Blagosklonny et al., 1996). Importantly, paclitaxel-induced Raf-1 activation promotes the phosphorylation of Bcl-2, inactivating its antiapoptotic activity (Blagosklonny et al., 1996; Wang et al., 1996), suggesting that Raf-1 and Bcl-2 monitor the integrity of spindle assembly and chromosome separation (Haldar et al., 1997).

There have been several studies of paclitaxel-induced killing in human and rat brain tumor–derived cell lines (Cahan et al., 1994; Gupta et al., 1997; Helson et al., 1993; Silbergeld et al., 1995; Terzis et al., 1997) and xenografts (Riondel et al., 1992). We have extended these previous reports by characterizing paclitaxel cytotoxicity in four human glioma- and three medulloblastoma-derived cell lines that differ in susceptibility to methylating and chloroethylating agents to assess the variability of sensitivity among brain tumor lines, determine the contribution of P-gp–mediated drug efflux to paclitaxel resistance, and compare paclitaxel and alkylating agent sensitivity.

Materials and Methods

Drugs

Paclitaxel was obtained from the Division of Cancer Treatment, National Cancer Institute, NIH, as a 6-mg/mL (10.5 mM) solution in 50% polyoxyethylated castor oil (Cremphor EL) and 50% absolute ethanol. Immediately before use, paclitaxel was diluted in sterile dimethylsulfoxide so that a constant volume was added at all doses. Nimodipine (Miles, West Haven, Conn.) and verapamil (Sigma, St. Louis, Mo.) were dissolved in absolute ethanol and sterile dimethylsulfoxide, respectively, at a final concentration of 800 μM. The final concentration of ethanol was no greater than 0.5%, while the final concentration of dimethylsulfoxide was 0.25%. These concentrations of solvents had no effect on cell growth or colony-forming ability.

Cell Culture

The medulloblastoma-derived lines UW228-1, UW228-2, and UW228-3 were established and characterized in our laboratory (Keles et al., 1995). The glioma lines SF763, SF767 (Berens et al., 1990), and U-373 MG (Ponten and MacIntyre, 1968) were from the American Type Culture Collection (Rockville, Md.). SNB19 (Gross et al., 1988) was provided by Dr. Richard Morrison, University of Washington. The CHO-derived lines $AuxB_1$ and CH^RC5 (Ling and Thompson, 1974) were the generous gift of Dr. Victor Ling, University of Toronto. The brain tumor lines were grown in Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum (HyClone, Logan, Utah), 2 mM L-glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin, whereas $AuxB_1$ and CH^RC5 were grown in α-minimum essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin. All lines were propagated as monolayers at 37°C in 5% CO_2 and 95% humidified air.

Determination of growth rate was carried out in 35-mm petri dishes inoculated with 2 mL of supplemented medium containing 1.5×10^4 cells. At 24-h intervals, medium was removed and saved, and cells were released by trypsinization. The cells were returned to the medium and pelleted by centrifugation at 800*g* for 5 min at 4°C. The pellet was resuspended in 1 mL of ice-cold phosphate-buffered saline, and the total cell number was determined using a hemacytometer. Ploidy and cell cycle characteristics of exponentially growing cells were determined by flow cytometry at the Flow Cytometry Laboratory, Department of Pathology, University of Washington.

Cytotoxicity Assay

Paclitaxel sensitivity was determined by clonogenic assay as described previously (Bobola et al., 1995a). Briefly, 12-well trays were inoculated with 2 mL of medium containing 750 cells and incubated overnight. The cultures were then incubated with paclitaxel for 4, 8, 24, and 48 h, were washed free of residual drug with phosphate-buffered saline, and were incubated in fresh, drug-free medium for 10–14 days. Colonies were fixed and stained with methanol containing 0.5% methylene blue to aid counting. Colony-forming efficiency (number of colonies formed/number of cells plated) ranged from 20 to 50%.

Paclitaxel sensitivity was determined by analysis of survival curves (log surviving fraction vs. dose) using standard methods (Harm, 1980). A curve-fitting program (CA-Cricket Graph III, Computer Associates,

Table 1. Taxol sensitivity of human glioma- and medulloblastoma-derived cell lines

	4 h			8 h			24 h		
Cell line	Fraction[a]	D_{37}[b] (nM)	LD_{10}[b] (nM)	Fraction[a]	D_{37}[b] (nM)	LD_{10}[b] (nM)	Fraction[a]	D_{37}[b] (nM)	LD_{10}[b] (nM)
Gliomas									
SNB19	0.50	55 ± 14	2524 ± 135	0.55	36 ± 10	1715 ± 35	1.0	7.7 ± 0.35	18 ± 2.6
	0.50	1410 ± 130	—	0.45	1170 ± 211	—			
U-373 MG	0.45	44 ± 10	798 ± 44	0.70	34 ± 4.0	362 ± 0.7	1.0	6.7 ± 1.6	15 ± 3.9
	0.55	438 ± 95	—	0.30	301 ± 46	—			
SF767	0.50	52 ± 12	1413 ± 35	0.55	43 ± 4.9	1135 ± 100	1.0	2.5 ± 0.16	3.9 ± 0.70
	0.50	895 ± 243	—	0.45	763 ± 88	—			
SF763	0.40	63 ± 15	1678 ± 35	0.45	43 ± 6.5	1442 ± 44	1.0	9.8 ± 0.93	13 ± 2.1
	0.60	944 ± 193	—	0.55	812 ± 30	—			
Medulloblastomas									
UW228-1	0.45	65 ± 4.7	1717 ± 97	0.70	59 ± 8.0	1098 ± 35	1.0	4.4 ± 0.35	10 ± 0.70
	0.55	930 ± 100	—	0.30	601 ± 121	—			
UW228-2	0.75	49 ± 15	1671 ± 25	0.65	36 ± 4.4	1160 ± 21	1.0	4.6 ± 0.35	10 ± 0.88
	0.25	561 ± 99	—	0.35	889 ± 216	—			
UW228-3	0.60	34 ± 2.8	1334 ± 62	0.75	20 ± 1.9	424 ± 0.7	1.0	4.6 ± 0.35	11 ± 0.70
	0.40	879 ± 163	—	0.25	452 ± 84	—			

D_{37} and LD_{10} were derived from survival curves, including those in Fig. 1, as described in MATERIALS AND METHODS.

[a]Proportion of total cells.

[b]Mean ± SD.

Islandia, NY) was used to generate a survival curve for each cell line from a minimum of three independent experiments (i.e., a minimum of nine determinations per drug concentration). Both linear curves, indicative of uniform drug sensitivity throughout the population, and biphasic curves, indicative of two subpopulations that differ in sensitivity, were observed. Paclitaxel sensitivity is reported as the parameters D_{37} and LD_{10}, which are derived from survival curves and are sufficient to reconstruct them. D_{37}, a measure of the rate of cell killing, is the drug concentration necessary to reduce survival by 63% on the exponential portion(s) of the survival curve. LD_{10} is the drug dose that reduces survival of the total population to 10%. For biphasic curves, LD_{10} reflects D_{37} for each subpopulation and the fraction of cells in each subpopulation. Graphical derivation of D_{37} and LD_{10} from survival curves is presented in detail in Bobola et al. (1995a).

Results

Time Dependence of Cytotoxicity

We assayed the paclitaxel sensitivity of seven human brain tumor–derived cell lines by quantitating survival of colony-forming ability after exposure of adherent, exponentially proliferating cells. Table 1 summarizes the survival parameters D_{37} and LD_{10} derived from survival curves such as those in Fig. 1. Notably, cytotoxicity increased for all lines with prolonged drug exposure. After incubation with paclitaxel for 4 h, all lines displayed biphasic survival curves, indicating two subpopulations that differ in sensitivity. The more resistant subpopulation comprised 25–60% of the total and had D_{37} from 9- to 26-fold greater than that of the more sensitive subpopulation (Table 1). Doubling the exposure time to 8 h still produced biphasic survival curves and had only a modest effect on killing, as evidenced by a 1.7-fold mean reduction of LD_{10} (range from 1.2- to 3.1-fold). For most lines, increase in cytotoxicity was limited by failure to appreciably decrease the fraction of more resistant cells and/or D_{37} in one or both subpopulations. In contrast, the twofold or greater decrease in LD_{10} for U-373 MG and UW228-3 (Fig. 1) was accompanied by a 1.5-fold or greater reduction in the fraction of more resistant cells and in the D_{37} for both subpopulations. Incubation with paclitaxel for 24 h, however, had a very pronounced effect on cytotoxicity. In all lines, the more resistant subpopulation was eliminated, producing linear survival curves. This, together with a 7- to 37-fold reduction in D_{37}, reduced LD_{10} from 52- to 365-fold compared with cells treated for 4 h. Interestingly, the relative sensitivity of the lines also changed as treatment was increased to 24 h. This transition is best illustrated by U373 MG, which had the lowest LD_{10} after treatment for 4 and 8 h but the second highest LD_{10} after exposure for 24 h (Table 1). Incubation with paclitaxel for 48 h did not enhance cytotoxicity (data not shown).

Correlation of Paclitaxel Cytotoxicity with DNA Content, Cell Cycle Fraction, and Growth Rate

Ploidy, cell cycle distribution, and doubling time for the cell lines are shown in Table 2. Regression analysis revealed no statistically significant correlations between LD_{10}, after 24-h exposure to paclitaxel and ploidy (r=–0.526), or fraction of cells in G_1 (r=0.0399), S

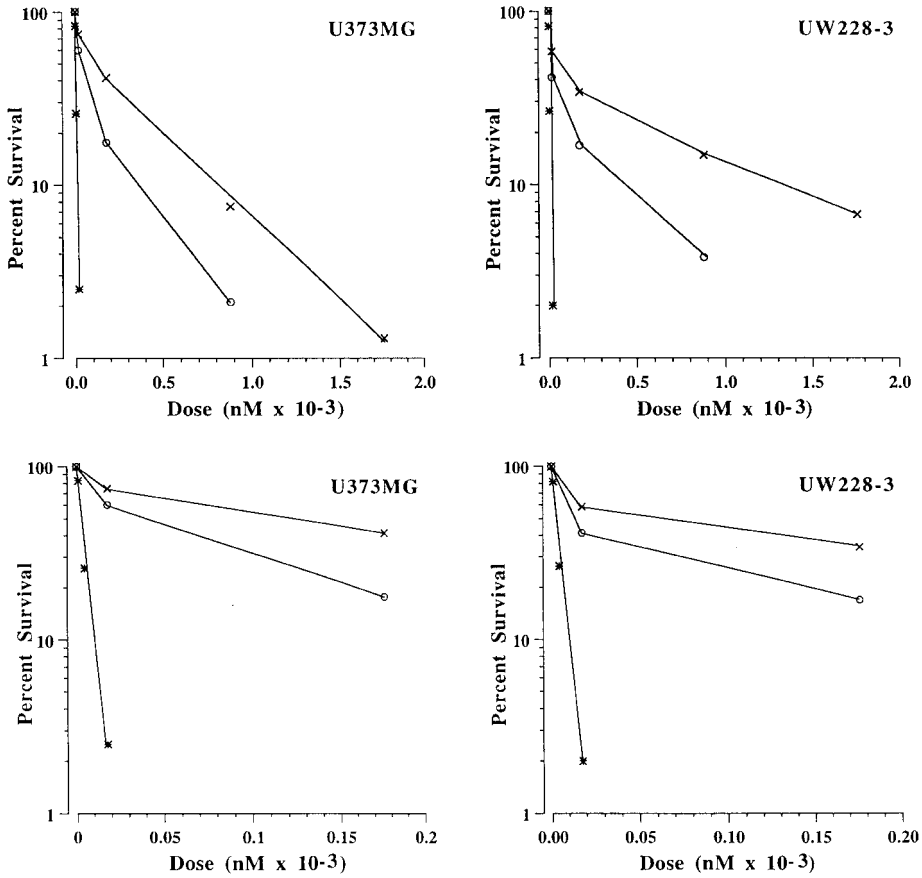

Fig. 1. Examples of paclitaxel-induced killing of human brain tumor cell lines. Each point is the average of three independent experiments (nine determinations for each dose). Percent survival is the quotient of the number of colonies at a given drug dose divided by the number of colonies for controls treated with solvent alone multiplied by 100. Standard deviations were <20% of the mean. U-373 MG and UW228-3 were derived from a glioblastoma and a medulloblastoma, respectively. O, 4-h exposure; X, 8-h exposure; S, 24-h exposure.

(r=–0.155), and G_2/M (r=0.267) phase. Interestingly, there was a statistically significant inverse correlation between LD_{10} and doubling time (r=–0.814, P=0.025). The correlation is exemplified by comparison of SNB19 and SF767, which differ fivefold in LD_{10} after 24-h exposure (18 and 3.9 µM; Table 1) and twofold in doubling time (17.7 and 33.4 h; Table 2). No correlation between LD_{10} and DNA content, cell cycle fraction, and doubling time was observed for cells treated for 4 or 8 h.

Potentiation of Cytotoxicity by Calcium Channel Blockers

Although increasing time of exposure greatly increased paclitaxel cytotoxicity, it did not eliminate differences in sensitivity among the lines. Drug efflux mediated by P-gp is a known mechanism of resistance to paclitaxel in human tumor-derived cell lines (Helson et al., 1993; Ise et al., 1996; Mechetner et al., 1998; Parekh et al., 1997) and could account for the differential drug sensitivity. Notably, P-gp–mediated resistance can be suppressed by calcium channel blockers, including verapamil and nimodipine (Ford and Hait, 1990). To examine directly the contribution of P-gp to the fivefold range in paclitaxel resistance, we treated the brain tumor cell lines for 24 h with paclitaxel in the presence of 2 µM verapamil or 2 µM nimodipine. As illustrated in Fig. 2 and summarized in Table 3, LD_{10} was not reduced appreciably with either calcium channel blocker. In contrast, both verapamil and nimodipine reduced LD_{10} seven- to eightfold in two CHO lines, $AuxB_1$ and CH^RC5 (Table 3), in which resistance to hydrophobic cytotoxic compounds such as paclitaxel is mediated by P-gp (Kartner et al., 1985). In accord, preliminary experiments revealed only faint or absent immunostaining for P-gp in the brain tumor lines, while CH^RC5 was strongly immunopositive (data not shown).

Comparison of Paclitaxel and Alkylating Agent Sensitivity

The LD_{10} of our brain tumor lines to the chloroethylating agent BCNU and the methylating agents streptozotocin, temozolomide, and *N*-methyl-*N*′-nitro-nitrosoguanidine, determined in earlier studies (Bobola et al., 1995a, 1995b, 1996), are shown in Table 4. The difference in LD_{10} among the seven lines varies from approximately 2.5-fold for streptozotocin to over 10-fold for *N*-methyl-*N*′-nitro-nitrosoguanidine, a range comparable to the fivefold range in paclitaxel sensitivity. Examination of Table 4 revealed no apparent relationship between sensitivity to

Table 2. Growth characteristics and DNA content

Cell line	Doubling time (h)	% G_1 phase	% S phase	% G_2 phase	Ploidy
UW228-1	25.2	47	43	10	1.6
UW228-2	33.4	49	27	24	1.7
UW228-3	29.0	51	34	15	1.7
SNB19	17.7	44	32	24	1.8
U-373 MG	22.7	50	32	18	2.1
SF763	33.4	49	33	18	2.8
SF767	23.2	20	37	23	1.9

paclitaxel and to any of the alkylating agents. In accord, regression analysis revealed no correlation between sensitivity to paclitaxel and susceptibility to BCNU, streptozotocin, temozolomide, or *N*-methyl-*N'*-nitro-nitrosoguanidine. The lack of correlation is exemplified by SF767 and UW228-1, which differ by threefold in their sensitivity to BCNU but have similar LD_{10} for paclitaxel.

Discussion

Despite advances in surgical technique, radiation therapy, and chemotherapy, the prognosis for primary brain tumors remains unfavorable (Berger et al., 1994; Prados and Russo, 1998). In vitro studies have indicated that the effectiveness of chloroethylating and methylating agent-based chemotherapies is limited by intrinsic and acquired resistance mediated, at least in part, by DNA repair activities (Bobola et al., 1995a, 1995b, 1996). Management is further complicated by diffuse infiltration of surrounding normal brain by tumor cells early in the course of the disease and by the recurrence of low-grade tumors as more aggressive high-grade neoplasms. Because most primary brain tumors recur within 2 cm of the tumor margin as seen on imaging studies (Wallner et al., 1989), agents that can control local recurrence after radiotherapy and alkylating agent-based chemotherapy will likely have a significant impact on survival. The clinically demonstrated activity of paclitaxel against a number of solid tumors (Hajek et al., 1996), including those resistant to DNA-damaging agents (Ezcurdia et al., 1997; Gore et al., 1995; Ravdin, 1995; Woo et al., 1996), suggests its use in the treatment of recurrent brain tumors.

We observed an approximately fivefold range of LD_{10} for paclitaxel among the seven brain tumor lines (Table 1), a variability comparable to that we previously observed for chloroethylating and methylating agents (Table 4; Bobola et al., 1995a, 1995b, 1996). The range and magnitude (4–20 nM) of LD_{10} after 24-h exposure to paclitaxel is comparable to that reported by Cahan et al. (1994) and Gupta et al. (1997) for five different human glioma and medulloblastoma cell lines. LD_{10} did not differ between glioma (12 ± 6.1 nM) and medulloblastoma (10 ± 0.58 nM) cell lines (mean ± SD for 24-h exposure), suggesting that both tumor types share common determinants of sensitivity. Importantly, the paclitaxel concentrations that produce one log of kill are comparable to those observed in the cerebrospinal fluid of brain tumor patients after a 3-h infusion of paclitaxel (Glantz et al., 1995).

All of the lines we studied showed biphasic responses after incubation with paclitaxel for 4 and 8 h, as evidenced by a subpopulation comprising 40–75% of cells that are 9- to 25-fold less susceptible to killing than the more sensitive subpopulation (Table 1 and Fig. 1). Increasing exposure to 24 h eliminated the more resistant subpopulation, reducing LD_{10} 50- to 400-fold compared with cells treated for 4 h. The presence of a more resistant fraction of cells, the size of which is dependent on

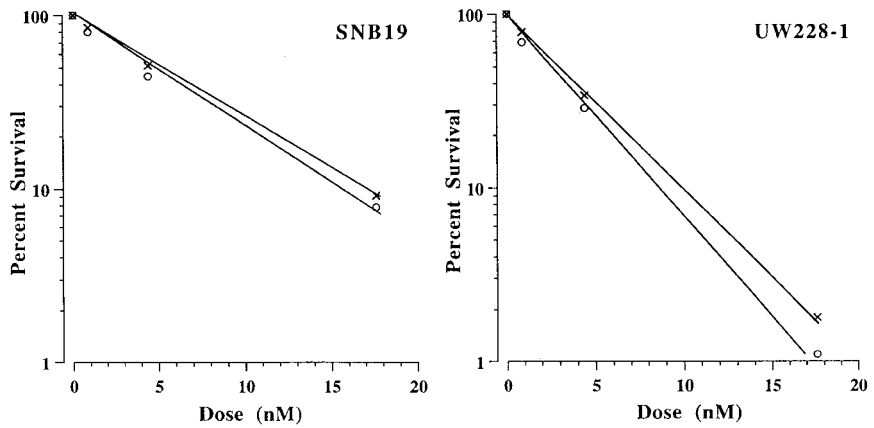

Fig. 2. Effect of calcium channel blockers on paclitaxel sensitivity of the glioblastoma line SNB19 and the medulloblastoma line UW228-1. X, paclitaxel alone; O, paclitaxel concurrently with nimodipine. Essentially identical results were obtained for paclitaxel with verapamil (data not shown). See legend to Fig. 1 for details.

Table 3. Effect of 2 mM nimodipine or verapamil on Taxol cytotoxicity

Cell line	LD_{10} (nM)[a]		
	Taxol	Taxol + nimodipine	Taxol + verapamil
Gliomas			
SNB19	18 ± 2.6	17 ± 2.8	15 ± 1.8
U-373 MG	15 ± 3.9	18 ± 3.2	18 ± 2.5
SF763	13 ± 2.1	13 ± 2.3	12 ± 2.3
SF767	3.9 ± 0.70	3.2 ± 0.18	2.8 ± 0.18
Medulloblastomas			
UW228-1	10 ± 0.70	9.0 ± 0.35	9.3 ± 3.3
UW228-2	10 ± 0.88	12 ± 2.5	12 ± 1.8
UW228-3	11 ± 0.70	12 ± 1.1	12 ± 0.70
CHO			
$AuxB_1$	54 ± 19	9.0 ± 1.8	9.0 ± 1.8
CH^RC5	6380 ± 2039	967 ± 141	931 ± 264

LD_{10} was derived from survival curves, including those in Fig. 2, as described in MATERIALS AND METHODS.

[a]Mean ± SD.

Table 4. Comparison of Taxol and alkylating agent sensitivities

Line	LD_{10} (mM)				
	Taxol	BCNU	STZ	TMZ	MNNG
SF767	0.0039	129	3000	973	9.4
UW228-1	0.010	197	5700	1037	22
UW228-1	0.010	117	3800	972	19
UW228-3	0.011	213	3800	1048	17
SF763	0.013	366	5300	789	11
U-373 MG	0.015	84	4900	1007	9.9
SNB19	0.018	49	2200	360	0.8

BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; STZ, streptozotocin; TMZ, temozolomide; MNNG, N-methyl-N′-nitro-nitrosoguanidine.

duration of paclitaxel exposure, has been reported for many human tumor cell lines (Baguley et al., 1995; Liebmann et al., 1993), including those derived from brain tumors (Cahan et al., 1994; Gupta et al., 1997; Helson et al., 1993; Silbergeld et al., 1995). The linear dose response and greater sensitivity displayed by our asynchronously growing lines treated for 24 h likely reflects a large majority of cells transiting G_2 and subsequently being arrested at the G_2/M interface and undergoing apoptosis. This mechanism is supported by the observation that nanomolar concentrations of paclitaxel do not effect progression through G_1 and S phases (Horwitz et al., 1986), and by the report that greater paclitaxel resistance in ovarian carcinoma cell lines is associated with longer doubling times (Baguley et al., 1995).

Paradoxically, we found a statistically significant inverse correlation between LD_{10} and doubling time for our lines (i.e., greater resistance is associated with faster growth). Conceivably, the more rapidly proliferating lines might express a compensatory resistance mechanism(s) that eliminates the expected greater sensitivity to paclitaxel associated with faster growth (Baguley et al., 1995). Drug efflux mediated by P-gp contributes to paclitaxel resistance in vitro and in vivo (e.g., Mechetner et al., 1998). Notably, focal regions of tumor cells immunopositive for P-gp have been detected in a minority of gliomas (Feun et al., 1994) and medulloblastomas (Tishler et al., 1992). Concurrent treatment with ≥100-fold molar excess of nimodipine or verapamil, inhibitors of P-gp–mediated drug efflux (Ford and Hait, 1990), had no appreciable effect on paclitaxel sensitivity, reducing LD_{10} no more than 40% (Table 3). In contrast, the same treatment decreased LD_{10} six- to sevenfold in the P-gp–expressing CHO lines $AuxB_1$ and CH^RC5. These results demonstrate that P-gp–mediated resistance does not account for the fivefold range of sensitivity or the inverse correlation between LD_{10} and doubling time among our lines. Other documented determinants of resistance to paclitaxel that might function in brain tumor cell lines include overexpression of the 190-kDa multidrug resistance protein (Lautier et al., 1996) and increased expression of tubulin isotypes (Jaffrezou et al., 1995; Kavallaris et al., 1997; Ranganathan et al., 1998) or mutated tubulin monomers (Giannakakou et al., 1997) that bind paclitaxel less avidly. Absence of Raf-1 also abrogates paclitaxel-induced apoptosis (Blagosklonny et al., 1996).

Resistance to one cytotoxic agent can be accompanied by decreased sensitivity to unrelated drugs (Jensen et al., 1997). For example, therapy with cis-platinum can produce resistance to other tumoricidal compounds (Yang and Page, 1995). We observed no correlation between sensitivity to paclitaxel and susceptibility to killing by chloroethylating and methylating agents, including BCNU and temozolomide, which are currently used in the chemotherapy of malignant gliomas (Prados and Russo, 1998). This suggests that glioma and medulloblastoma lines do not frequently express unrecognized mechanisms that could promote cross-resistance between paclitaxel and alkylating agents. In accord, gliomas recurring after alkylating agent–based chemotherapy are responsive to paclitaxel (Chamberlain and Kormanik, 1995, 1997).

The maximum tolerated dose and toxicity of paclitaxel in patients with recurrent malignant gliomas have been evaluated in phase I and phase II trials (Prados et al., 1996; Chang et al., 1998). Small prospective trials have reported modest efficacy when paclitaxel alone is given to patients with gliomas recurring after radiotherapy and alkylating agent–based chemotherapy (Chamberlain and Kormanik, 1995, 1997). Notably, in all brain tumor trials, paclitaxel was administered as a 3- to 4-h infusion, which has been observed to produce peak cerebral spinal fluid concentrations ranging from 5 to 83 nM 3–5 h after beginning infusion (Glantz et al., 1995). However, paclitaxel has a half-life in plasma of 4–6 h (Arbuck et al., 1993), which would reduce peak concentration 16- to 64-fold within 24 h, resulting in levels that are less than the LD_{10} for the brain tumor lines we studied (Table 1). Paclitaxel is metabolized in human liver by the cytochrome P450 isoform CYP3A4 (Marre et al., 1996). Notably, elevation of CYP3A4 activity has been observed in patients

treated with methylprednisolone (Monsarrat et al., 1998) or barbiturates (Royer et al., 1996), indicating that agents used to control edema and seizures associated with brain tumors may accelerate paclitaxel metabolism, reducing peak concentrations and half-life in plasma.

Our results suggest that the efficacy of paclitaxel against malignant brain tumors may be increased if it is given continuously for at least 24 h, either by infusion or interstitial implantation of a biodegradable polymer (Walter et al., 1994). Moreover, continuous infusion may permit maintenance of tumoricidal concentrations at lower doses, thereby reducing toxicity to the patient. Our observations have two other clinical implications. First, the apparent lack of cross-resistance between paclitaxel and alkylators suggests that use of both in combination may be more effective than using either alone, as observed for ovarian cancer treated with paclitaxel and cisplatin (McGuire et al., 1997). Notably, loss of p53, which is frequently observed in astrocytic gliomas, is associated with increased resistance to DNA-damaging agents but not to microtubule-disrupting agents (Iwadate et al., 1998). Second, paclitaxel may be effective against recurrent medulloblastomas for which there is currently no therapy that can produce long-term remission (Cohen and Packer, 1996). Further clinical trials are necessary to fully evaluate the potential of paclitaxel in the therapy of malignant brain tumors.

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