Taxol Sensitizes Human Astrocytoma Cells to Radiation

Roy B. Tishler,1 Charles R. Geard, Eric J. Hall, and Peter B. Schiff3

Center for Radiological Research, Department of Radiation Oncology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Abstract

Taxol is a chemotherapeutic drug which acts by stabilizing microtubules, preventing normal mitosis and resulting in a block of the cell cycle at G2 and M. The drug is isolated from the yew, Taxus sp. L., and is currently being evaluated in a series of Phase II and Phase III clinical trials. Taxol blocks cells in the most radiosensitive phases of the cell cycle and thus could act as a cell cycle-specific radiosensitizer. We report the results of combined taxol-radiation exposures in the human Grade III astrocytoma cell line, G18. Taxol is a potent inhibitor of G18 cell division; a concentration of 10 nM is cytostatic for a cell population observed for at least two doubling times. Cell survival curves for G18 cells showed a significant concentration-dependent interaction between taxol and radiation. Treatment of G18 cells with a fixed taxol concentration and radiation dose showed the interaction to be dependent on the duration of taxol exposure and consequently the fraction of cells in the G2 or M phase of the cell cycle. The sensitizer enhancement ratio for 10 nM taxol at 10% survival is 1.8 and, for 1 nM taxol, it is 1.2. These results suggest that appropriate combinations of taxol have a more than additive interaction in human tissue culture and may have a role in clinical protocols.

Introduction

Taxol is the prototype drug of a new class of antineoplastic agents that targets microtubules. It is isolated from the bark of the western yew, Taxus brevifolia. Its complex structure and antitumor activity in rodents were described in 1971 (1). On a molecular level, the drug is a potent microtubule stabilizing agent and promoter of microtubule assembly in vitro (2), in contrast to agents such as colchicine and vinblastine which inhibit microtubule assembly. Tissue culture studies have shown that the cellular effect of taxol is to increase the fraction of cells in the G2 or M phase of the cell cycle (3). Taxol is known to be a potent cytotoxic agent against a range of human malignant cell types using cell culture and xenograph model systems (4), while studies in humans have demonstrated taxol’s ability to increase the mitotic index in a variety of tissues (5). Clinical trials have demonstrated that taxol is an active agent in salvage treatment for epithelial ovarian cancers (6–8) and that it has activity against breast cancer (9) and melanoma (10,11). Based on taxol’s established mechanism of action, in particular its effect on the distribution of cell populations in the cell cycle, and the known cell cycle stage-dependent sensitivity of cells to ionizing radiation (12–15), we hypothesized that taxol could function as a radiosensitizer. We tested this hypothesis in an astrocytoma cell line, where a combination such as this may have clinical application. In addition, this is a class of tumor where the role of taxol alone has not been extensively examined.

Materials and Methods

The human astrocytoma cell line (G18) was established in culture from a surgical specimen from the Neurological Institute of New York of the Columbia-Presbyterian Medical Center and has been shown to be relatively radioresistant (16). Cells were grown in modified Eagle’s medium with Hanks’ balanced salts (GIBCO) and 10% fetal calf serum (Hyclone) with 12.5 ml of SerXtend (Hana Biological) per 500 ml of serum. Medium was supplemented with modified Eagle’s medium-l-glutamine, essential amino acids, nonessential amino acids, vitamins, and gentamicin (GIBCO). During survival curve incubations, the medium was supplemented with penicillin and streptomycin. Cells were grown in plastic flasks with loosely capped tops, in incubators with 5% CO2, and subcultured 1–2 times per week at a 1:20 ratio.

For cell kinetic studies, G18 cells were allowed to attach to 60-mm dishes, and then taxol was added at different concentrations. Control cells were in exponential growth phase with a doubling time of approximately 24 h. At 48 h cells were trypsinized and counted.

Taxol (NSC 125973) was obtained from the National Cancer Institute drug program. A stock solution of 10 mM was prepared in DMSO and kept at −40°C until thawed for use. Cells were acutely irradiated with a 137Cs irradiator (Atomic Energy of Canada; Model GC40) at a dose rate of 1.12 Gy/min. Irradiation times ranged from 0.9 to 7.1 min.

For survival assays, cells were plated in 100-mm dishes with 10 ml of medium and allowed to attach for about 20 h. The number of cells per dish was chosen such that 100 to 200 colonies would survive after a specified treatment. Following an incubation period with a specified drug concentration (or DMSO alone), cells were acutely irradiated, medium was removed by aspiration, and 10 ml of medium were added back. Samples with unirradiated controls were also prepared in the same way. Dishes were then incubated for 10 days. Three plates were prepared for each concentration and dose of irradiation in triplicate experiments. Cells were fixed in 75% methanol/25% acetic acid and stained with crystal violet. Survival was determined from the number of cells per dish normalized by unirradiated controls treated with the same concentration of taxol or DMSO.

Cells for flow cytometry were washed with Hanks’ balanced salts, trypsinized, washed in normal medium, resuspended in cold methanol, and refrigerated. Cells were resuspended in 50 μg/ml of propidium iodide prior to flow cytometry using a Coulter EPICS 752 dual laser. Flow studies measured the number of cells versus DNA content and allowed for the determination of the fraction of cells in each phase of the cell cycle.

Results and Discussion

Significant morphological effects of taxol on astrocytoma cells have been demonstrated at these concentrations (17). G18 cell replication after a 48-h exposure to taxol was inhibited in a concentration-dependent manner (Fig. 1). Whereas untreated cells underwent two population doublings in 48 h (Fig. 1A), cell exposure to 1 nM taxol over this time period led to about...
taxol sensitizes human astrocytoma cells to radiation

Fig. 1. Effect of taxol on astrocytoma cell growth. In A, cells were plated into 60-mm dishes and allowed to attach. At time t = 0, there were ~2 x 10^6 cells per dish. The population doubling time was ~24 h. In B, data are presented for replicate experiments exposed to specified taxol concentrations for 48 h. □, control and taxol-treated cells; ●, DMSO alone.

one population doubling while 10 nM taxol exposure resulted in no overall increase in cell number (Fig. 1B). That is, it appears that a 10 nM taxol concentration completely impedes normal cell division.

The rationale for combining ionizing radiation and taxol therapeutically is that cells in the G2 or M phase of the cell cycle are relatively more radiosensitive than are cells in other phases of the cell cycle. This variation in cell cycle sensitivity has been demonstrated for a number of tissue systems (18). Sinclair (14) presented data showing how the shape of the radiation survival curve changed for cells in different phases of the cell cycle. Clonogenicity results for 24-h treatments with different concentrations of taxol alone, for radiation alone, and for combined treatments of taxol and radiation at 24 h are shown in Fig. 2A, while results relative to radiation alone are shown in Fig. 2B. Control plating efficiencies during the course of these experiments ranged from 53 to 72%. It can readily be seen (Fig. 2A) that taxol alone is dose dependently cytotoxic, with less than 0.1% clonogenic capacity at 100 nM, about 5% at 10 nM, and about 90% at 1 nM. Constant treatment with taxol (i.e., for the 10-day cell incubation period) again resulted in about 90% clonogenicity at 1 nM but less than 0.01% clonogenicity at 10 nM compared with 5% after a 24-h treatment (results not shown), indicating a time- and concentration-dependent mode of action. Previous work (17) with 10 nM taxol indicated that drug exposure times in excess of 8 h were necessary to elicit significant cytotoxicity. The relative increase in radiosensitivity is demonstrated by the change in the shape of the survival curves at higher taxol concentrations. This change is more clearly demonstrated in Fig. 2B, where the survival curves are normalized by the plating efficiency of cells treated with the same taxol concentration and without radiation, i.e., survival for taxol alone is defined as 100% for each of the curves. The relative decrease in survival for a given radiation dose is apparent.

Results obtained for acute radiation alone confirm the relative radioresistance of these human Grade III astrocytoma cells (16). Combined treatment studies indicate that radiation is relatively more cytotoxic as the concentration of taxol is increased (Fig. 2). The enhancement of the response to radiation by taxol, based on the cell cycle principles noted above, is seen in the concentration range where kinetic and cell cycle effects were observed. At the higher taxol concentration of 10 nM, the drug itself demonstrates a significant degree of cytotoxicity. For concentrations in the 1 nM range, there is no significant cell death without the radiation, but there is increased cytotoxicity for a given dose of radiation. Based on the terminology of Steele (19), taxol would thereby be classified as a radiosensitizer. The sensitizer enhancement ratio for 10 nM taxol at 10% survival is approximately 1.8 and, for 1 nM taxol, it is about 1.2.

Experimental data supporting the proposed mechanism of interaction between taxol and radiation are shown in Fig. 3. The longer cells are exposed to 10 nM taxol, the more cells accumulated in the G2 or M phase of the cell cycle (3). Plating efficiency decreases as the incubation time with 10 nM taxol increases (see Fig. 3A). As the number of cells in G2 or M increases, the relative sensitivity of the cell population to radiation also increases. Fig. 3B shows the time-dependent increase in cell sensitivity to radiation, which is similar to the time-

Fig. 2. Radiation survival curves with different concentrations of taxol. Clonogenicity for astrocytoma cells expressed as a function of taxol concentration and radiation dose. In A, survivals are expressed on an absolute scale, expressed relative to unirradiated controls without taxol. The effect of taxol alone on cell survival can be seen as the change in survival at 0 Gy. The interaction of taxol and radiation on the cells is demonstrated by the change in shape of the curves at higher taxol concentrations. □ and ○, untreated and DMSO-treated cells. There was no effect of DMSO alone. Treatment with 0.1 nM taxol also resulted in no difference from control (data not shown). In B, survival curves are presented with survival at a particular radiation dose expressed relative to unirradiated controls with the same concentration of taxol. This emphasizes the change in shape of the survival curves. Points, mean; bars, SE.
TAXOL SENSITIZES HUMAN ASTROCYTOMA CELLS TO RADIATION

Fig. 3. Cytotoxicity and enhancement of radiation sensitization as a function of time of exposure to 10 nM taxol. Cells were exposed to 10 nM taxol for the indicated time periods, and clonogenecity was assessed with and without 6-Gy irradiation. A, plating efficiency, or effect of taxol alone, on cell survival. For DMSO-treated controls, the plating efficiency is relatively stable, perhaps increasing slightly as a function of time, whereas for the taxol-treated cells, there is clearly a decrease in plating efficiency as a function of treatment time. B, survival of cells treated with 10 nM taxol and 6 Gy of radiation. Survival of irradiated cells is expressed relative to unirradiated cells treated with taxol for the same length of time. Therefore, the change in cell survival seen as a function of time of taxol exposure is a measure of the increased interaction between the two treatments. A relative decrease in cell survival and therefore an increase in the interaction between taxol and radiation measured at time (i.e., the data presented in Fig. 3A). Thus, these data represent the relative increase in the interaction between taxol and radiation. This effect therefore depends on the perturbation of the cell cycle distribution of cells and is not a result of direct interaction between the two modalities.

The potential for the combination of taxol and radiation as being clinically useful is based on a number of factors. First, the experiments presented here were performed at readily clinically achievable taxol levels, predominantly 10 nM or less. Clinical trials have shown that drug concentrations of 200 nM can be maintained for greater than 30 h using a 6-h infusion of 230 mg/m² of taxol (20). Peak concentrations of about 4000 nM were achieved in this trial. Regimens of this type have been used with acceptable levels of taxol-related toxicity. The proposed role of this combination in the treatment of CNS cancers will depend on the access of drug to tumor. Delivery of taxol to the CNS has not been investigated nor has the efficacy of the drug for the treatment of primary CNS tumors been examined. In terms of the applicability of the combination of radiation and taxol to other tumor sites, the distribution of drug may be somewhat more predictable since there is no barrier to drug access. The fact that the drug effectively gets into other body compartments is demonstrated by studies which showed an increased number of mitotic cells in normal tissues after taxol treatments (5).

We have documented that taxol is cytotoxic to a human astrocytoma cell line at relatively low drug concentrations. The data also show a potential beneficial effect by combining taxol and radiation, which could be pursued both in vitro and in clinical trials.

References

Taxol Sensitizes Human Astrocytoma Cells to Radiation


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