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Human Brain Tumor Xenografts in Nude Mice as a Chemotherapy Model*

DAVID P. HOUCHENS,†‡ ARTEMIO A. OVEJERA,† SYLVA M. RIBLET† and DONALD E. SLAGEL§

†*Battelle Memorial Institute, 505 King Avenue, Columbus, OH 43201, U.S.A. and §Department of Surgery, University of Kentucky, Lexington, KY 40506, U.S.A.*

Abstract—*Two human brain tumors which were previously established in nude mice were used to determine antitumor efficacy of various therapeutic agents. These tumors were a medulloblastoma (TE-671) and a glioma (U-251) with mass doubling times of 3.5 and 5.5 days respectively as subcutaneous implants in nude mice. Intracranial (i.c.) tumor challenge was accomplished by inoculating tissue culture-grown cells of either tumor into the right cerebral hemisphere to a depth of 3 mm. Median survival time (MST) in untreated mice with 10^5 i.c. injected TE-671 cells was approximately 30 days and 53 days in the U-251 tumor. With 2×10^5 U-251 tumor cells the MST was 27–31 days. Groups of mice which had been inoculated with tumor were treated with various doses and schedules of antineoplastic compounds by the i.p. route. The TE-671 tumor responded to AZQ treatment with an increase in life span (ILS) of 37% compared to untreated controls and an ILS of 30% with CCNU treatment. BCNU and PCNU were ineffective. With the U-251 tumor BCNU produced an ILS of >60%, with 75% cures, >112% ILS with PCNU and 49% ILS with CCNU. Neither tumor responded to procarbazine, PALA, dianhydrogalactitol, D-O-norleucine or dibromodulcitol. The U-251 tumor was treated on various schedules and doses with BCNU and found to respond well on late as well as early treatment. A new drug (rapamycin) being investigated by the NCI was found to be very effective against the U-251 tumor. This model system should prove valuable in assessing the effects of various chemotherapeutic modalities against brain tumors.*

INTRODUCTION

THE PROGNOSIS of brain neoplasia is generally poor. Although tumors of the brain and cranial meninges account for only 1.5% of the total number of cancers in the U.S., the median survival time for all stages and types of these tumors is less than 1 yr, and therapeutic approaches developed to date have not significantly improved this survival pattern since 1950 [1].

Treatment of these tumors may include one modality or a combination of surgery, chemotherapy, radiation and/or immunotherapy. Data indicate some significant effects with certain chemotherapeutic agents [2–4] against experimental animal brain tumors. However, several questions have been raised by various investigators as to the most appropriate tumor type and implant site to use in these systems. It is generally accepted that intracerebrally implanted gliomas more closely approximate human brain tumors, although the transplanted tumors may have a different blood supply than primary tumors. These concerns are valid as reflected by the fact that the murine ependymoblastoma model has a high rate of 'cures' with nitrosureas, which is not seen with human brain tumor patients [5]. Numerous clinical trials have been performed using a variety of chemotherapeutic agents to treat brain neoplasia in humans [6, 7]. Both single-agent therapy studies [8] and combination treatment [9] have given some promise of control, but 'cures' are rarely achieved.

With the advent of the growth and treatment of human tumor xenografts in athymic nude mice [10] various high-priority drugs have been

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‡To whom requests for reprints should be addressed.

evaluated by the National Cancer Institute (NCI) against human breast, colon and lung tumors as a part of the NCI tumor panel [11]. Shapiro *et al.* [12] reported the transplantation of seven human brain tumors from patients, both subcutaneously and intracerebrally, in nude mice. They showed a difference to chemotherapy response of the different tumors. Such an observation has been reported by others with subcutaneously implanted colon tumor xenografts of the same pathological type [13] and with melanoma xenografts [14].

In the present study we report on the use of long-term tissue culture lines of human glioma and medulloblastoma implanted into nude mice for evaluating reponse to a variety of chemotherapeutic agents, including those which have been used in the treatment of human brain tumors. This was to determine if such a model could be used in the selection of drugs for clinical trials.

MATERIALS AND METHODS

Six- to eight-week-old female nude mice on a random-bred NIH Swiss background supplied by the Mammalian Genetics and Animal Production Branch of NCI were used in this study. They were maintained in autoclaved cages under polyester filters, given autoclaved feed and water *ad libitum* and kept in a laminar flow rack in quarters separate from other animals.

The human tumors used in this study were a cerebellar medulloblastoma, TE-671, which was originally established in tissue culture from a 6-yr-old female without prior treatment by McAllister *et al.* in California [15] and furnished as a tumor line in nude mice by Dr. Beatrice Lampkin, Children's Hospital of Cincinnati, and a glioblastoma multiforme, U-251, which was originally established from a 75-yr-old male by Pontén [16] in Sweden and furnished by Dr. Darell Bigner of Duke University. Tissue cultures of the TE-671 and U-251 lines were maintained as monolayers and grown at 37°C, 8% CO_2 in Eagle's minimum essential medium supplemented with 5% calf serum and L-glutamine. Tumors were also maintained as xenografts by subcutaneous implantation of a 3-mm^3 fragment in the right subaxillary region of nude mice.

The drugs used in this study were supplied by the Developmental Therapeutics Program, NCI. They were as follows: D-*O*-norleucine (DON) (NSC-7365); procarbazine (NSC-77213); 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU) (NSC-95466); 1,4-cyclohexadiene-1,4-dicarbamic acid,2,5-bis(1-aziridinyl)-3,6-dioxo-diethyl ester (AZQ) (NSC-182986); *N*-(phosphonacetyl)-L-aspartate, tetrasodium salt (PALA) (NSC-224131); rapamycin (NSC-226080); and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (NSC-409962). BCNU was dissolved in 95% ethanol (10% of final volume) and physiological saline. Methyl CCNU and CCNU were dissolved in 95% ethanol (10% of final volume), Emulphor (10% of final volume) and physiological saline. All other drugs were dissolved or suspended in physiological saline. All drugs were administered at the dosages and schedules indicated and were selected based on murine tumor model data from this laboratory and from the NCI and not from clinical treatment schedules.

For intracranial tumor implantation the tissue-cultured tumor cells were mechanically harvested by scraping, washed two times in Hank's balanced salt solution (HBSS) at 1000 revs/min in a clinical centrifuge and resuspended in serum-free HBSS to give a concentration of $1\text{–}2 \times 10^5$ viable (trypan blue exclusion) cells per 0.025 ml. The mice were placed under anesthesia with sodium pentobarbital and the tumor implanted in the right cerebral hemisphere with a 26-gauge needle fitted with a sleeve that allowed only a 3-mm penetration.

All mice were observed daily and median survival time was determined for each group. The percentage increase in life span (ILS) was determined by the formula $\frac{T\text{-}C}{C} \times 100$, where T and C are the median survival time for the treated group and control group respectively. An ILS of >25% was considered as indicative of activity as described by NCI protocols for evaluation of chemotherapeutic agents in a murine brain tumor [2].

RESULTS

Table 1 shows the median survival time of the intracranial tumor-implanted control animals from four experiments each for TE-671 and U-251 tumors. All mice that died with both TE-671 and U-251 inoculation exhibited neurological symptoms such as limb paralysis for several days before death. Additionally, most animals showed some enlargement of the head, with definable tumor masses extruded through the needle tract in the skull and under the scalp.

Tables 2 and 3 show the results of experiments with TE-671 and U-251 respectively; treated with various antineoplastic compounds.

With the TE-671 (Table 2) CCNU was toxic at the 30 mg/kg dose but was somewhat effective in prolonging life at the 20 mg/kg dose, while 15 mg/kg was ineffective. If the treatment was delayed 10 days after implantation the 20 mg/kg dose was not effective. AZQ was toxic at 40, 20 and 15 mg/kg, but produced a 37% ILS when

Table 1. Deaths in untreated control nude mice inoculated intracranially with human medulloblastoma or glioma cells

Tumor*	Experiment No.	No. of mice	MST† (days)
TE-671 medulloblastoma	1	5	34.0
	2	11	31.0
	3	9	27.0
	4	5	24.0
		30	29.4 (average)
U-251 glioma	1	5	52.0
	2	11	57.0
	3	10	56.5
	4	12	47.0
		38	53.0 (average)

*Each mouse was injected with 1×10^5 tumor cells.
†MST = median survival time.

*Table 2. Effects of various antineoplastic compounds on an intracranially implanted human medulloblastoma (TE-671) in nude mice**

Drug†	NSC No.	Dose (mg/kg/inj.)	Schedule	First day of treatment	Increase in life span‡ (%)
D-*O*-Norleucine	7365	40	Q4D × 3	3	0
		20		3	15
Procarbazine	77213	500	Q4D × 3	3	23
		250		3	0
		500		10	0
CCNU	79037	30	single	3	0 (toxic)§
		20	injection	3	30
		15		3	0
		20		10	0
PCNU	95466	10	single injection	1	0
Dibromodulcitol	104800	200	Q4D × 3	3	19
		100		3	15
		200		10	0
Dianhydrogalactitol	132313	4	Q4D × 3	3	3
AZQ	182986	40	Q4D × 3	3	0 (toxic)§
		20		3	0 (toxic)§
		15		3	0 (toxic)§
		7.5		3	37
		7.5		10	0
PALA	224131	500	Q4D × 3	3	0
		400		3	15
BCNU	409962	30	single	1	0
		15	injection	1	0

*Five mice per group implanted with 1 ± 10^5 tumor cells in 0.025 ml volume.
†Drugs injected intraperitoneally.
‡Increase in life span—percentage median survival time of treated group divided by median survival time of untreated tumor control group minus 100. Active if >25%.
§Toxic—mice died with a decrease in median survival time compared to control mice.

*Table 3. Effects of various antineoplastic compounds on an intracranially implanted human glioma (U-251) in nude mice**

Drug†	NSC No.	Dose (mg/kg/inj.)	Schedule	First day of treatment	Increase in life span‡ (%)	Survivors/ total
D-*O*-Norleucine	7365	40	Q4D × 3	3	0	0/6
		20		3	0	0/5
Procarbazine	77213	500	Q4D × 3	3	23	1/5
		250		3	7	0/5
CCNU	79037	30	single	3	0 (toxic)§	2/5
		20	injection	3	49	0/5
		15		3	4	0/5
PCNU	95466	15	single	3	0 (toxic)§	1/5
		10	injection	3	44	2/5
		15		7	0 (toxic)§	0/6
		10		7	> 112	3/6
Dibromodulcitol	104800	200	Q4D × 3	3	12	1/6
		100		3	12	0/5
Dianhydrogalactitol	132313	4	Q4D × 3	3	2	0/5
AZQ	186986	40	Q4D × 3	3	0 (toxic)§	0/5
		20		3	0 (toxic)§	0/5
PALA	224131	500	Q4D × 3	3	0	1/5
		400		3	0	0/6
BCNU	409962	30	single	1	44	2/5
		15	injection	1	> 61	3/4
		30		7	8	0/6
		15		7	25	0/6

* Five or six mice per group implanted with 1 ± 10^5 tumor cells in 0.025 ml volume.

† Drugs injected i.p.

‡ Increase in life span—percentage median survival time of treated group divided by median survival time of untreated tumor control group minus 100. Active if >25%.

§ Toxic—mice died with a significant decrease in median survival time compared to control mice.

administered at 7.5 mg/kg/injection every 4 days, starting on the third day after implantation. If treatment was delayed until 10 days after implantation there was no increase in life span. No other drugs produced any significant increase in life span and there were no mice cured or that were long-term survivors.

With the U-251 (Table 2) CCNU was very effective at the 20 mg/kg dose, producing a 49% ILS. At 10 mg/kg 3 days after tumor implant PCNU produced a 44% ILS and two survivors out of five mice. When the treatment was delayed until day 7 50% of the mice were long-term survivors and the ILS was >112%. BCNU was also very effective when administered at 15 mg/kg 1 day after tumor implant and produced an ILS of >61% with three survivors out of four mice, but was only minimally effective at the 15 mg/kg dose when treatment was delayed until day 7. The top doses of CCNU and PCNU were toxic and both doses of AZQ were toxic. No other drugs were effective.

In order to determine if late tumor stage treatment was as effective as early treatment and that, in fact, established tumor was being treated and the drug effects seen were not just tumor prevention, the experiment shown in Table 4 was performed. Mice were implanted with 2×10^5 U-251 cells in order to reduce the time to death from that seen with 1×10^5 cells. After tumor inoculation the mice were randomized and treated with BCNU at either 7, 14 or 21 days at the dose indicated in the table. The BCNU for this experiment was a clinical formulation (Bristol). The mice were observed daily for death for a period of 88 days after tumor inoculation. Antitumor activity was seen with the 40 mg/kg dose with treatment at both day 7 and day 14. At the 20 mg/kg dose a greater increase in life span was seen on day 14 or 21 treatment than on day 7

*Table 4. Effect of BNCU on an intracranially implanted human glioma (U-251) in nude mice**

Group	Treatment day	Dose† (mg/kg)	MST‡ (days)	ILS§ (%)
1	7	40	55	77
2		20	42	35
3		10	36	15
4		5	38	22
5	14	40	56	80
6		20	48	55
7		10	42	35
8		5	38	22
9	21	20	50	61
10		10	41	32
11		5	41	32
12	—	—	31	—

*Seven mice per treatment group implanted with 2×10^5 cells in 0.025 ml volume. Group 12 (control) had 16 mice.
†BCNU given i.p.
‡MST = median survival time of group.
§ILS = increase in life span as defined in Tables 2 and 3.

treatment. The 10 mg/kg dose was active only at day 14 and day 21 and the 5 mg/kg dose showed activity only on the day 21 treatment schedule.

A new drug, rapamycin, an inhibitor of DNA synthesis (Randall K. Johnson, personal communication) which was shown to have efficacy against the Zimmerman ependymoblastoma by the Developmental Therapeutics Program of the NCI, was tested against the TE-671 and U-251 tumors (Table 5). As can be seen, there was no effect with any dose of the drug on the TE-671 medulloblastoma, while there was a very pronounced effect on the ILS in the U-251 glioma model. No toxicity was seen at any of the three doses of drug which were used in this study.

Table 5. Effect of rapamycin (NSC-226080) on life span of nude mice inoculated intracranially with TE-671 medulloblastoma or U-251 glioma cells

Tumor*	Drug dose† (mg/kg/inj.)	MST‡ (days)	ILS§ (%)
TE-671	800	17.5	0
	400	21.5	0
	200	21.5	0
	—	22.5	—
U-251	800	49.0	78
	400	40.5	47
	200	46.0	67
	—	27.5	—

*Female nude mice inoculated with 1×10^5 cells of TE-671 and 2×10^5 cells of U-251 tumor.
†Drugs given i.p. on days 2, 6 and 10 after tumor inoculation.
‡MST = median survival time.
§ILS = increase in life span as defined in Tables 2 and 3.

DISCUSSION

The results presented in this study indicate the usefulness of such a model in the assessment of chemotherapy against human brain tumors. The results further confirm the report of Shapiro *et al.* [12] that drugs may be readily evaluated in this system and, further, that both dosage and schedule changes can be incorporated. Additionally, the death time can be adjusted by changing the number of cells implanted in the i.c. model. Currently we are implanting the U-251 tumor at 2×10^5 cells and the median survival time is in the range of 27–32 days. Although the model reported in the present study would not be feasible for extensive screening of compounds because of the special care and costs associated with nude mice, it offers a real potential in the evaluation of high-priority drugs for phase II or III clinical trials. Since large numbers of tumor cells can be grown in tissue culture with high viability and tumorigenicity maintained, the reproducibility of the system is very good. There is little variation from one experiment to another in the death time and the small differences are no more than those seen in solid murine tumor models.

The fact that nitrosoureas were selected as active by the U-251 tumor demonstrates that clinically useful drugs are selected by the system. Of course, gliomas from different patients may not have responded to this treatment. Bullard *et al.* [17] reported a study with the subcutaneous growth of U-251 and two other gliomas and their subsequent treatment with BCNU. BCNU produced consistent volume reduction of the U-

251 tumor in their study and somewhat less tumor reduction in the other tumors.

The fact that late treatment of tumors, as seen in Table 4, was as effective as early treatment would indicate that this model is actually a measure of drug activity on existing tumor and not just prevention of tumor growth.

The experiment with the new drug, rapamycin (Table 5), shows the possible usefulness of this model in developing new drugs. This drug is now being used in pre-clinical toxicology trials for the NCI and we are conducting combination radiation–chemotherapy studies with rapamycin in the U-251 model.

The nude mouse–human brain tumor model described can also be used for other studies of combined modality therapy. Slagel *et al.* [18] used this model with the TE-671 and U-251 tumors to evaluate combination chemoradiotherapy and found marked synergism with combination therapy compared to either modality alone when using procarbazine against TE-671 and BCNU against U-251.

In conclusion, the studies reported in this paper demonstrate the usefulness of the human brain tumor xenograft model for evaluation of antineoplastic effect of chemotherapeutic agents. Degrees of response from negative to highly effective can be seen based on drug, dose and schedule. Such a model could aid in the development of therapy for clinical trials.

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