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Short communication

Cytotoxicity of taxol in vitro against human and rat malignant brain tumors

Mitchell A. Cahan[1], Kevin A. Walter[1], O. Michael Colvin[2], Henry Brem[1, 2]

[1] Department of Neurological Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA [2] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

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Abstract. Taxol is a novel antitumor alkaloid that has shown clinical activity against several tumors, including ovarian and breast carcinoma and melanoma. To evaluate taxol's potential as a therapy for malignant brain tumors, we measured the sensitivity of four human (U87, U373, H80, and D324) and two rat (9L, F98) brain-tumor cell lines to taxol. The cells were exposed to taxol in vitro using a clonogenic assay. Log cell kill (LD_{90}) occurred at concentrations of 42 (9L), 25 (F98), 19 (H80), 7.2 (U373), 9.1 (U87), and 3.9 n*M* (D324) when cells were continuously exposed to taxol for 6–8 days. The human cell lines were uniformly more sensitive to taxol than were the rat lines. The duration of exposure had a significant effect on taxol's cytotoxicity. When cells were exposed to taxol for 1 h the LD_{90} increased to 890 n*M* for the 9L rat line and 280 n*M* for the human U373 line. On the basis of these results, we conclude that taxol has significant potency in vitro against malignant brain tumors and that the activity occurs at concentrations of taxol that have previously been shown to be effective for several tumors against which the drug is currently being evaluated clinically.

Introduction

Taxol, a novel antitumor alkaloid that functions by promoting tubulin polymerization [6], has received extensive attention from both the scientific and the lay communities. Indeed, taxol was one of the most cited topics in oncology in 1992 [16]. The reason for this attention is that taxol has demonstrated significant activity in clinical trials against a number of human tumors, including ovarian and breast carcinoma and melanoma [12].

Correspondence to: Dr. Henry Brem, Johns Hopkins University School of Medicine, 817 Hunterian, 725 N. Wolfe Street, Baltimore, MD 21205, USA

There have been only a few reports, however, investigating taxol's possible role as a treatment for malignant brain tumors; it has been reported to inhibit the growth of human brain tumor xenografts implanted subscapularly in nude mice [9] and to act as a radiation sensitizer for gliomas in vitro [18].

Neither of these reports presents a quantitative analysis of brain-tumor cell sensitivity to taxol. However, such data are important for determining whether human brain-tumor cells are sensitive to taxol at concentrations that are physiologically attainable and that have correlated with good clinical efficacy in other types of tumors. Therefore, the purpose of the present study was to evaluate the cytotoxicity of taxol in vitro against four human (H80, D324, U87, U373) and two rat (9L, F98) malignant brain-tumor lines.

Materials and methods

Cell culture. Tumor sensitivity to taxol was measured by the clonogenic assay [10, 15] with rat glioma (9L, F98), human glioma (H80, U87, U373), and human medulloblastoma (D324) cell lines. Cells were grown and propagated in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, L-glutamine, penicillin, and streptomycin and incubated at 37°C in an atmosphere containing 5% CO_2. At the start of each assay, 600 tumor cells in 2 ml of medium were plated on Falcon 6-well tissue-culture plates (Becton-Dickinson, Lincoln Park, N. J.). After incubating for 24 h, the medium was removed from the plates and replaced with 2 ml of medium containing taxol and 0.1% dimethylsulfoxide (DMSO). The treatment solutions were prepared as described by Roytta et al. [14]. The taxol treatment solution was then either replaced with fresh taxol-free media containing 0.1% DMSO after 1 or 24 h or was left on the plates for the 6- to 8-day incubation period. At the end of the incubation period the plates were stained with a solution containing 0.63 g of Coomassie blue (Sigma), 125 ml of methanol, 87 ml of H_2O, and 38 ml of acetic acid. The colonies on each plate were counted and the result was expressed as a percentage of the colonies formed on control plates not exposed to taxol. Plating efficiencies for control plates ranged from 20% to 25%. A range of drug concentrations was applied to each set of cells. The percentage of cell kill values for the taxol treatments were plotted as a function of the drug concentration used. The concentration of drug necessary to produce 1 log of cell kill (LD_{90}) was interpolated

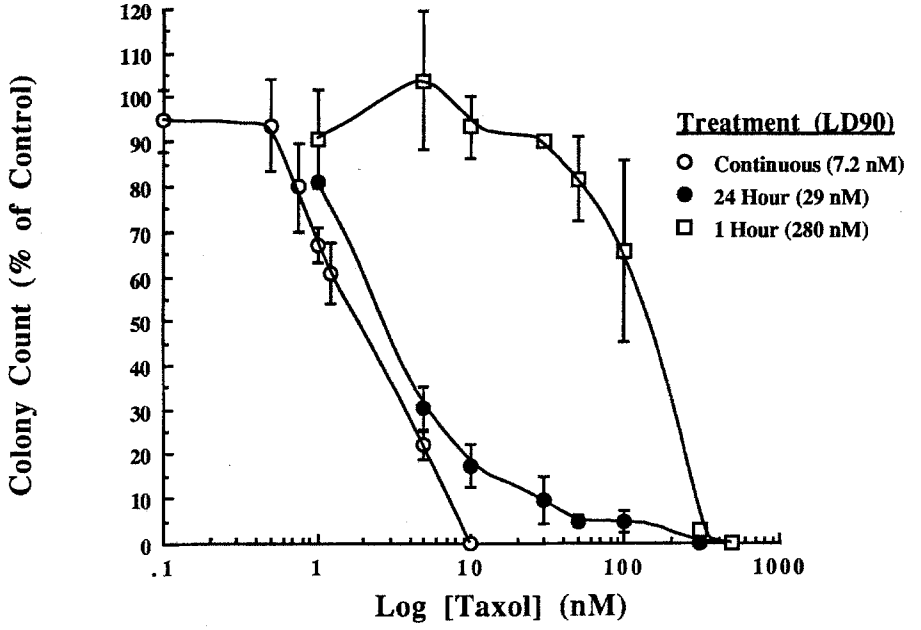

Fig. 1. Taxol cell kill as determined by clonogenic assay versus the human glioma U373 cell line with 1-h, 24-h and continuous (6- to 8-day) exposure to the drug. Increased duration of exposure to taxol decreases the amount of drug necessary to effect log cell kill (LD_{90}). Error bars represent standard deviations of triplicate measurements

Table 1. LD_{90} values for taxol against malignant brain tumor cell lines as determined by clonogenic assay

Treatment duration	LD_{90} concentration (n*M*) for					
	9L	F98	U373	H80	U87	D324
1 h	890		280			
24 h	100		29			
6–8 days	42	25	7.2	19	9.1	3.9

Values are means of triplicate measurements
Cells were exposed to taxol for 1 or 24 h or continuously for 6–8 days.

from the graph. Graphs were prepared and analyzed using Cricket Graph v 1.3.2 (Cricket Software, Malvern, Pa). Each determination was done in triplicate.

Chemicals. Taxol for these experiments, which was provided by the NCI (NSC 125973/55), was used without further purification and stored as a bulk solid at 4°C. A 1-m*M* stock solution of taxol in DMSO was prepared and kept at –20°C until thawed for use. F98 glioma cells carried in our laboratory for 5 years were initially provided by Dr. Joseph Goodman, Department of Neurosurgery, Ohio State University, Columbus, Ohio. 9L glioma cells carried in our laboratory for 8 years were originally obtained from Dr. Marvin Barker of the University of California, San Francisco, California. D324 (DAOY) [7] was provided by Dr. Henry Friedman, Division of Pediatric Hematology and Oncology, Department of Pediatrics, Duke University, Durham, North Carolina. U373, U87 [1], and H80 (U251) [3] were obtained from the American Type Culture Collection, Rockville, Maryland.

Results

The effects of taxol on colony formation in vitro in the rat glioma (9L, F98), human glioma (U87, U373, H80), and human medulloblastoma (D324) tumor lines are shown in Table 1. All of the cell lines were sensitive to the drug when exposed continuously to taxol for 6–8 days. Log cell kill (LD_{90}) occurred at values ranging between 3.9 (D324) and 42 n*M* (9L). The human tumor lines were uniformly more susceptible to taxol than were the rat lines. Log cell kill occurred at nanomolar taxol concentrations for three (U87, U373, H80) of the four human lines studied, whereas 4–10 times these amounts were required for the rat lines (9L, F98).

The duration of exposure to the drug significantly affected taxol's potency in vitro (Table 1). After exposure of cells to taxol for only 1 h, the LD_{90} increased by factors of more than 20 for the 9L line and 40 for the U373 lines as compared with the values recorded for the continuous (6- to 8-day) exposure. Cells exposed to taxol for 24 h gave LD_{90} values between those obtained for 1-h and continuous exposure. For example, for the human U373 line, Fig. 1 shows that the LD_{90} for 1-h exposure was 280 n*M*, that for 24-h exposure was 29 n*M*, and that for continuous exposure was 7.2 n*M* for the human U373 line.

Taxol has previously been shown to be stable in cell-culture medium [8]. It equilibrates with its equipotent epimer, 7-epitaxol, but undergoes insignificant (<10%) hydrolysis to inactive compounds. The potency of the taxol solutions should therefore not have diminished during the course of the 6- to 8-day incubation.

Discussion

The prognosis for patients with glioblastoma remains poor; the median survival from the time of diagnosis is less than 1 year. Although several chemotherapeutic regimens have been developed in recent years, including carmustine, PCV (procarbazine, lomustine, and vincristine), and others, the overall prognosis has not changed significantly. Thus, there is a need for promising new approaches and treatments. Since clinical studies have shown that taxol is effective against a number of tumors, including ovarian and breast

carcinoma and melanoma, we investigated the effects of this agent on brain-tumor cell lines in vitro [12].

We showed that taxol is highly potent in vitro against the rat and human brain-tumor cell lines examined. Log cell kill occurred at nanomolar concentrations of the drug, which is consistent with the reports of taxol's activity against other malignancies in vitro. For example, nanomolar concentrations of taxol have been found to be cytotoxic in vitro against ovarian, breast, lung, and prostatic cancer and melanoma [5, 13, 14]. Furthermore, taxol has demonstrated efficacy against each of these tumors in clinical trials [11, 12]. Brain tumors therefore appear to be as sensitive to taxol in vitro as other tumor lines that are currently being treated with taxol in clinical trials. Thus, taxol merits serious consideration as a new chemotherapeutic agent for malignant brain tumors.

We demonstrated that cell sensitivity to taxol concentration increased significantly with increasing duration of exposure to the drug (from 1 h to 1 week) in vitro. This finding is consistent with previous investigators' reports on taxol's action in vitro against other malignancies and is not surprising, considering taxol's cytotoxic mechanism [6, 13]. Taxol arrests the cell cycle during the late G_2 or M phase but does not slow cell progression through the preceding stages of cell replication [6]. Increasing the duration of exposure to taxol allows more cells in a given sample to enter the cell-cycle phases during which taxol is active [13]. With shorter periods of exposure to the drug, a greater proportion of cells exist entirely outside the taxol-sensitive G2 and M phases during the treatment interval.

To maximize the clinical efficacy of taxol, therefore, a drug delivery protocol that could maintain an elevated concentration of drug for an extended period would be desirable. To date, the protocols developed in clinical phase I trials have generally involved a single 1- to 24-h infusion repeated every 2–3 weeks or a 1- to 6-h infusion given once a day for 5 days. The elimination half-lives determined in these studies indicate that taxol is cleared relatively rapidly with an elimination half-life. $t_{1/2\beta}$, of between 1.3 and 8.6 h [12]. Since 93.5% of a drug is eliminated after four half-lives, most of the taxol is dissipated in these regimens at between 5 and 26 h after its administration. Our results indicate, however, that taxol's potency increases in vitro by a factor of 2–4 when cells are exposed to taxol for more than 24 h. To achieve the optimal cell kill in vivo, therefore, methods of extending the exposure of a tumor to taxol should be addressed.

One method of extending the duration of exposure of a tumor to a drug is to deliver the drug interstitially to the tumor [2]. Controlled-infusion pumps and biodegradable polymer devices are currently being developed to deliver drugs in such a sustained fashion to tumors of the central nervous system [17]. Furthermore, interstitial delivery minimizes the systemic drug levels and side effects of an agent. Delivering taxol locally to a tumor could therefore be an effective method of prolonging tumor exposure to taxol while minimizing taxol's dose-limiting systemic side effects, such as neutropenia.

Interstitial drug delivery also bypasses the limitations of the blood-brain barrier. Presently, it is unclear how well taxol crosses the blood-brain barrier. We have successfully used interstitial polymer delivery clinically with the antitumor agent carmustine (BCNU) and found that we could deliver it in active form for up to 3 weeks from a biodegradable polyanhydride polymer matrix while minimizing systemic exposure [2, 4].

Taxol is a novel chemotherapeutic agent that has produced clinical responses against several forms of human cancer. Herein we show that the in vitro sensitivities of human brain-tumor cells are similar to those of human tissues that have shown a clinical response to taxol.

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