Original Article

Phase II Study of Antineoplastons A10 (NSC 648539) and AS2-1 (NSC 620261) in Patients With Recurrent Glioma

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• *Objective*: To assess the pharmacokinetics, toxicity, and efficacy of antineoplastons A10 (NSC 648539) and AS2-1 (NSC 620261).

• *Design*: We initiated a phase II trial in order to determine whether evidence of antitumor activity of A10 and AS2-1 could be documented.

• *Material and Methods*: Patients with anaplastic astrocytoma or glioblastoma multiforme recurring after radiation therapy were eligible for enrollment in the trial. Patients received escalating doses of A10 and AS2-1 by multiple intermittent intravenous injections with use of a portable programmable pump to the target daily dose of 1.0 g/kg for A10 and of 0.4 g/kg for AS2-1.

• *Results*: Nine patients were treated, in six of whom the treatment response was assessable in accordance with protocol stipulations. No patient demonstrated tumor regression. Reversible grade 2 or 3 neurocortical toxicity, consisting of transient somnolence, confusion, and exacerbation of an underlying seizure disorder, was noted in five patients. Mean steady-state plasma concentrations of phenylacetate and phenylacetylglutamine after escalation to the target doses of A10 and AS2-1 were 177 ± 101 μg/mL and 302 ± 102 μg/mL, respectively. Patients who exhibited confusion tended to have higher phenylacetate levels.

• *Conclusion*: Although we could not confirm any tumor regression in patients in this study, the small sample size precludes definitive conclusions about treatment efficacy. Antineoplaston-related toxicity was acceptable in most patients with appropriate dose modification, although severe neurocortical toxicity may occur. Steady-state plasma concentrations of phenylacetate with use of A10 and AS2-1 were similar to those reported with use of similar doses of phenylacetate alone.

Mayo Clin Proc 1999;74:137-145

CT = computed tomographic; HPLC = high-performance liquid chromatographic; MRI = magnetic resonance imaging; PA = phenylacetate; PAG = phenylacetylglutamine

The term "antineoplastons" is used to describe mixtures of peptides, amino acid derivatives, and organic acids that serve as components of a theoretical natural defense system against human cancers and other human diseases.[1] A10 (a 1:4 ratio of phenylacetylisoglutamine and phenylacetylglutamine [PAG]) and AS2-1 (a 1:4 ratio of PAG and phenylacetic acid) are two such antineoplastons (code designations: NSC 648539 and NSC 620261, respectively) used by Burzynski and associates[2] in the treatment of patients with recurrent anaplastic astrocytoma or glioblastoma multiforme. In 1991, a team of researchers from the National Cancer Institute reviewed available clinical information from seven patients selected by Burzynski from his clinical experience. From this review, the investigators determined that presumptive evidence of antitumor activity was available, and the National Cancer Institute proposed that phase II trials be conducted.[3]

In addition, preclinical evidence indicates that phenylacetate (PA) may be an active inhibitor of astrocytic tumor growth, and potential mechanisms have been reported.[4-11] Neither the clinical efficacy nor the pharmacokinetic activity of A10 and AS2-1 with use of the antineoplaston protocol advocated by Burzynski has been studied previously by independent investigators in prospectively designed trials. Therefore, we initiated the following phase II trial in an effort to determine the effectiveness and pharmacokinetics of A10 and AS2-1.

SUBJECTS AND METHODS

Patient Eligibility

Before enrollment in the trial, all patients provided written informed consent. Adult patients with histologic proof of anaplastic astrocytoma or glioblastoma multiforme and computed tomographic (CT) or magnetic resonance imaging (MRI) evidence of tumor growth after radiation therapy

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This study was supported in part by grants U01 CA 69912, M01 RR 00585, P30 CA 15083, and CM 07311-04 from the National Institutes of Health, Public Health Service, and by the Linse Bock Foundation.

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were eligible for the trial if the maximal diameter of the contrast-enhancing portion of the tumor was less than or equal to 5 cm and if no evidence was noted of multifocal tumor or of leptomeningeal or systemic metastatic involvement. All patients had performance scores of 0 or 1 (scale established by the Eastern Cooperative Oncology Group), a life expectancy of at least 4 months, and geographic access to follow-up. Patients with such small tumors and good performance scores constitute a minority of all patients with recurrent malignant glioma, albeit a group with a relatively good prognosis. These criteria substantially reduced patient accrual to this trial. Because of slow patient accrual, the eligibility criteria were modified after the first seven patients had been enrolled, in order to allow patients with tumors larger than 5 cm or with multifocal tumors to participate. At least 1 month must have elapsed after the end of radiation therapy, and at least 6 weeks since the end of nitrosourea chemotherapy, for patients to participate. Patients must have been receiving a fixed dose of corticosteroids (or no corticosteroids) for at least 1 week before the baseline scan, and treatment commenced within 1 week after the baseline scan.

Patients were excluded from the trial for any of the following factors: prior or concurrent treatment with antineoplaston, PA, or phenylbutyrate; leukocyte count less than 2.0 x 10^9/L; absolute neutrophil count less than 1.5 x 10^9/L; platelet count less than 100 x 10^9/L; hemoglobin less than 10 g/dL; total bilirubin greater than or equal to 2.0 mg/dL; creatinine greater than or equal to 2.0 mg/dL; abnormal serum electrolytes; pregnancy or nursing; uncontrolled infection; prior malignant condition other than carcinoma in situ of the uterine cervix; New York Heart Association class III or IV; history of congestive heart failure; myocardial infarction within the previous year; angina necessitating medication; chronic obstructive pulmonary disease; poor medical or psychiatric risk that would, in the opinion of the investigator, make therapy with an investigational drug unwise; or concurrent chemotherapy.

Patient Assessments

Within 7 days before study enrollment, patients underwent physical examination and a review of their medical history, Folstein and Folstein Mini-Mental State Examination, complete blood cell count with differential, reticulocyte count, prothrombin time and activated partial thromboplastin time, serum chemistry and electrolyte panel analysis, anticonvulsant drug level study, serum pregnancy test in women of childbearing potential, 24-hour urine collection for pharmacokinetic studies (described subsequently), brain CT or MRI scan with use of a contrast agent, posteroanterior and lateral chest roentgenography, and electrocardiography. Pretreatment laboratory tests for patients at Mayo Clinic Rochester also included quantitative immunoglobulin levels, quantitative T- and B-lymphocyte assays, and a Wright-stained peripheral blood smear.

During the first 7 to 10 days of treatment, patients were hospitalized for observation and additional laboratory monitoring, including a complete blood cell count with differential, serum electrolytes and chemical studies, determination of anticonvulsant drug levels, and serum sampling for pharmacokinetic studies. Assays for quantitative determination of T and B lymphocytes on day 8 were performed for patients treated at Mayo Clinic Rochester.

Every 4 weeks, patients returned for review of their medical history, physical examination, Folstein and Folstein Mini-Mental State Examination, complete blood cell count with differential, serum electrolyte and chemistry panels, serum sampling for pharmacokinetic studies, and brain CT or MRI scan with use of contrast medium. The patient or home caregiver completed a home toxicity monitoring record to determine interval toxicities and a home medication record to monitor concomitant medications.

Treatment Protocol

Before treatment, patients had a double-lumen central venous catheter inserted into the subclavian vein. Patients received gradually escalating doses of the two antineoplastons by multiple intermittent intravenous injections administered with use of a portable programmable pump, as described in Table 1. Treatment began in the hospital in order to assess the patient's tolerance to therapy, to measure the pharmacologic variables, and to train the family in the use of the pump and replacement of the infusion bags. After hospitalization, patients continued to receive intermittent intravenous treatment at home by using the portable programmable pump. Subsequent administration continued at the target dose of each antineoplaston or at the highest dose tolerated by the patient if the patient was unable to tolerate the target dose. Patients continued to receive treatment until tumor progression was evident or side effects became intolerable.

Dose modifications were prescribed by protocol, based on published toxicities of antineoplastons A10, AS2-1, and PA. All toxicity grades were based on the National Cancer Institute Common Toxicity Criteria. The following dose modification criteria were used: leukocytes 0.5 to 1.0 x 10^9/L or platelets 50 to 100 x 10^9/L, decrease both agents 50%; leukocytes less than 0.5 x 10^9/L or platelets less than 50 x 10^9/L, discontinue treatment; nausea or vomiting greater than or equal to grade 3, decrease agents 50%; neurocortical toxicity greater than or equal to grade 3, decrease agents 25%; grade 3 or worse hepatic toxicity,

Table 1.—**Summary of Treatment Protocol With Antineoplastons**

Agent	Day	Daily dose (g/kg)	Route*	Retreatment schedule
A10	1	0.24	Intermittent intravenous infusion administered during a 15-minute period every 30 minutes through a central venous catheter with use of a programmable ambulatory infusion pump	Daily until progression or excessive toxicity
	2	0.48		
	3	0.72		
	4 and following	1.00		
AS2-1	1	0.12	Intermittent intravenous infusion administered during a 15-minute period every 30 minutes through a central venous catheter with use of a programmable ambulatory infusion pump beginning 15 minutes after initiation of A10 treatment	Daily until progression or excessive toxicity
	2	0.24		
	3	0.36		
	4 and following	0.40		

*Treatment was administered through a double-channel infusion pump into a central venous catheter. One channel delivered A10 for 15 minutes every 30 minutes alternating with the second channel delivering AS2-1 for the next 15 minutes every 30 minutes.

discontinue treatment for 2 days and then resume at 50% dose; any grade allergic skin reaction, discontinue treatment; hypokalemia less than or equal to 3.5 mEq/L, replace with potassium chloride as clinically necessary and record amount needed; hyperuricemia greater than 12 mg/dL, add allopurinol 300 mg/dL; and any other grade 3 or 4 toxicity, withhold treatment until toxicity is less than or equal to 1 and then resume treatment with the dose decreased 25%.

Assessment of Treatment Efficacy

Response criteria were based on objective tumor measurements. Neurologic status was assessed independently. *Complete response* was defined as complete disappearance of all contrast-enhancing tumor on neuroimaging studies for a minimal duration of 4 weeks. *Partial response* was defined as more than 50% reduction in the sum of the products of the greatest perpendicular diameters of all measurable lesions in comparison with the corresponding baseline evaluation, maintained for 4 weeks or longer, with no concomitant increase in size of any lesion or appearance of new lesions. *Stable disease* was defined as less than 50% change in the sum of the products of the perpendicular diameters of the tumors in comparison with the baseline evaluation. This state must have been maintained for a minimum of 12 weeks to qualify for stable disease.

Design of Protocol

Patients were stratified by histologic type—that is, anaplastic astrocytoma versus glioblastoma multiforme—as determined by the World Health Organization criteria.[12] Within each stratum, a modification of the two-stage phase II clinical trial design proposed by Fleming[13] was used. Initially, 15 adequately treated patients were to be assessed in each stratum. If less than one major response (complete or partial regression) was observed per stratum, it would be concluded that the true response rate within that stratum is less than 20% with 96% confidence (one-tailed analysis), and no further patients would be entered onto that stratum. If, however, 1 or more major responses were observed in either stratum, 20 more patients would be accrued to reach a final sample size of 35 adequately treated patients in that stratum. If 4 or more responses were observed among the 35 patients per stratum, the evidence would be considered sufficient to conclude that the antineoplaston regimen used was active and merited further study within that diagnostic stratum. This design would detect a true response rate equal to 20% with a 91% probability and would allow only a 7% probability of early termination of the treatment if the regimen were truly active. It would reject a response rate of 5% with 92% probability that the regimen was truly inactive if 35 patients were treated and with a 74% probability that the regimen was truly inactive if 15 patients were treated.

Pharmacokinetic Studies

Chemicals.—Antineoplastons A10 and AS2-1 were supplied by the Burzynski Research Institute to the participating institutions through the National Cancer Institute, National Institutes of Health. Reagent grade and high-performance liquid chromatographic (HPLC) grade solvents were obtained from commercial sources and used as received. Phenylacetic acid, sodium phenylacetate, phenylacetylchloride, and glutamine were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). PAG was synthesized by acylation of glutamine with phenylacetylchloride in a solution of tetrahydrofuran and 5% sodium bicarbonate. The proton magnetic resonance spectrum of the product recrystallized from chloroform-methanol-hexane was consistent with the desired compound.

Table 2.—**Patient Characteristics and Results of Treatment With Antineoplastons*†**

Case	Age (yr) and sex	Tumor histologic type	Date of initial diagnosis	Date of first recurrence	First Rx after recurrence	Date of second recurrence	AN Rx			Survival status	Survival time (days) after AN Rx begun
							Date begun	Duration (days)	Reason stopped		
1	36 M	AA	7/17/90	2/22/94	Surg Rx + PCBZ	6/29/94	7/6/94	16	Prog	Dead	43
2	41 F	GBM	1/22/93	9/15/94	Gamma knife	11/28/94	12/1/94	25‡	Prog	Dead	377
3	58 M	GBM	1/3/94	5/2/94	AN	...	6/9/94	20	Toxicity	Dead	359
4	62 F	GBM	8/1/94	11/14/94	BCNU, VP-16, CDDP	2/95	4/17/95	37	Toxicity	Dead	221
5	30 M	AA	6/4/93	2/1/94	AN	...	3/16/94	66	Prog	Dead	152
6	46 M	GBM	8/2/93	9/12/94	Gamma knife	10/24/94	10/27/94	24	Prog	Dead	490
7	30 M	GBM	8/9/94	2/27/95	AN	...	4/3/95	9	Toxicity	Dead	80
8	49 F	AOA	9/22/93	1/94	Surg Rx	2/94	3/21/94	50	Prog	Dead	206
9	49 F	GBM	11/18/91	10/92	PCV	2/94	3/15/94	15	Prog	Dead	157

*AA = anaplastic astrocytoma; AN = antineoplaston; AOA = anaplastic oligoastrocytoma; BCNU = carmustine; CDDP = cisplatin; GBM = glioblastoma multiforme; PCBZ = procarbazine; PCV = procarbazine, CCNU (lomustine), and vincristine; Prog = tumor progression; Rx = treatment; Surg = surgical; VP-16 = etoposide.

†Dates are shown as month/day (if known)/year.

‡Pump failed twice and then treatment was discontinued for 3 days because of infection; number reflects actual days of treatment.

Specimens.—Blood samples (7 mL) were collected in heparinized tubes and immediately chilled in an ice water bath. Plasma was separated by centrifugation (3,000 rpm for 10 minutes) in a centrifuge maintained at 4°C, transferred to a plastic tube, and frozen at –20°C until HPLC analysis. Blood specimens were obtained immediately before the daily infusion schedule was begun and at 15 and 30 minutes after onset of the daily infusion schedule on days 1, 3, 5, 8, and 15. Therefore, samples were obtained each day just before the initiation of the sequential pulsed injections of antineoplaston A10 and AS2-1; immediately after the first 15-minute infusion of antineoplaston A10; and immediately after the first 15-minute infusion of antineoplaston AS2-1. Blood samples were also obtained at monthly intervals while the patient received the treatment.

Urine was collected in separate plastic containers during the 24 hours before treatment was begun and during the following periods after initiation of treatment: 0 to 24, 48 to 72, and 96 to 120 hours. After collection, urine samples were mixed thoroughly, the total volume was recorded, and a 10-mL aliquot was transferred to a plastic tube, capped, and frozen at –20°C until HPLC analysis.

Sample Preparation and Analysis.—Plasma concentrations of PA and PAG for patients treated at Mayo Clinic Rochester and Memorial Sloan-Kettering Cancer Center were determined by a modification of the HPLC assay developed by Brusilow.[14] In brief, proteins were precipitated from plasma with cold methanol after addition of 3-(4-hydroxyphenyl)propionic acid as the internal standard. The aqueous-methanol supernatant was injected onto an HPLC system that consisted of a Novapak C18 (150- by 3.2-mm inside diameter, 4-μm) analytical column protected with a Newguard RP18 (15- by 3.2-mm inside diameter, 7-μm) guard column. The mobile phase was composed of acetonitrile:77 μM potassium phosphate, pH 3.1 (8:92, vol/vol) delivered at 1.0 mL/min. PA, PAG, and 3-(4-hydroxyphenyl)propionic acid were detected by ultraviolet absorption at 220 nm. Plasma concentrations of PA, PAG, and phenylacetylisoglutamine for patients treated at the National Cancer Institute were determined by the HPLC assay developed by Thibault and associates.[15]

RESULTS

Accrual and Patient Characteristics

Between September 1993 and April 1995, nine patients were enrolled in this study: two from Memorial Sloan-Kettering Cancer Center, two from the National Cancer Institute, and five from Mayo Clinic Rochester. Seven patients entered the trial before and two patients after March 1995, when eligibility criteria were adjusted to allow inclusion of patients with multifocal disease or tumors larger than 5 cm in maximal diameter of contrast enhancement. The trial was closed in June 1995 because of slow patient accrual. More than 8 additional years would have been needed to complete the study as originally planned. Patient characteristics are summarized in Table 2. Antineoplaston therapy was used after the first tumor recurrence in three patients and after the second recurrence in six patients. One patient (case 8), whose tumor was an anaplastic oligoastrocytoma and who had received no irradiation before antineoplaston therapy, was considered ineligible for response assessment on the basis of protocol

Table 3.—**Summary of Neurocortical Toxicity Data Associated With Antineoplaston Therapy***

Case	Neurocortical toxicity: Present	Neurocortical toxicity: Day first noted	Neurocortical toxicity: Maximal day	Mean PA Css (μg/mL)†	Dose adjusted	Symptoms resolved
1	No	...	...	71	No	...
2	Yes (gr 3)	7	8	348	Yes (↓25%)	Yes (+ DXM)
3	Yes (gr 3)	6	17	192	Yes (DC)	Yes
4	No	...	...	210	No	...
5	No	...	...	69	No	...
6	Yes (gr 2, seizures)	5	5	312	Yes (↓25%)	Yes (+ DXM)
7	Yes (gr 3)	10	11	140	Yes (DC)	Yes
8	Yes (gr 2)	8	8	160	No	Yes (+ narcotics)
9	Yes (gr 1, headache)	16	18	87	Yes (DC)	Yes

*Css = steady-state plasma concentration; DC = discontinued; DXM = dexamethasone; gr = grade of toxicity; PA = phenylacetate.
†Determined from levels obtained on days 5-8 of treatment.

guidelines. A second patient (case 6), who underwent stereotactic radiosurgery as a component of his initial radiation therapy, was found to have radiation necrosis at surgical intervention after completion of antineoplaston therapy, and the response to treatment was not assessable. A third patient (case 9) had a baseline scan more than 7 days before initiation of therapy and was ineligible for assessment of response, in accordance with the established protocol. Thus, six of the nine patients were assessable for treatment response according to the protocol. All nine patients are included in the assessments of toxicity and pharmacologic studies.

Toxicity

The most severe toxicity, noted in six of the nine patients (Table 3), was neurocortical and consisted of excessive somnolence, somnolence plus confusion, and increased frequency of underlying focal motor seizures. MRI scans revealed increased cerebral edema in two patients without an increase in contrast enhancement. In the patient who also had the highest plasma concentration of PA (case 2), an electroencephalogram disclosed focal delta slowing over the area of the tumor plus diffuse bilateral slow abnormalities, findings suggestive of a superimposed diffuse metabolic encephalopathic process. Two patients (cases 2 and 6) had their antineoplaston infusion temporarily discontinued and received dexamethasone; the symptoms resolved within 48 hours. Treatment was resumed with a 25% decrease in dose, as stipulated in the protocol, without recurrence of neurocortical toxicity. A third patient (case 3) had confusion that persisted until the antineoplaston infusion was discontinued because of a suspected allergic skin reaction. Within a few days, the confusion cleared, and he returned to his baseline mental status without the addition of corticosteroids. Treatment was not resumed in that patient, in accordance with protocol guidelines for the suspected presence of an allergic reaction. The onset of somnolence plus confusion occurred between treatment days 5 and 7, after the target dose of antineoplastons had been reached on day 4. Steady-state plasma PA concentrations were generally higher in the five patients who experienced grade 2 or 3 neurocortical toxicity (348, 312, 192, 160, and 140 μg/mL; median, 192) than in the four patients who had grade 0 or 1 neurocortical toxicity (210, 87, 71, and 69 μg/mL; median, 79). In the two patients (cases 2 and 6) who resumed treatment at a lower dose, plasma PA concentrations were subsequently lower (76 and 129 μg/mL, respectively).

Other toxic effects included nausea and vomiting, headache, myalgia, and edema (Table 4). These toxicities were usually mild to moderate with the exception of headaches, which were severe in two patients. In one patient (case 3), severe cutaneous erythema, pruritus, and facial edema developed. Treatment was discontinued. Two days later, the erythema was unchanged despite administration of antihistamines. He subsequently discontinued the use of phenytoin, which he had been taking (without incident) in order to control seizures for 5 months before enrollment in the current study. The allergic reaction resolved a few days later, and treatment with antineoplastons was not resumed. In another patient (case 4), treatment was discontinued because of anasarca of the extremities and face that was refractory to diuretics. After discontinuation of antineoplaston infusions, the edema resolved.

Table 4.—**Summary of Types and Severity of Toxic Effects Associated With Antineoplaston Therapy**

Toxicity	No. of patients			
	Grade 1	Grade 2	Grade 3	Total
Neurocortical findings	1	2	3	6
Headache	1	...	2	3
Nausea ± vomiting	3	2	1	6
Anorexia	1	...	...	1
Edema	2	1	...	3
Allergic reaction	...	1	...	1
Thrombocytopenia	3	...	...	3
Dizziness or light-headedness	2	...	...	2
Fatigue	2	...	...	2
Myalgia	2	...	...	2
Neurosensory effects	1	...	...	1
Urinary hesitancy	1	...	...	1
Leukopenia	1	...	...	1

Efficacy

Three patients (cases 2, 5, and 6) had reoperation for tumor after treatment with antineoplastons (Table 5). Pathologic examination revealed recurrent tumor in one patient (case 2), recurrent tumor plus radionecrosis in one (case 5), and only radionecrosis in one (case 6). Because this last patient underwent stereotactic radiosurgery before antineoplaston therapy, he is considered ineligible for response assessment. None of the six assessable patients (or three ineligible patients) exhibited CT or MRI scan evidence of tumor regression associated with antineoplaston treatment.

All six assessable patients had scan evidence of tumor progression during antineoplaston treatment ranging from 16 to 66 days, after which treatment was discontinued. Three patients (cases 3, 4, and 7) discontinued treatment because of toxicity, and follow-up scans obtained 16, 16, and 15 days later, respectively, revealed tumor progression. The mean time to treatment failure (either progression or unacceptable toxicity) was 29 days, and the mean time to tumor progression was 33 days (Table 2).

Survival

All nine patients died. The median and mean survival times were 5.2 and 7.2 months, respectively. One patient (case 5) died of sepsis related to complications of chemotherapy administered after discontinuation of antineoplaston treatment. All other study patients died of tumor progression.

Pharmacologic Studies

We assessed the pharmacokinetics of PA and PAG in all nine patients who received antineoplastons by the intermittent infusion schedule outlined in Table 1. Antineoplaston dosages were increased stepwise from the starting level of 0.24 g/kg daily for A10 and 0.12 g/kg daily for AS2-1 to the target level of 1.0 g/kg daily for A10 and 0.4 g/kg daily for AS2-1 during a 4-day period. After 24-hour administration of 0.48 g/kg daily of A10 and 0.24 g/kg daily of AS2-1 (level 2), the mean plasma concentrations (± standard deviations) of PA and PAG were 47 ± 20 μg/mL and 109 ± 37 μg/mL, respectively. Steady-state plasma concentrations of PA and PAG were achieved after 24-hour administration of the target level of 1.0 g/kg daily of A10 and 0.4 g/kg daily of AS2-1. The mean plasma concentrations of PA and PAG were 177 ± 101 μg/mL and 302 ± 102 μg/mL, respectively. Low steady-state plasma concentrations (14 to 35 μg/mL) of phenylacetylisoglutamine were noted in two patients who also had phenylacetylisoglutamine determined during administration of antineoplastons. The plasma concentrations of PA decreased by 30% and 67%, respectively, in the two patients who had their doses of A10 and AS2-1 reduced because of toxicity.

The urinary excretion of PA and PAG was determined in all nine patients. Minimal PA (1% or less) was detected in urine during the collection intervals. Substantial amounts of PAG were recovered in the urine. When recovery was calculated on the basis of total doses of A10 and AS2-1 administered during the collection period, the amount of PAG in the urine represented approximately 100% recovery of the administered PAG and PA.

Immunologic Studies

Four patients had quantitative determinations of B lymphocytes and T lymphocytes at baseline and on day 8 of treatment. No patient exhibited substantial changes in total lymphocytes or any subset, including T lymphocytes,

Table 5.—**Summary of Additional Treatment After Antineoplaston Therapy***

Case	Surg Rx after AN Rx	Pathologic condition	Chemotherapy after AN Rx	Drugs	Best response
1	No	...	No	...	...
2	Yes	Grade 4 glioblastoma multiforme	Yes	CCNU, VCR, PCBZ	Partial regression
3	No	...	Yes	PCBZ	Stable disease
4	No	...	No	...	...
5	Yes	Radionecrosis; anaplastic astrocytoma suspected (infiltrating tumor)	Yes	PCBZ	Died of sepsis
6	Yes	Dec. 13, 1994: extensive radionecrosis, no viable tumor. Sep. 19, 1995: grade 4 fibrillary astrocytoma	?	...	...
7	No	...	No	...	...
8	No	...	No	...	Progression
9	No	...	Yes	CCNU, PCBZ, VCR	Progression

*AN = antineoplaston; CCNU = lomustine; PCBZ = procarbazine; Rx = treatment; Surg = surgical; VCR = vincristine.

helper or suppressor B lymphocytes, or NK (natural killer) cells.

Drug Preparation

Antineoplastons were found to have a particularly foul odor, and careful preparation under a hood was necessary. Furthermore, accidental spillage occurred in one instance, after which the area had to be evacuated for several days. In addition, hives developed in one pharmacy technician in the evening after her first exposure to antineoplastons. The drug did contact her skin. Because she had never experienced hives previously, we concluded that the skin reaction was a possible adverse effect of contact with the compounds.

DISCUSSION

In addition to information from Burzynski and associates,[2] certain preclinical data suggest that PA may inhibit proliferation of certain malignant cell lines and induce morphologic changes of differentiation. Samid and colleagues[4] reported rapid decline in *myc* oncogene expression, growth arrest, and granulocyte differentiation when promyelocytic leukemia cells were exposed to clinically achievable concentrations of PA. Subsequently, Samid and coworkers[5] noted growth arrest in conjunction with diminished DNA synthesis as well as cell maturation and reversion to a nonmalignant phenotype in glioblastoma cell lines U87, A172, U373, U343, and HS683 treated with PA. Stockhammer and associates[6] also described morphologic changes of differentiation, including increased glial fibrillary acidic protein-positive processes in U-251G and C6 glioma cells treated with PA. In other studies, AS2-1 inhibited cell proliferation in malignant melanoma A375, prostate adenocarcinoma PC3, and erythroleukemia K562 cell lines.[7] In vivo, when Fischer rats received intracerebral inoculations of 9L gliosarcoma cells and were subsequently treated with either saline or continuous infusions of PA, significantly less tumor growth was evident in the rats that received PA.[8]

Several mechanisms by which PA may inhibit tumor growth have been proposed recently. Danesi and colleagues[9] reported inhibition of protein isoprenylation in prostate cancer cell lines, and Hudgins and coworkers[10] reported inhibition of protein prenylation with PA in prostate carcinoma, glioblastoma, and melanoma cell lines. Liu and associates[11] described increased biosynthesis and secretion of transforming growth factor-α in human melanoma cells treated with PA. Thus, several investigators have verified growth-inhibitory properties in vitro as well as potential mechanisms of action of PA.

PA has been known to be toxic in humans since 1919.[16] Exposure to PA has been associated with brain damage, which occurs in patients with phenylketonuria.[17,18] PA is detoxified by conversion to PAG, the urinary metabolite of PA.[14]

Thibault and colleagues[19] reported results of a phase I trial of intravenously administered PA, including pharmacokinetic data in patients with advanced cancer. In that trial, one of six patients with recurrent glioblastoma multiforme had symptomatic but not radiographic improvement. Central nervous system toxicity consisting of lethargy and confusion was noted at the higher dose level in

that phase I study. Central nervous system toxicity was correlated with higher PA plasma concentrations and was reversible when treatment was discontinued in all patients.

Because of the small cohort of patients treated in this trial, limited conclusions can be drawn. Why was accrual to this study so slow? First, only a minority of patients with recurrent anaplastic astrocytoma or glioblastoma multiforme have tumors smaller than 5 cm in maximal diameter. Second, few patients have an Eastern Cooperative Oncology Group performance score of 0 or 1. Third, patient acceptance of this study was hampered by the requirement for double-lumen port placement and catheter care, hospitalization, the prospect of continuous infusion therapy, and the effort necessary to learn to change intravenous fluid bags and to operate a programmable double-channel infusion pump. The investigators recognized the narrow eligibility criteria before the study was initiated; however, the level of patient enthusiasm anticipated for this trial did not materialize. Representatives of the drug manufacturer objected to the expanded eligibility criteria that allowed inclusion of patients with multifocal disease or those with tumors larger than 5 cm in maximal diameter. Despite efforts to negotiate expanded eligibility criteria, we were unable to reach agreement with the drug manufacturer; hence, the trial was discontinued.

The antineoplaston-related toxicity was acceptable in most patients, although some had severe transient neurocortical toxic effects consisting of somnolence, confusion, and exacerbation of underlying seizures. This toxicity was reversible with discontinuation of therapy, and lower doses of drug were subsequently administered to some patients without recurrence of the adverse effects. Moderate to severe neurocortical toxicity, especially confusion, was associated with higher plasma concentrations of PA, as noted in phase I trials of PA in patients with advanced cancer. Thibault and associates[19] also observed confusion and lethargy in patients, sometimes preceded by emesis in the patients treated with high doses of continuous intravenous infusions of PA. Those investigators reported that the mean serum PA concentration associated with neurotoxicity was 1,078 μg/mL in three patients treated at high dose levels. Five of our patients with serum concentrations of 152 μg/mL or higher experienced somnolence and confusion. In one patient with a peak PA plasma concentration of 208 μg/mL, confusion did not develop. No patient with peak PA plasma concentrations of less than 208 μg/mL experienced confusion, but two patients with PA plasma concentrations of less than 190 μg/mL had somnolence. Because plasma PA levels were not scheduled to be determined during episodes of toxicity, exact correlations are not possible. In general, doses that produce plasma PA levels of less than 150 μg/mL seem less likely to cause neurotoxicity in this population of patients with brain tumors.

In our study, hives developed in one patient and one pharmacy technician in association with antineoplaston treatment or administration; this reaction was possibly related to contact with the agents. Severe edema necessitated discontinuation of therapy in one patient. Other toxic effects, such as headache, anorexia, nausea, vomiting, and fatigue, were generally mild to moderate and manageable with symptomatic care.

As the mechanism of action for the antitumor activity of PA becomes more clearly delineated, an issue that arises is whether to administer the chemically defined drug, PA, rather than the antineoplaston mixture. Antineoplastons are mixtures of chemicals developed on the basis of the theory that the body contains nonimmunologic, protective substances that could be isolated from the urine.[20] To assess the potential value of antineoplastons as a delivery vehicle for PA, we were interested in comparing the steady-state plasma concentration values for PA by the two approaches to treatment. The dose of PA administered in the antineoplaston mixture in this trial is comparable to the dose of PA administered as the chemically pure form. Our target doses of 1.0 g/kg daily of A10 and 0.4 g/kg daily of AS2-1 resulted in a daily dose of 320 mg/kg of PA. In comparison, Thibault and coworkers[19] administered daily doses of 266 ± 40 mg/kg of PA by continuous infusion with use of adaptive control dosing in a phase I trial to evaluate the toxicity of PA. The mean steady-state plasma concentration of PA associated with administration of target doses of antineoplastons was 177 μg/mL. Administration of a mean daily dose of 266 ± 40 mg/kg of PA resulted in a steady-state plasma concentration of 178 ± 85 μg/mL.[19] Therefore, comparable doses of PA, whether administered in the antineoplaston mixture or as pure PA, yield similar plasma concentrations of PA.

The other major component of antineoplastons, PAG, is a urinary metabolite of PA that is formed by conjugation of PA with glutamine by the liver enzyme phenylacetyl coenzyme A:glutamine acyltransferase. PAG does not have documented antiproliferative activity.[4] Soltysiak-Pawluczuk and Burzynski,[21] however, have reported that PAG augments PA activity through inhibition of glutamine transport into cells. Administration of 880 mg/kg daily of PAG during infusion of the target doses of A10 and AS2-1 in this study yielded a high steady-state plasma concentration of PAG (301 μg/mL). When PA was administered alone, PAG concentrations were 188 ± 55 μg/mL. Theoretically, PAG may inhibit PA clearance by product inhibition of PA conjugation. In our study, however, high concentrations of PAG had no apparent effect on the pharmacokinetics of PA because similar PA plasma concentra-

tions were achieved whether PA was administered in the antineoplaston mixture (with PAG) or in the chemically pure form.[19] Additionally, essentially all the administered PA was excreted in the urine as the metabolite, PAG. Therefore, the antineoplaston mixtures may not be needed for the administration of PA because PA is the only component with documented antiproliferative activity and the pharmacokinetics of PA are not altered by coadministration of PAG and phenylacetylisoglutamine.

The efficacy of antineoplastons A10 and AS2-1 remains uncertain. We were unable to document efficacy in any of six assessable patients. Similarly, Thibault and colleagues[19] treated six patients with recurrent glioblastoma with 2-week continuous intravenous infusions of PA in a phase I study and documented no objective tumor regressions. One patient remained clinically stable for 9 months during therapy. Details sufficient to assess the effect of therapy in that single case are not reported. In a subsequent phase I study, a partial regression was reported in one of seven patients with glioma treated twice daily with PA.[22] Preliminary data of a phase II trial have been presented in abstract form, which suggest possible activity of infusional PA in patients with recurrent glioma;[23] however, the study is ongoing, and final results have not been published. Pending publication of these results, no studies of antineoplastons A10 and AS2-1, PA, or phenylbutyrate published in the peer-reviewed medical literature have documented meaningful clinical activity of this class of compounds in patients with recurrent astrocytoma. Therefore, we conclude that evidence of beneficial activity of these drugs in patients with recurrent high-grade astrocytoma is insufficient to recommend this therapy outside the performance of a properly designed, independently monitored clinical trial. Furthermore, these data support limiting future clinical evaluation to PA.

ACKNOWLEDGMENT

We acknowledge the contributions of the following: Zachary B. Kramer, M.D., and Harish K. Malhotra, M.D., for assistance with patient care; Marc K. Rosenblum, M.D., and Bernd W. Scheithauer, M.D., for neuropathology review; Pam G. Bagniewski, B.S., for pharmacologic assays; Regina Berkery, R.N., Ester C. Dantis, R.N., Nancy Edmonds, R.N., Raymond J. Miller, M.S., Jill R. Piens, R.N., Faye Spiers, R.N., Debra Thomas, R.N., Michael Torelli, R.N., and the Mayo Clinic General Clinical Research Center staff for drug preparation and administration; Thomas E. Witzig, M.D., for quantitative lymphocyte assays; Jean Hanson, C.R.A., and Martin P. Kleber, M.A., for data management; and Frank S. Lieberman, M.D., Randall Milliken, M.D., Ph.D., Jerome B. Posner, M.D., and Charles W. Young, M.D., for scholarly advice and encouragement.

REFERENCES

1. **Green S.** 'Antineoplastons': an unproved cancer therapy. *JAMA* 1992;267:2924-2928
2. **Burzynski SR, Kubove E, Burzynski B.** Phase II clinical trials of antineoplaston A10 and AS2-1 infusions in astrocytoma. In: Adam D, Buchner T, Rubinstein E, editors. *Recent Advances in Chemotherapy.* Munich: Futuramed Publishers; 1991. pp 2506-2507
3. Antineoplastons: request for phase II trials in CNS malignancies. *CTEP Lett* 1992 May;10:10
4. **Samid D, Shack S, Sherman LT.** Phenylacetate: a novel nontoxic inducer of tumor cell differentiation. *Cancer Res* 1992;52:1988-1992
5. **Samid D, Ram Z, Hudgins WR, Shack S, Liu L, Walbridge S, et al.** Selective activity of phenylacetate against malignant gliomas: resemblance to fetal brain damage in phenylketonuria. *Cancer Res* 1994;54:891-895
6. **Stockhammer G, Manley GT, Johnson R, Rosenblum MK, Samid D, Lieberman FS.** Inhibition of proliferation and induction of differentiation in medulloblastoma- and astrocytoma-derived cell lines with phenylacetate. *J Neurosurg* 1995;83:672-681
7. **Samid D, Yeh T-J, Shack S.** Interferon in combination with antitumourigenic phenyl derivatives: potentiation of IFNα activity in-vitro. *Br J Haematol* 1991;79(Suppl 1):81-83
8. **Ram Z, Samid D, Walbridge S, Oshiro EM, Viola JJ, Tao-Cheng JH, et al.** Growth inhibition, tumor maturation, and extended survival in experimental brain tumors in rats treated with phenylacetate. *Cancer Res* 1994;54:2923-2927
9. **Danesi R, Nardini D, Basolo F, Del Tacca M, Samid D, Myers CE.** Phenylacetate inhibits protein isoprenylation and growth of the androgen-independent LNCaP prostate cancer cells transfected with the T24 Ha-ras oncogene. *Mol Pharmacol* 1996;49:972-979
10. **Hudgins WR, Shack S, Myers CE, Samid D.** Cytostatic activity of phenylacetate and derivatives against tumor cells: correlation with lipophilicity and inhibition of protein prenylation. *Biochem Pharmacol* 1995;50:1273-1279
11. **Liu L, Hudgins WR, Miller AC, Chen LC, Samid D.** Transcriptional upregulation of TGF-alpha by phenylacetate and phenylbutyrate is associated with differentiation of human melanoma cells. *Cytokine* 1995;7:449-456
12. **Kleihues P, Burger PC, Scheithauer BW.** *Histological Typing of Tumours of the Central Nervous System.* 2nd ed. Berlin: Springer-Verlag; 1993
13. **Fleming TR.** One-sample multiple testing procedure for phase II clinical trials. *Biometrics* 1982;38:143-151
14. **Brusilow SW.** Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. *Pediatr Res* 1991;29:147-150
15. **Thibault A, Figg WD, McCall N, Samid D, Myers CE, Cooper MR.** A simultaneous assay of the differentiating agents, phenylacetic acid and phenylbutyric acid, and one of their metabolites, phenylacetylglutamine, by reversed-phase, high performance liquid chromatography. *J Liquid Chromatogr* 1994;17:2895-2900
16. **Sherwin CP, Kennard KS.** Toxicity of phenylacetic acid. *J Biol Chem* 1919;40:259-264
17. **Loo YH, Potempska A, Wisniewski HM.** A biochemical explanation of phenyl acetate neurotoxicity in experimental phenylketonuria. *J Neurochem* 1985;45:1596-1600
18. **Potempska A, Loo YH, Wisniewski HM.** On the possible mechanism of phenylacetate neurotoxicity: inhibition of choline acetyltransferase by phenylacetyl-CoA. *J Neurochem* 1984;42:1499-1501
19. **Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, et al.** A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. *Cancer Res* 1994;54:1690-1694
20. **Burzynski SR.** Antineoplastons: history of the research (I). *Drugs Exp Clin Res* 1986;12(Suppl 1):1-9
21. **Soltysiak-Pawluczuk D, Burzynski SR.** Cellular accumulation of antineoplaston AS2-1 in human hepatoma cells. *Cancer Lett* 1995; 88:107-112
22. **Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, et al.** Phase I study of phenylacetate administered twice daily to patients with cancer. *Cancer* 1995;75:2932-2938
23. **Prados MD, Spence A, Schold C, Robbins I, Mehta M, Berger M, et al.** A phase II trial of phenylacetic acid for recurrent malignant glioma: preliminary report of the North American Brain Tumor Consortium [abstract]. *Program Proc Am Soc Clin Oncol* 1996;15:156