Anti-Cancer Drugs 1995, **6**, pp. 270–276

In vitro assessment of Taxol for human glioblastoma: chemosensitivity and cellular locomotion

Daniel L Silbergeld, Michael R Chicoine and Cynthia L Madsen

Department Neurological Surgery, Washington University School of Medicine, Campus Box 8057, 660 South Euclid Avenue, St Louis, MO 63110-1093, USA. Tel: (+1) 314 362-3457; Fax: (+1) 314 362-2107.

Although the antineoplastic efficacy of Taxol against a variety of tumors has been established, it has only recently been used for malignant brain tumors. We evaluated *in vitro* chemosensitivity of glioblastoma to Taxol and the affect of Taxol on glioblastoma cell locomotion. The clonogenic assay was used to evaluate the chemosensitivity of five human glioblastomas and the C6 rat glioma. Cells exposed to Taxol (0–250 nM) were suspended in agar in capillary tubes. Following incubation, colonies were counted to determine percent survival. All six cell lines demonstrated sensitivity to Taxol (LD_{50} 1 nM to >250 nM). However, even at concentrations exceeding those achievable clinically, all cell lines had surviving cells, indicating a saturation threshold for Taxol cytotoxicity. Cell locomotion was evaluated using the radial dish assay to determine the rate of egress of cells from a region of high cell density to the periphery. Increasing Taxol concentration caused increased locomotion in all six cell lines ($p < 0.0001$). Although Taxol has significant cytocidal impact, it increases *in vitro* locomotion of glioblastoma cells. These findings suggest that the clinical use of Taxol for glioblastoma may slow the growth of bulk disease, but may also lead to increased tumor invasion.

Key words: Cell locomotion, chemosensitivity, glioblastoma, motility, Taxol.

Introduction

Taxol® (Paclitaxel) is a new antineoplastic agent with proven antimitotic and antitumor activity against advanced and refractory ovarian, breast, lung, and head and neck cancers.[1–5] The drug was first extracted from the bark of the Pacific Yew tree (*Taxus brevifolia*) in 1963 by Wani and Wall.[6] However, the supply of this drug was initially limited due to restricted supply of the bark extract which required destruction of the tree for acquisition. Despite this, the National Cancer Institute (NCI) conducted a broad screening program to assess the efficacy of Taxol in the 1960s.[4] In the 1970s, Schiff *et al.* described the unique mechanism of Taxol's cytotoxicity.[7] Unlike other antimitotic agents, Taxol acts by promoting microtubule assembly into a meta-stable structure which the cell cannot disassemble.[8–10] Microtubules become arranged in parallel 'bundles' in G_0, G_1, S and early G_2 phases, or form abnormal mitotic 'asters' during the G_2–M interface that are enucleated without centrioles and can replace the normal bipolar mitotic spindles.[4] Another factor that slowed the use of Taxol was its relative insolubility, requiring the use of a Cremophor® EL vehicle which elicited a high rate of hypersensitivity reactions.[11] Pretreatment with steroids and antihistamines was found to greatly reduce the incidence of this side effect. Despite these problems, Taxol entered phase I clinical trials in the US in the 1980s. Currently, Taxol is successfully used for a number of systemic neoplasms and is undergoing phase II trials for malignant brain tumors.

Primary malignant brain tumors are rarely curable. It is the motile invading cells from these tumors, which cannot be surgically extirpated, that are responsible for tumor recurrence following radical resection,[12] and also these cells that, by unknown mechanisms, can lead to progressive neurologic dysfunction without evidence of mass effects of recurrence of bulk disease.[13,14] Although current treatments are aimed at tumor control within the region of bulk disease and/or the area of brain adjacent to the tumor, it is now clear that malignant brain tumors extend microscopically through much of the neural axis at the time of diagnosis.[15–18] Cytoskeletal assembly is required for cellular motility as well as mitosis. Because Taxol affects the cellular cytoskeletal system, we hypothesized that Taxol may affect brain tumor cell motility as well as having an anti-mitotic affect. Alterations in tumor cell egress from bulk disease could potentially affect

This work was supported in part by the McDonnell Center for Cellular and Molecular Biology Award 26275D (DLS) and KO8 NS01730 from the National Institutes of Health (DLS).

Correspondence to DL Silbergeld

© *1995 Rapid Communications of Oxford Ltd*

patients' neurologic status and length of survival.

Previous preclinical investigations of Taxol have demonstrated its antimitotic potency with a number of systemic cancers (leukemia, colon adenocarcinoma, mammary carcinoma, osteogenic sarcoma, melanoma, lung carcinoma, ovarian carcinoma).[1,5,19–23] We utilized the human capillary clonogenic assay to evaluate the susceptibility of a number of human glioblastomas to Taxol. A newly developed radial dish assay was used to evaluate the impact of Taxol on human brain tumor cell locomotion.

Materials and methods

Acquisition of human brain tumor specimens

Surgical specimens were obtained during routine craniotomies for resection of previously untreated supratentorial glioblastomas in adults. The extent of resection and standard neurosurgical craniotomy methods were not altered in any way for this investigation. Specimens were split, with one part sent for culture (described below) and one part for routine histopathological evaluation, including staining with hematoxylin and eosin (H&E) and immunocytochemical staining for glial fibrillary acidic protein (GFAP) expression (all tumors used expressed GFAP). Astrocytoma specimens were graded using a three tier system.[24–26]

Establishment of human malignant brain tumor cell lines

The techniques for culturing and maintaining cell lines of malignant brain tumors have been previously reported.[27–32] Briefly, fresh surgical specimens were transported from the operating room in sterile saline, minced to a slurry with crossed scalpels, centrifuged at 250 *g* at 4°C for 10 min, and resuspended in calcium- and magnesium-free Hank's balanced salt solution (HBSS) containing a triple enzyme mixture of 0.04% collagenase, 0.02% deoxyribonuclease and 0.2% neutral protease. The cell suspension was then incubated at 37°C for 45 min, washed twice in Dulbecco's modified Eagle's medium (DMEM) via centrifugation at 250 *g* at 4°C for 12 min, and resuspended in DMEM with 15% heat inactivated fetal calf serum (FCS), 0.2 mM glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml neomycin. Then 10 ml of cell suspension was plated in 75 ml Falcon T-75 tissue culture flasks at a density of 2.5–5 × 10^5 cells/ml. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and stored in a dimethylsulfoxide solution, in liquid nitrogen, for later use (viability after thawing and re-plating was typically 93–99%). All experiments used low passage cell lines ($P < 8$).

Clonogenic stem cell assay for determination of chemosensitivity profiles

The clonogenic assay evaluates the ability of tumor stem cells to form colonies in agar following exposure to a cytotoxic agent.[33] Two thirds of tumors found to be sensitive to a given cytotoxin *in vitro* ultimately respond to the agent clinically, whereas one-tenth of those predicted to be resistant show a clinical response.[34–41] Hence, tumor cell lines shown to be drug resistant with the clonogenic assay will probably not respond clinically; cell lines shown to be sensitive probably will respond.

The methodology used has been previously described.[34,35] Cells near confluency (mid-logarithmic growth phase) in T-75 flasks were washed with calcium- and magnesium-free HBSS, then exposed to 3 ml of trypsin solution (315 U/ml of trypsin activity and 0.2 M EDTA) for 60 s at 37°C. The cells were then resuspended in 7 ml of DMEM with 10% FCS and centrifuged at 250 *g*, 4°C, for 5 min, and resuspended in DMEM with 10% FCS at 2.5 × 10^5 cells/ml. The cells were then exposed to Taxol for 60 min at 37°C, then centrifuged at 250 *g*, 4°C, for 5 min. The supernatant was discarded and the cells washed with 1 ml of HBSS, via centrifugation at 250 *g*, 4°C, for 5 min, then resuspended in 0.9 ml of double-enriched DMEM (15% heat-inactivated horse serum, 2% heat-inactivated bovine serum, 2 units insulin/ml, 0.4 mM L-glutamine, 0.4 mM L-asparagine). To each 0.9 ml of cell suspension, 0.1 ml of 2.5% agar (Sea Plaque agar, FMC BioProducts, melted and then cooled to 37°C in a water bath) was added, and then loaded into capillary tubes, prepared using 100 μl pipettes (Clay Adams, #4625) cut to a length of 9.75 cm. Following capillary tube loading, the ends of the tubes were occluded with hematocrit clay, placed in labeled borosilicate tubes in a rack at a 45° angle and incubated at 37°C. On day 14, capillary tubes were removed from the incubator and the ends removed with a glass cutter. A piece of rubber tubing attached to a rubber bulb was fixed to a micropipette tip on a micropipette and the agar/cell suspension was gently blown onto a microscope slide and examined with light microscopy. Colonies (>50 cells and

>50 μm) were then counted and the data plotted to compare colony growth for each concentration of Taxol used.

Determination of *in vitro* cell motility utilizing the radial dish assay

A simple technique was previously developed in our laboratory[28] to specifically evaluate *in vitro* cell locomotion from a region of high cell density (as in bulky tumor) to a region of lower cell density (as in surrounding brain). Human brain tumor cells (2×10^4) suspended in 1 ml of feeding media (DMEM with 15% FCS) were plated in the center of an 8 cm diameter round Petri dish. After 12 h at 37°C, in a humidified 5% CO_2 atmosphere, the medium was discarded. A 2 cm circular zone of plated cells, in the center of the Petri dish, was thus established. The attached cells were washed and then fed with 10 ml of feeding media containing the specific substance(s) to be evaluated. The effect upon motility of various substances was evaluated by adding relevant concentrations to the feeding media.[28] Increasing serum concentration increased cell locomotion. Cytochalasin B, which blocks cytoskeletal assembly, halted cell movement. Fibronectin coating on the Petri dish surface did not alter cell locomotion. After initial experiments[28] demonstrated that hydroxyurea, a cell cycle inhibitor, had no effect upon brain tumor cell motility, hydroxyurea (10 mM) was added to all plates tested with the radial dish assay to maintain a constant number of cells per plate for the duration of each experiment.

Motility was determined by counting daily (in triplicate) the number of cells per random high power field (HPF, ×20) at predetermined distances from the perimeter of the central zone (0–1, 1–2 and 2–3 cm). The area of each concentric field of cells increases with increasing distance from the center of the Petri dish. To calculate the total number of cells in each concentric ring from the mean number of cells counted per HPF, the surface area of each ring (which increases with increased distance from the central zone) was used to determine cell density. The average distance traveled each day by the cells was computed by multiplying the number of cells (the mean of the three cell counts) in each concentric ring. Each count is 'weighted' relative to the area of its respective ring to determine cell density (weighted cell count = *WCC*). The central cell zone is not weighted (cell counts are multiplied by 1):

Area of the central cells = $A_{CC} = \pi r^2 = \pi(1\text{ cm})^2 = \pi$

Weighting factor$_{CC} = A_{CC}/A_{CC} = 1$

Each of the concentric rings are then weighted:

Area of 0–1 cm ring = $A_1 = (\pi(2\text{ cm})^2 - A_{CC}) = 4\pi - \pi = 3\pi$

Weighting factor$_1 = A_1/A_{CC} = 3$

WCC_1 = 3(mean cell count per ×20 field in the 0–1 cm ring)

Area of 1–2 cm ring = $A_2 = (\pi(3\text{ cm})^2 - (A_1 + A_{CC})) = 9\pi - 4\pi = 5\pi$

Weighting factor$_2 = A_2/A_{CC} = 5$

WCC_2 = 5(mean cell count per ×20 field in the 1–2 cm ring)

Area of 2–3 cm ring = $A_3 = (\pi(4\text{ cm})^2 - (A_1 + A_2 + A_{CC})) = 16\pi - 9\pi = 7\pi$

Weighting factor$_3 = A_3/A_{CC} = 7$

WCC_3 = 7(mean cell count per ×20 field in the 2–3 cm ring)

The *WCC* for each concentric ring multiplied by the distance traveled in centimeters, divided by the total number of cells counted, is the average distance traveled (*ADT*):

$$ADT = \frac{0\text{ cm} \times WCC_{\text{center}} + 0.5\text{ cm} \times WCC_1 + 1.5\text{ cm} \times WCC_2 + 2.5\text{ cm} \times WCC_3}{\text{Total } WCC_{\text{center+1+2+3}}}$$

Data were analyzed using the SAS PROC GLM as a randomized-block factorial type RBF design[42] with average distance traveled by the cells measured at intervals for each of the five concentrations of Taxol teste.

The kinetics of cell movement in the radial dish assay are mathematically most similar to directional (i.e. driven) movement. Directional movement is described by the equation $x = vt$ (x is the mean distance from the point of origin, v is the linear velocity and t is the time).[43] This is a linear relationship, as was found with the cell movement in the radial dish. Alternatively, isotropic random movement is described by the equation $\{x^2\} = 4Dt$ (x^2 is the mean square distance from the point of origin, D is the diffusion coefficient and t is the time)[43] which is non-linear. The directional movement in the radial dish assay is most likely driven by the higher cell density in the central zone compared with the periphery. Therefore, tumor cell density alone is an important factor in tumor cell locomotion.

Table 1. Capillary clonogenic assay chemosensitivity testing results

Taxol concentration (nM)	C6	WU17	WU18	WU20	WU29	WU31
0	100	100	100	100	100	100
0.25	81	90	88	78	72	63
0.5	74	92	77	66	70	58
1.0	52	71	58	52	64	45
10	43	67	58	51	48	43
100	26	53	43	41	45	29
250	30	56	37	10	43	24

Numbers represent the percent of cells surviving (i.e. forming colonies in agar) with escalating concentrations of Taxol. At higher concentrations, a plateau of cytocidal impact is reached where additional drug does not decrease cell survival.

Results

Capillary clonogenic assay

Table 1 shows the surviving fraction of cells (i.e. those cells that formed colonies) following exposure to increasing concentrations of Taxol. Although all cell lines demonstrated sensitivity to Taxol, the degree of sensitivity varied along the six cell lines. Even at high Taxol concentrations, all cell lines had surviving cells, indicating a saturation threshold for Taxol cytotoxicity. Tumor cells exposed to higher concentrations of Taxol formed intracellular cytoplasmic inclusions as previously described with other tumor types.[10]

Radial dish assay

Cells exposed to Taxol demonstrated a dose-dependent increase in locomotion from the central region of the dish to the periphery (the computed F statistic with degrees of freedom 4 and 102 was 7.29, with $p < 0.0001$) (Table 2). Very few of the C6 cells survived when the Taxol concentration was 1000 nM, so motility for this cell line could only be evaluated at lower concentrations. This dose-dependent increase in motility was a linear relationship (the computed F statistic with degrees of freedom 4 and 102 was 17.35, with $p < 0.0001$).

Discussion

Malignant astrocytomas are diffusely infiltrative and rapidly fatal.[18,44–52]. Malignant human astrocytoma cells are exceedingly motile. The extent of tumor cell infiltration and the highly motile nature of astrocytoma cells *in vivo* has been documented by Bernstein *et al.*[53,54] Labeled astrocytoma cells were implanted in rat forebrain. Within 7 days of implantation, tumor cells were found throughout the central nervous system. Bulk disease was identified only at the site of injection, while distant cells remained single. Westermark *et al.*[55] demonstrated the marked motility of human malignant glioma cells *in vitro*. Evaluation of phagokinetic tracks of cells grown on colloidal gold deposits revealed increased cell motility with exposure to epidermal growth factor (EGF). Engebraaten[56] showed that the rate at which glioblastoma cells migrate from spheroids is increased by the addition of specific cytokines. The affects of EGF were blocked with the addition of an antibody to the EGF receptor.[57]

Optimal treatment of malignant astrocytomas should address both the bulk tumor and the diffusely invading tumor cells. Taxol induces cells to assemble microtubules into cycle-specific metastable structures (bundles or asters) which the cells cannot disassemble.[4] Since microtubule assembly is required for many cell activities including mitosis and locomotion, we tested the hypothesis that Taxol

Table 2. Average distance (cm) traveled in 7 days by glioblastoma cells in culture in the presence of escalating concentrations of Taxol: the six cell lines show a trend towards increased locomotion with increasing Taxol concentration ($p < 0.0001$)

Taxol concentration (nM)	C6	WU17	WU18	WU20	WU29	WU31
0	1.222521	0.118018	0.491288	0.812278	0.528338	0.998535
1.0	1.383273	0.168021	0.578815	0.499193	0.577321	1.281442
10	1.324196	0.240205	0.717347	0.874215	0.519721	1.156528
100	1.405435	0.350992	0.408132	1.273227	0.572494	1.360001
1000	cell death	0.315363	0.863941	1.166667	0.707185	1.363745

is cytocidal and alters the rate of tumor cell locomotion.

Previous studies[34,41] have demonstrated that cell lines that are insensitive to a drug *in vitro* are almost always insensitive to the drug *in vivo*. However, cell lines that are sensitive to a drug *in vitro* are not necessarily sensitive to the drug *in vivo*. Thus, *in vitro* assays are most predictive of treatment failures, not successes. The six cell lines tested showed significant, though not 100% lethal, response to Taxol using an established *in vitro* method. Clinically, high-dose Taxol (250 mg/m^2) can achieve a plasma level of approximately 100 nM.[58] Helson *et al.*[19] performed *in vitro* chemosensitivity testing of two glioblastomas, two neuroblastomas and two primitive neuroectodermal tumors. They demonstrated that sensitivity varied among the six cell lines tested and that at a 'saturation threshold' can be attained. When Taxol concentration exceeds this saturation level, no additional cytotoxic effect exists. This raises the possibility of a 'saturable microtubular target'.[19] This concept is supported by the work of Leibmann *et al.*[20] who demonstrated a saturation threshold as low as 50 nM using eight human tumors (including one grade three astrocytoma).

Brain tumor cell invasion into surrounding normal brain is a complex process that involves three coordinated events: cell movement from the parent tumor into the surrounding extracellular matrix, hydrolysis of matrix components and movement through the matrix.[59] With invasion, the normal balance between proteinase activation (activation of metalloproteinases, cysteine proteinases and serine proteinases) and proteinase inhibition (by tissue inhibitors of metalloproteinases, cystatin, and plasminogen activator inhibitors) is disrupted.[38,60,61] Cytochalasin B blocks cytoskeletal assembly and halts all cell locomotion in the radial dish assay.[28] In contrast, Taxol induces cytoskeletal assembly, possibly providing a rigid lattice for attachment of contractile mechano-proteins. Even at relatively high Taxol concentrations (1000 nM), surviving cells exhibited increased cellular velocity. Results obtained with the radial dish assay reached statistical significance with the cell lines tested with $p < 0.0001$.

Using a human prostate tumor cell line that invades and metastasizes in immunodeficient mice, Stearns and Wang[62] showed that Taxol inhibits secretion of two type IV collagenases and a gelatinase. Furthermore, Boyden chamber studies showed that Taxol inhibited invasion of cells into matrigel. Therefore, although glioblastoma cells showed increased locomotion, they may or may not have altered ability to invade into normal brain. The prostate tumor cell line invades and metastasizes in immunodeficient mice, while brain tumor cell lines do not typically metastasize systemically, indicating a fundamental difference in the types of tumors investigated and possibly in the types of collagenases and gelatinases produced or required for invasion.

Conclusions

Although the development of Taxol began three decades ago, only recently has this drug been used in patients with primary malignant brain tumors. Phase II trials with this drug are now ongoing. Our results indicate that Taxol is a potent antimitotic agent at low concentrations, but reaches a saturable threshold at higher concentrations. Taxol enhances brain tumor cell locomotion in a dose–response fashion. This is suggestive that Taxol may impact tumor cell invasion *in vivo*.

Acknowledgments

The authors are grateful to Elliot Elson for technical expertise and invaluable advice, Ralph G Dacey, Jr and Thomas Woolsey for support and guidance, and Jack D Baty for assistance with statistical evaluation of data.

References

1. Arbuck SG, Canetta R, Onetto N, *et al.* Current dosage and schedule issues in the development of paclitaxel (Taxol). *Semin Oncol* 1993; **4** (suppl 3): 31–9.
2. Cheson BD. Clinical trials referral resource. Clinical trials with taxol. *Oncology* 1992; **6**: 67–70.
3. Cheson BD. Clinical trials referral resource. Update on taxol trials. *Oncology* 1993; **7**: 66–7.
4. Donehower RC, Rowinsky EK. An overview of experience with taxol (paclitaxel) in the U.S.A. *Cancer Treat Rev* 1993; **19** (suppl C): 63–78.
5. Pazdur R, Kudelka AP, Kavanagh JJ, *et al.* The taxoids: paclitaxel (taxol) and docetaxel (taxotere) *Cancer Treat Rev* 1993; **19**: 351–86.
6. Wani MC, Wall ME. 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.
7. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly *in vitro* by taxol. *Nature* 1979; **22**: 665–7.
8. Caplow M, Zeeberg B. Dynamic properties of microtubules at steady state in the presence of taxol. *FEBS Lett* 1982; 319–24.

9. Horwitz SB, Lothstein L, Manfredi JJ, *et al.* Taxol: mechanisms of action and resistance. *Ann NY Acad Sci* 1986; 733–44.
10. Jordan MA, Toso RJ, Thrower D, *et al.* Mechanisms of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc Natl Acad Sci USA* 1993; **90**: 9552–6.
11. Webster L, Linsenmeyer M, Millward M, *et al.* Measurement of cremophor EL following taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug-resistant phenotype. *J Natl Cancer Inst* 1993; **85**: 1685–90.
12. Nazzaro JM, Neuwelt EA. The role of surgery in the management of supratentorial intermediate and high-grade astrocytomas in adults. *J Neurosurg* 1990; **73**: 331–44.
13. Silbergeld DL, Rostomily RC, Alvord EC. Causes of death in 117 cases of supratentorial glioblastoma in adults. *J Neurosurg* 1991; **74**: 337.
14. Silbergeld DL, Rostomily RC, Alvord EC. The causes of death in patients with glioblastoma is multifactorial: clinical factors and autopsy findings in 117 cases of supratentorial glioblastoma in adults. *J Neuro-Oncol* 1991; **10**: 179–85.
15. Salazar OM, Rubin P. The spread of glioblastoma multiforme as a determining factor in radiation treated volume. *Int J Radiat Oncol Biol Phys* 1976; **1**: 627–37.
16. Burger PC, Dubois PJ, Schold SC, *et al.* Computerized tomographic and pathologic studies of the untreated, quiescent and recurrent glioblastoma multiforme. *J Neurosurg* 1983; **58**: 159–69.
17. Burger PC, Heinz ER, Shibata T, *et al.* Topographic anatomy and CT correlations in untreated glioblastoma multiforme. *J Neurosurg* 1988; **68**: 698–704.
18. Burger PC, Vollmer RT. Histologic factors of prognostic significance in the glioblastoma multiforme. *Cancer* 1980; **46**: 1179–86.
19. Helson L, Helson C, Malik S, *et al.* A saturation threshold for taxol cytotoxicity in human glial and neuroblastoma cells. *Anti-Cancer Drugs* 1993; **4**: 487–90.
20. Liebmann JE, Cook JA, Lipshultz C, *et al.* Cytotoxic studies of paclitaxel (taxol) in human tumor cell lines. *Br J Cancer* 1993; **68**: 1104–9.
21. Rogers BB. Taxol: a promising new drug of the '90s. *ONF* 1993; **20**: 1483–9.
22. Rowinsky EK, Burke PJ, Karp JE, *et al.* Phase I and pharmacodynamic study of taxol in refractory acute leukemias. *Cancer Res* 1989; **49**: 4640–7.
23. Sharma A, Mayhew E, Straubinger RM. Antitumor effect of taxol-containing liposomes in a taxol-resistant murine tumor model. *Cancer Res* 1993; **53**: 5877–81.
24. Burger PC, Vogel FS, Green SB, *et al.* Glioblastoma multiforme and anaplastic astrocytoma: pathologic criteria and prognostic implications. *Cancer* 1985; **56**: 1106–11.
25. Nelson JS, Tsukada Y, Schoenfeld D, *et al.* Necrosis as a prognostic criterion in malignant supratentorial, astrocytic gliomas. *Cancer* 1983; **52**: 550–4.
26. Schiffer D, Chio A, Giordana MT, *et al.* Prognostic value of histologic factors in adult cerebral astrocytoma. *Cancer* 1988; **61**: 1386–93.
27. Ali-Osman F, Spence A, Berger MS, *et al.* Primary cultures of human glial tumor cells and their relationship to histopathology. *J Neuropathol Exp Pathol* 1987; **46**: 371.
28. Chicoine MR, Silbergeld DL. Assessment of brain tumor cell motility *in vivo* and *in vitro*. *J Neurosurg*; in press.
29. Freshney RI. Tissue culture of glioma of the brain. In Thomas, Graham, eds. *Brain tumors: scientific basis, clinical investigation, and current therapy.* 1980: 21–50.
30. Silbergeld DL, Ali-Osman F. Glioblastoma induced transformation of cultured normal endothelial cells. *J Neurosurg* 1990; **72**: 343A.
31. Westermark R, Ponten J, Hugosson R. Determinants for the establishment of permanent tissue culture lines from human gliomas. *Acta Pathol Microbiol Scand* 1973; **81**: 791–805.
32. Wroblewska Z, Delvin M, Gilden DH, *et al.* Human brain in tissue culture. *J Comp Neurol* 1975; **161**: 307–16.
33. Kapp JP, Perkins E, Tucker E. Preservation of brain tumor specimens for drug sensitivity testing. *Neurosurgery* 1989; **24**: 810–2.
34. Ali-Osman F, Beltz PA. Optimization and characterization of the capillary human tumor clonogenic assay. *Cancer Res* 1988; **48**: 715–24.
35. Ali-Osman F, Giblin J, Dougherty D, *et al.* Application *in vivo* and *in vitro* pharmacokinetics for physiologically relevant drug exposure in a human tumor clonogenic assay. *Cancer Res* 1987; **47**: 3718–24.
36. Ali-Osman F, Rosenblum ML, Giannini DD, *et al.* Potential antiglioma activity of 9-hydroxy-22-*N*-methyllipticine as determined by pharmacological and human tumor clonogenic cell studies. *Cancer Res* 1985; **45**: 2988–92.
37. Mann BD, Kern DH, Guliano AE, *et al.* Clinical correlations with drug sensitivities in the clonogenic assay. *Arch Surg* 1982; **117**: 33–6.
38. Rosenblum ML, Deen DF, Hoshino T, *et al.* Comparison of clonogenic cell assays after *in vivo* and *in vitro* treatment of 9L gliosarcoma. *Br J Cancer* 1980; 41 (suppl): 307–8.
39. Salmon SE. Development and applications of a human tumor colony assay for chemosensitivity testing. *Rec Results Cancer Res* 1984; **94**: 8–16.
40. Shoemaker RH, Wolpert-DeFilippes MK, Kern DH. Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985; **45**: 2145–53.
41. Thomas DGT, Darling JL, Paul EA, *et al.* Assay of anticancer drugs in tissue culture: relationship of relapse free interval (RFI) and *in vitro* chemosensitivity in patients with malignant cerebral glioma. *Br J Cancer* 1985; **51**: 525–32.
42. SAS Institute Inc. *SAS/STAT user's guide*, 4th edn (Version 6), Vol. 2. Cary, NC: SAS Institute 1989: 846.
43. Sheetz MP, Turney S, Qian H, *et al.* Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements. *Nature* 1989; **340**: 284–88.
44. Barnard RO, Geddes JF. The incidence of multifocal cerebral gliomas. *Cancer* 1987; **60**: 1519–31.
45. Burger PC, Mahaley MS, Dudka L, *et al.* The morphologic effects of radiation administered therapeutically for intracranial gliomas. *Cancer* 1979; **44**: 1256–72.
46. Concannon JP, Kramer S, Berry R. The extent of intracranial glioma at autopsy and its relationship to techniques used in radiation therapy of brain tumors. *Am J Radiol* 1960; **84**: 99–107.
47. Davis L, Martin J, Goldstein SL, *et al.* A study of 211 patients with verified glioblastoma multiforme. *J Neurosurg* 1949; **6**: 33–44.

48. EROTC brain tumor study group. Effect of CCNU on survival, rate of objective remission and duration of free interval in patients with malignant gliomas—final evaluation. *Eur J Cancer* 1978; **14**: 851–6.
49. Netsky MG, August B, Fowler W. The longevity of patients with glioblastoma multiforme. *J Neurosurg* 1950; **7**: 261–9.
50. Roth JG, Elvidge AR. Glioblastoma multiforme: a clinical study. *J Neurosurg* 1960; **17**: 736–50.
51. Salford LG, Brun A, Nirfalk S. Ten-year survival among patients with supratentorial astrocytomas grade III and IV. *J Neurosurg* 1988; **69**: 506–9.
52. Scanlon PW, Taylor WF. Radiotherapy of intracranial astrocytomas: analysis of 417 cases treated from 1960 through 1969. *Neurosurgery* 1979; **5**: 301–8.
53. Bernstein JJ, Goldberg WJ, Laws ER. Human malignant astrocytoma xenografts migrate in rat brains: a model for central nervous system cancer research. *J Neurosci Res* 1989; **22**: 134–43.
54. Bernstein JJ, Goldberg WJ, Laws ER. Immunocytochemistry of human astrocytoma cells xenografted into rat brain. *Neurosurgery* 1989; **42**: 541–6.
55. Westermark B, Magnusson A, Heldin C. Effect of epidermal growth factor on membrane motility and cell locomotion in cultures of clonal glioma cells. *J Neurosci Res* 1982; **8**: 491–507.
56. Engebraaten O, Bjerkvig R, Pedersen PH, *et al.* Effects of EGF, bFGF, NGF and PDGF(bb) on cell proliferative, migratory and invasive capacities of human brain-tumour biopsies *in vitro*. *Int J Cancer* 1993; **53**: 209–14.
57. Lund-Johansen M, Bjerkvig R, Humphrey PA, *et al.* Effect of epidermal growth factor on glioma cell growth, migration, and invasion *in vitro*. *Cancer Res* 1990; **50**: 6039–44.
58. Jamis-Dow CA, Klecker RW, Sarosy G, *et al.* Steady-state plasma concentrations and effects of taxol for a 250 mg/m^2 dose in combination with granulocyte-colony stimulating factor in patients with ovarian cancer. *Cancer Chemother Pharmacol* 1993; **33**: 48–52.
59. Rusciano D, Burger MM. Mechanisms of metastasis. In: Levine AJ, Schmideck HH, eds. *Molecular genetics of nervous system tumors*. New York: Wiley-Liss 1993: 357–69.
60. Monod L, Diserens AC, Jongeneel CV, *et al.* Human glioma cell lines expressing the common acute lymphoblastic leukemia antigen (cALLa) have neutral endopeptidase activity. *Int J Cancer* 1989; **44**: 948–51.
61. Apodaca G, Rutka JT, Bouhana K, *et al.* Expression of metalloproteinases and metalloproteinase inhibitors by fetal astrocytes and glioma cells. *Cancer Res* 1990; **50**: 2322–9.
62. Stearns ME, Wang M. Taxol blocks processes essential for prostate tumor cell (PC-3 ML) invasion and metastases. *Cancer Res* 1992; **52**: 3776–81.

(Received 14 November 1994, accepted 15 December 1994)