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Increased 9-aminocamptothecin dose requirements in patients on anticonvulsants

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Abstract *Background*: High grade astrocytomas remain uniformly fatal despite aggressive surgery and radiotherapy. As existing chemotherapeutic agents are of limited benefit, clinical trials are underway to screen new drugs, such as 9-aminocamptothecin (9-AC), for activity in high grade astrocytomas. *Purpose*: This study was designed to estimate the efficacy of 9-AC in patients with newly diagnosed glioblastoma multiforme and recurrent high grade astrocytomas. The planned dose of 9-AC for this trial was 850 μg/m^2 per 24 h as a 72-h continuous intravenous infusion every 2 weeks. This was the maximum tolerated dose (MTD) on this schedule in multiple phase I studies in patients with systemic malignancies. However, we found this dose subtherapeutic in our patient population. As a result, the purpose of the study was altered to determine the MTD. *Methods*: A group of 32 patients were studied using 850 μg/m^2 per 24 h with a provision to escalate to 1000 μg/m^2 per 24 h if the first three cycles of 9-AC were without significant hematologic toxicity. Once it was determined that myelosuppression did not occur in patients on anticonvulsants, dose escalations were initiated using the continual reassessment method. Dose escalations were conducted independently in newly diagnosed and recurrent patients and in those taking and not taking hepatic enzyme-inducing anticonvulsants. Pharmacologic studies were conducted during the first cycle of 9-AC. Toxicity was determined using the NCI common toxicity criteria and efficacy was assessed using serial volumetric brain scans. *Results*: 9-AC was administered to 59 patients, 31 with newly diagnosed glioblastoma multiforme and 28 with recurrent high grade astrocytomas. No grade III–IV myelosuppression was noted in the 29 patients (128 cycles) on phenytoin, carbamazepine, phenobarbital, and/or valproic acid who received 850 μg/m^2 per 24 h. In contrast, two of three patients (five cycles) who were not taking anticonvulsants developed grade IV myelosuppression. Steady-state total 9-AC plasma levels were lower in patients on anticonvulsants (median 25.3 n*M*) than in patients who were not taking anticonvulsants (median 76.5 n*M*). Dose escalations performed in 27 additional patients determined the MTD in patients taking anticonvulsants to be 1776 μg/m^2 per 24 h for patients with newly diagnosed tumors and 1611 μg/m^2 per 24 h for patients with recurrent disease. *Conclusions*: We describe a new and unexpected drug interaction between 9-AC and anticonvulsants. This is similar to recent findings with paclitaxel, and suggests that higher than "usual" doses of some chemotherapeutic agents are required in patients on anticonvulsants. Prospectively defined dose escalations and pharmacologic studies are essential for the careful evaluation of new chemotherapeutic agents in patients with brain tumors.

Key words Primary brain tumor · Glioblastoma multiforme · High grade astrocytoma · 9-Aminocamptothecin · Anticonvulsants

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Introduction

More than 50% of the 15 000 primary brain tumors diagnosed each year in the United States are high grade astrocytomas. These are often referred to as anaplastic astrocytomas (grade III astrocytomas) or glioblastoma

multiforme (Grade IV astrocytomas). The incidence of and the mortality from these tumors appear to be rising, particularly among the elderly.[15] Despite aggressive surgical resection, radiation therapy, and chemotherapy, the prognosis for patients with high grade astrocytomas remains poor. Long-term survivors are rare and the median survival is usually less than 1 year.

Systemic chemotherapy for malignant gliomas has been shown to be of marginal benefit [12, 41]. The most active agents in this disease are the nitrosoureas, procarbazine, and cisplatin. Combination chemotherapy has not been shown to more efficacious than single agents, except perhaps in patients with anaplastic astrocytomas treated with procarbazine, CCNU and vincristine [24]. New and more effective chemotherapeutic agents are needed to improve the outcome for these patients.

In an effort to foster the evaluation of new therapeutic approaches for these malignancies, the National Cancer Institute (NCI) funded CNS Consortia in 1994. The New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium is screening new agents for activity in adults with measurable newly diagnosed and recurrent high grade astrocytomas using volumetric neuro-imaging studies to define responses. It has recently completed a study of paclitaxel and discovered that patients on hepatic enzyme-inducing antiepileptic drugs (EIAED) require 170% of the conventional maximum tolerated dose (MTD) of this agent to achieve blood levels and toxicities that are comparable to those who are not taking these agents [11]. This is probably a consequence of induction of the hepatic P450 system which is known to play a major role in the metabolism of paclitaxel.

This report describes the NABTT CNS Consortium's experience with another promising new antineoplastic agent, 9-aminocamptothecin (9-AC), in patients with high grade astrocytomas. As this agent has no known metabolites, it was assumed that the MTD for patients with primary brain tumors who are receiving EIAED and patients with systemic cancers would be virtually identical. However, our studies have shown that the MTD in patients receiving EIAED is approximately twice that recommended by the NCI based on phase I studies in patients with systemic malignancies. This report describes the toxicity and pharmacology of the dose escalation (phase I) portion of two clinical trials of 9-AC in patients with high grade astrocytomas. Studies of the efficacy of this agent in these tumors are currently under evaluation by the NABTT CNS Consortium at the new MTDs defined by the results reported here.

Methods

The 59 patients discussed in this report were on two similar clinical trials which were conducted simultaneously by the NABTT CNS Consortium. The first was for adults with newly diagnosed glioblastoma multiforme while the second was for patients with recurrent anaplastic astrocytoma or glioblastoma multiforme. Patients on each protocol were to receive 9-AC at a dose of 850 $\mu g/m^2$ per day for 3 days as a continuous intravenous infusion to assess the response rate of high grade astrocytomas to this chemotherapeutic agent. Once it became apparent that the MTD suggested by the NCI's Cancer Therapy Evaluation Program (NCI/CTEP) was associated with minimal toxicity in this patient population, dose escalations were initiated using the continual reassessment method (CRM).

Each of these protocols and all subsequent protocol amendments were reviewed and approved by NCI/CTEP and the institutional review board of each participating institution (Brown University, Columbia University, Henry Ford Hospital, Johns Hopkins University, Massachusetts General Hospital, Moffitt Cancer Center, Northwestern University, and Wake Forest University). Informed consent was obtained from each patient participating in these studies.

Protocol for adults with newly diagnosed glioblastoma multiforme

Eligibility criteria

To be eligible for the newly diagnosed protocol patients had to: (1) be 18 years of age or older, (2) have a histologically confirmed supratentorial grade IV astrocytoma (glioblastoma multiforme), (3) have measurable contrast-enhancing tumor on the postoperative pretreatment MRI or CT scan, (4) have received no prior radiation therapy, chemotherapy, hormonal therapy, immunotherapy or therapy with biologic agents for the brain tumor, (5) have a Karnofsky status $\geq 60\%$, (6) have normal hematologic, renal and liver function (absolute neutrophil count $\geq 1500/mm^3$, platelets $\geq 100\,000/mm^3$, creatinine ≤ 1.7 mg/dl, total bilirubin ≤ 1.5 mg/dl, transaminases not more than four times above the upper limits of the institutional norm), (7) be able to give informed consent, (8) be free of other serious medical problems which could jeopardize the ability of the patient to safely receive the 9-AC, (9) have no active concomitant malignancy other than curatively treated carcinoma of the cervix in situ or basal cell carcinoma of the skin, (10) not be pregnant or breastfeeding and be willing to follow acceptable birth control methods to avoid conception, and (11) have no prior history of hemorrhagic cystitis. Patients with uncontrolled hypertension, angina pectoris, or cardiac arrhythmias or with known hypersensitivity to *E. coli* were excluded from this trial.

Study objectives

The original primary objective of this study was to estimate the response rate to 9-AC administered as a 72-h infusion prior to radiation in patients with newly diagnosed supratentorial glioblastoma multiforme. 9-AC was to be administered at a dose of 850 $\mu g/m^2$ per day, which was thought to be the MTD. In addition, this study was designed to assess the proportion of patients surviving more than 1 year following institution of therapy, to estimate the duration of disease-free progression and survival with this treatment regimen, to seek correlations between estimates of important pharmacokinetic parameters with toxicity and/or drug activity in this patient population, and to provide data on the toxicity of cranial irradiation following 9-AC.

Treatment plan

The treatment approach, which consisted of preirradiation chemotherapy, was modeled on the experience at Johns Hopkins using BCNU and cisplatin in newly diagnosed high grade astrocytomas [18]. The initiation of 9-AC therapy was permitted as soon after craniotomy or biopsy as medically appropriate. A postoperative pretreatment MRI or CT scan was obtained prior to beginning 9-AC. Patients were to receive six cycles of 9-AC, 2 weeks apart with volumetric scans performed before every other treatment cycle (i.e. prior to cycles one, three and five). Once hematologic toxicity from the sixth cycle of 9-AC had resolved, a preirradiation volumetric

scan was obtained and standard radiation therapy was administered. If the patient deteriorated or tumor volumetrics demonstrated progression, 9-AC was discontinued and radiation therapy was instituted immediately. All patients were followed clinically and radiologically every 2 months following completion of their radiation therapy until tumor progression was evident.

9-AC preparation and administration

9-AC was provided by the NCI. Each ampule of 9-AC contained 5 mg 9-AC diluted in 1 ml 5 mg/ml dimethylacetamide solution. These were stored at refrigerator temperatures in the intact ampules until the drug was needed. Each vial of 9-AC was accompanied by one 49-ml amber vial of diluent containing 51% polyethylene glycol 400 and 49% 0.01 *M* phosphoric acid buffer (PEG/PA). When 9-AC was needed, 1 ml of the 9-AC concentrate was added to the special diluent vial using a glass syringe. The resulting mixture contained 100 μg/ml of 9-AC in a 2% dimethylacetamide, 50% polyethylene glycol 400, and 48% 0.01 *M* phosphoric acid solution. The final solution of 9-AC was filtered through a 5 μm filter. The total 72-h dosing volume was administered using a CADD infusion pump. Drug sterility and stability studies had shown that 9-AC was stable for 72-h and the FDA approved the use of a single 72 h cassette for this purpose. 9-AC was diluted with additional PEG/PA diluent to reach a volume suitable for infusion with the CADD pump. Additional diluent was used to flush central venous catheters to displace any aqueous solution.

The starting dose of 9-AC for this study was 850 $\mu g/m^2$ per 24 h based on actual body weight administered continuously for 72 h every 2 weeks. This was the recommended phase II dose on an every-2-week dosing schedule based on phase I studies and updated phase II data [7, 32]. 9-AC was administered in the hospital or in an outpatient setting using a central venous catheter and a Pharmacia CADD pump. As this drug is not compatible with standard infusion fluids it was not administered with other fluids through an existing intravenous catheter.

Pharmacokinetics

Steady-state (Css) 9-AC plasma concentrations measuring total 9-AC levels (lactone plus carboxylate) were obtained during the first cycle of chemotherapy. One blood sample was obtained prior to drug administration. Following the institution of 9-AC, two samples were collected 1 h apart on days 2 and 3 of the infusion when 9-AC levels should have reached steady state. Each sample was collected in a 7-ml heparinized tube, immediately placed in an ice bath, centrifuged at 4 °C at 1000 *g* for 10 min, and the plasma was stored at −70 °C in a polypropylene tube. The specimens were mailed on dry ice to the NABTT Operations Office. The total 9-AC concentrations were determined in batches by a reversed-phase HPLC assay with post-column acidification in the Pharmacology Laboratory of The Johns Hopkins Oncology Center [33].

Supportive care issues

A dose of dexamethasone sufficient to control peritumoral edema was determined on clinical grounds for each patient before beginning the first cycle of 9-AC. An effort was made to keep the patient on this steroid dose until the next scan, as changing steroid doses can complicate the interpretation of response [16, 43]. Corticosteroid doses were tapered as clinically indicated if the patient responded to therapy as judged by serial scans. Antiemetics were administered as needed. Dexamethasone was not used as an antiemetic because of its potential effects on blood–brain barrier integrity.

Radiation therapy

Conventional radiation therapy was begun when serial MR or CT scans showed progression on 9-AC or after the patient completed six cycles of 9-AC. The radiation consisted of treatment to the tumor plus a generous margin to a total of 6000 cGy in 30 fractions. Only patients with supratentorial tumors were eligible for this study to ensure that the radiation techniques were similar. In patients with responsive or stable disease, treatment planning was performed on the basis of the initial on-study MR/CT scan. For patients progressing during 9-AC therapy, the neuroimaging study that showed progressive disease was used for treatment planning.

Dose-limiting toxicities, dose modifications, and the use of growth Factors

Major toxicities for this protocol were defined as nonhematologic or hematologic. The dose-limiting nonhematologic toxicities were grades III–IV in severity as specified by the NCI Common Toxicity Criteria. However, nausea and vomiting without sufficient antiemetic prophylaxis and alopecia were not considered major toxicities. Unresolved nonhematologic toxicities that delayed the subsequent course of chemotherapy by more than 7 days were also considered major toxic events. Hematologic toxicities were viewed as major toxicities if the absolute neutrophil count was less than 500/μl for 3 or more days, the platelet count was less than 25 000/μl, febrile neutropenia occurred, or if there was more than a 7-day delay in the subsequent course of chemotherapy because of continued myelosuppression.

If these major toxicities occurred, the dose of 9-AC would be decreased to 700 $\mu g/m^2$ per day. If major toxicities persisted at this dose the administered dose would be further reduced to 600 $\mu g/m^2$ per day. If there were only minimal toxicities (≤ grade I neutropenia or thrombocytopenia and/or ≤ grade II absolute neutrophil count) after three courses of 9-AC at 850 $\mu g/m^2$ per day, the 9-AC dose could be escalated to 1000 $\mu g/m^2$ per day. G-CSF was not used prophylactically in this protocol in accordance with the recommendation of the NCI/CTEP. Clinicians caring for patients on this protocol were permitted to use G-CSF to provide optimal care for patients with severe neutropenia in accordance with the 1994 ASCO guidelines for the use of these agents. Thus, G-CSF was used in an acute setting of neutropenia, but not with subsequent cycles or in lieu of a dose reduction.

Quality assurance

Serial MRI and CT scans for all patients treated at the MTD were required to be centrally reviewed. Likewise, the pathology of all responding patients were reviewed at the NABTT Central Operations Office.

Protocol for adults with recurrent high grade astrocytomas

Eligibility criteria

To be eligible for the recurrent disease trial patients had to: (1) be 18 years of age or older, (2) have histologically proven anaplastic astrocytoma or glioblastoma which was progressive or recurrent following radiation or chemotherapy, (3) have measurable disease, (4) have an estimated life expectancy of more than 2 months, (5) have received one or fewer prior chemotherapy regimens which could not include a topoisomerase inhibitor, (6) have a Karnofsky status of ≥60%, (7) have normal hematologic, renal and liver function (absolute neutrophil count ≥1500/mm^3, platelets ≥100 000/mm^3, creatinine ≤1.7 mg/dl, total bilirubin ≤1.5 mg/dl, transaminases not more than four times above the upper limits of the institutional norm), (8) be able to give informed consent, (9) be free of other serious concurrent infection or other medical illness which could jeopardize the ability of the patient to safely receive the 9-AC, (10) have no active concomitant malignancy other than curatively treated carcinoma of the cervix in situ or basal cell carcinoma of the skin, (11) not be pregnant or breastfeeding and be willing to follow acceptable birth control methods to avoid

conception, and (12) have no prior history of hemorrhagic cystitis. Patients with uncontrolled hypertension, angina pectoris, evidence of uncontrolled cardiac arrhythmias or known hypersensitivity to *E. coli* were excluded from this trial.

Study objectives

The primary objective of this study was to assess the response rate to 9-AC in adults with recurrent or progressive malignant glioma. The study was also designed to determine the time to progression, to seek correlations between steady state 9-AC levels with toxicity and to determine the toxicity of 9-AC in this patient population.

Treatment plan

Patients were to receive 9-AC as described in the newly diagnosed protocol and to continue on these treatments every 2 weeks until there was evidence of tumor progression on serial CT or MR images. The 9-AC preparation and administration, initial doses and dose modifications, 9-AC pharmacokinetics, supportive care, and quality assurance were conducted as described in the newly diagnosed protocol. As this group of patients had already received cranial irradiation, this was not a part of this protocol.

Dose escalation using the continual reassessment method

Patients on each of these protocols were to receive 9-AC at a fixed dose of 850 $\mu g/m^2$ per 24 h for 3 days as a continuous intravenous infusion to assess the response rate of high grade astrocytomas to this chemotherapeutic agent. Once it became apparent that this dose and the escalated dose of 1000 $\mu g/m^2$ per 24 h were associated with minimal toxicity in patients on anticonvulsants, dose escalations using a modified version of the CRM were initiated [26]. Aside from the dose escalations, the overall treatment plan was not altered.

Escalations occurred separately for each protocol (newly diagnosed and recurrent disease) and for patients who were and were not taking EIAED. For purposes of these studies, EIAED included phenytoin, carbamazepine, phenobarbital, primidone, felbamate, and valproic acid. Non-EIAED included gabapentin and lamotrigine. The use of dexamethasone was not factored into the dose escalations.

We employed a dose escalation scheme similar to the CRM recently proposed by Goodman et al. [14]. This was appealing because it was likely that the dose of 9-AC administered was not far from the true MTD. The modified CRM algorithm began with treatment of three patients at each dose level. Data from the patients who had received 800 and 1000 $\mu g/m^2$ per 24 h on these protocols and an initial dose projection using the CRM generated the next dose level of 1260 $\mu g/m^2$ per 24 h. After three patients were treated at this dose, all available data were modeled with a logistic dose response function. This model was used to calculate the dose associated with a toxicity rate of 30% from the first cycle of 9-AC. Dose increases were restricted to 1.5 times (150%) the maximum dose already administered. In this way, rapid dose escalations or testing high doses without a reasonable degree of clinical certainty concerning their safety were prevented. The process stopped and a new MTD was recommended for a formal determination of the efficacy of 9-AC in this patient population when the recommended dose using the CRM method changed by less than 10%.

This design is not routinely employed for phase I cytotoxic drug studies. However, it has been used for dose findings in several clinical trials and has been studied extensively by statistical simulation [26]. Furthermore, the design is particularly efficient when starting near the MTD and when a fair amount of dose response data are available. The design also permits de-escalation in the event that toxicities are observed more frequently than desired.

Results

Initial 32 patients on phase II protocols

The protocols for patients with newly diagnosed glioblastoma multiforme and for recurrent high grade astrocytomas began accruing patients at approximately the same time. The statistical section of these protocols specified a review after an initial cohort of patients to determine if there was sufficient efficacy for the trials to continue. The formal review for both of these protocols was conducted concurrently by the Central Operations Office of the NABTT CNS Consortium. A total of 32 patients were initially entered on these two protocols: 16 with newly diagnosed glioblastoma multiforme and 16 with recurrent high grade astrocytomas. The patients ranged in age from 27 to 75 years with a median age of 58 years; 64% were male and 88% were Caucasian. Only one partial response was reported from the treating institutions in a patient with a recurrent high grade astrocytoma. The low response rate to 9-AC in these patients suggested that further evaluation of this agent in high grade astrocytomas was unlikely to be productive.

A total of 133 cycles of 9-AC were administered to these 32 patients. The 16 patients with newly diagnosed glioblastoma multiforme received 50 cycles of 9-AC; 37 cycles were administered at a dose of 850 $\mu g/m^2$ per 24 h and 13 at 1000 $\mu g/m^2$ per 24 h. The 16 patients with recurrent disease received 83 cycles of 9-AC; 61 at 850 $\mu g/m^2$ per 24 h, 21 at 1000 $\mu g/m^2$ per 24 h, and 1 at 700 $\mu g/m^2$ per 24 h which was a dose reduction secondary to myelosuppression. Following the first cycle of 9-AC, only two of the 32 patients experienced significant toxicities. One patient had grade III–IV myelosuppression while the other had grade III non-hematologic toxicity. When myelosuppression was analyzed as a function of EIAED use, two of three patients (five cycles) who were not taking EIAED developed grade IV myelosuppression. In contrast, no grade III–IV myelosuppression was noted in the 29 patients (128 cycles) on phenytoin (15), carbamazepine (7), phenobarbital (2), and/or valproic acid (3).

Steady state total 9-AC levels were assessed using reversed-phase HPLC with post-column acidification in 23 patients on these two protocols. Of these patients, 20 were on EIAED. 9-AC levels were lower (mean 40 n*M*, median 25.3 n*M*, SD 28.6) in patients on EIAED than in patients not taking EIAED (mean 74.7 n*M*, median 76.5 n*M*, SD 10.1).

These findings prompted the temporary closure of these protocols and a review of the data with the NCI. The protocols were revised to include separate phase I dose escalations for patients who were taking specified EIAED and for those who were not as described above in the Methods section. Dose escalations and de-escalations were made using the modified CRM described above. Once the MTD was determined in patients taking

Table 1 Dose-limiting toxicities (DLT) observed with cycle one of 9-AC in patients with newly diagnosed glioblastoma multiforme in relation to dose and anticonvulsant status (*EIAED* enzyme-induced antiepileptic drugs)

Dose level ($\mu g/m^2$)	No. of patients	Grade III–IV nonhematologic	Febrile neutropenia	Grade IV neutropenia >3 days	Platelets <25 000 per μl	Patients with DLT per dose level (%)
850 (−EIAED)	6	1	0	0	0	17
850 (+EIAED)	14	0	0	0	0	0
1000 (+EIAED)	7	0	0	0	0	0
1260 (+EIAED)	4	0	0	0	0	0
1740 (+EIAED)	3	0	0	1	0	33
1865 (+EIAED)	4	0	1	1	0	50

and not taking EIAED in newly diagnosed glioblastoma multiforme and in recurrent high grade astrocytomas, a standard phase II evaluation of efficacy was to begin. The results of the phase I portion of these trials are described below. The phase II studies of 9-AC at the newly defined MTD are currently underway through the NABTT CNS Consortium and will be reported separately.

Patients with newly diagnosed glioblastoma multiforme

A total of 31 patients with newly diagnosed glioblastoma multiforme were treated with 9-AC on this research protocol. Six patients who were not taking EIAED received a total of 13 cycles of 9-AC at a dose of 850 $\mu g/m^2$ per 24 h (Table 1). Five of these patients were not taking any EIAED and one was receiving gabapentin. One of six patients developed grade III diarrhea during the first cycle of 9-AC which was a dose-limiting toxicity.

A total of 14 patients taking EIAED were treated at a dose of 850 $\mu g/m^2$ per 24 h. No hematologic or nonhematologic grade III–IV toxicities were observed at this dose. EIAED taken by these patients included phenytoin (eight), carbamazepine (four), and valproic acid (two). Seven patients received 9-AC at a dose of 1000 $\mu g/m^2$ per 24 h. All of these patients had been dose escalated from 850 $\mu g/m^2$ per 24 h. No grade III–IV toxicities were noted at 1000 $\mu g/m^2$ per 24 h. Four of these patients were on phenytoin, two were on valproic acid, and one was on carbamazepine.

The next dose selected by the CRM was 1260 $\mu g/m^2$ per 24 h. Four patients received this dose. All were receiving phenytoin and none experienced grade III–IV toxicities. The next dose level was 1865 $\mu g/m^2$ per 24 h. Two of the four patients at this dose developed dose-limiting toxicities. One had a febrile neutropenia and the second had grade IV myelosuppression lasting for over 3 days and grade II stomatitis. The next dose level selected by the CRM was 1740 $\mu g/m^2$ per 24 h. Three patients were treated at this dose level and all were receiving phenytoin. One of the three developed grade IV myelosuppression lasting for over 3 days. The dose-limiting toxicities for the newly diagnosed patients are presented Table 1. The data from these dose escalations were modeled using the CRM to establish an MTD of 1776 $\mu g/m^2$ per 24 h for previously untreated patients taking EIAED.

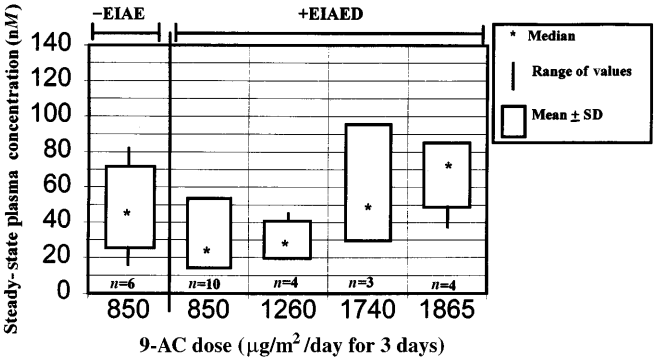

Fig. 1 Steady state plasma concentrations of 9-AC observed with cycle one of 9-AC in patients with newly diagnosed glioblastoma multiforme in relation to dose and anticonvulsant status (*EIAED* enzyme-inducing antiepileptic drugs)

Steady-state total 9-AC levels were obtained in six patients treated at 850 $\mu g/m^2$ per 24 h who were not taking EIAED, in ten patients treated at 850 $\mu g/m^2$ per 24 h while taking EIAED, in four patients treated at 1260 $\mu g/m^2$ per 24 h, in three patients treated at 1740 $\mu g/m^2$ per 24 h and in four patients treated at 1865 $\mu g/m^2$ per 24 h. The median and range of total 9-AC levels in patients taking or not taking EIAED are presented in Fig. 1.

Patients with recurrent high grade astrocytomas

A total of 28 patients with recurrent high grade astrocytomas received 9-AC on this protocol (Table 2). Three patients who were not taking EIAED received a dose of 850 $\mu g/m^2$ per 24 h. One patient developed grade IV myelosuppression.

Fifteen patients taking EIAED were treated at a dose of 850 $\mu g/m^2$ per 24 h. No hematologic or nonhematologic grade III–IV toxicities were observed. The EIAED these patients were taking were as follows: phenytoin (seven), carbamazepine (five), phenobarbital (three), and valproic acid (two). Six patients received 9-AC at a dose of 1000 $\mu g/m^2$ per 24 h after being dose escalated from

Table 2 Dose-limiting toxicities (DLT) observed with cycle one of 9-AC in patients with recurrent high grade astrocytomas in relation to dose and anticonvulsant status (*EIAED* enzyme-induced antiepileptic drugs)

Dose level ($\mu g/m^2$)	No. of patients	Grade III–IV nonhematologic	Febrile neutropenia	Grade IV neutropenia > 3 days	Platelets < 25 000 per μl	Patients with DLT per dose level (%)
850 (−EIAED)	3	0	1	0	0	33
850 (+EIAED)	15	0	0	0	0	0
1000 (+EIAED)	6	0	0	0	0	0
1260 (+EIAED)	6	0	0	1	0	17
1611 (+EIAED)	3	0	0	1	0	33

850 $\mu g/m^2$ per 24 h. One patient developed a grade III anemia. Four of these patients were receiving phenytoin and two were receiving carbamazepine. One of six patients treated at a dose of 1260 $\mu g/m^2$ per 24 h developed grade IV myelosuppression lasting for more than 3 days with the first cycle of 9-AC. All of these patients were receiving phenytoin and two patients were also taking a second EIAED (carbamazepine and valproic acid). Four patients were treated at a dose of 1611 $\mu g/m^2$ per 24 h; however the toxicities in one patient could not be assessed because of missing data. One of the three evaluable patients at this dose was receiving phenytoin alone, one was taking phenytoin and phenobarbital, and the third was receiving phenytoin and gabapentin. One of these patients developed grade IV neutopenia which persisted for more than 3 days. The dose-limiting toxicities for these patients are presented in Table 2 and for all patients on these protocols in Fig. 2. The MTD for previously treated patients taking EIAED was 1611 $\mu g/m^2$ per 24 h while the MTD for patients who were not taking EIAED has yet to be determined.

Steady-state total 9-AC levels were obtained in three patients treated at 850 $\mu g/m^2$ per 24 h who were not taking EIAED, in ten patients treated at 850 $\mu g/m^2$ per 24 h who were taking EIAED, in six patients treated at 1260 $\mu g/m^2$ per 24 h, and in four patients treated at 1611 $\mu g/m^2$ per 24 h. The steady-state total 9-AC levels in these patients are presented graphically in Fig. 3 in relation to dose and EIAED status. Dose-limiting toxicities were noted only in patients with mean total 9-AC levels above 50 n*M*.

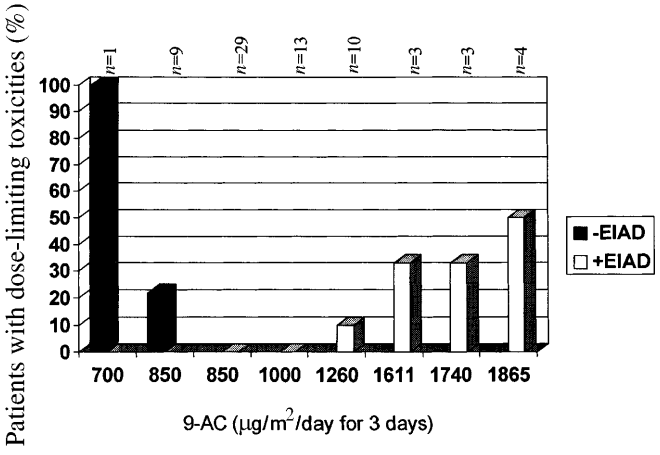

Fig. 2 Percentage of all patients (newly diagnosed and recurrent disease) with dose-limiting toxicities following one cycle of 9-AC in relation to dose and anticonvulsant status (*EIAED* enzyme-inducing antiepileptic drugs)

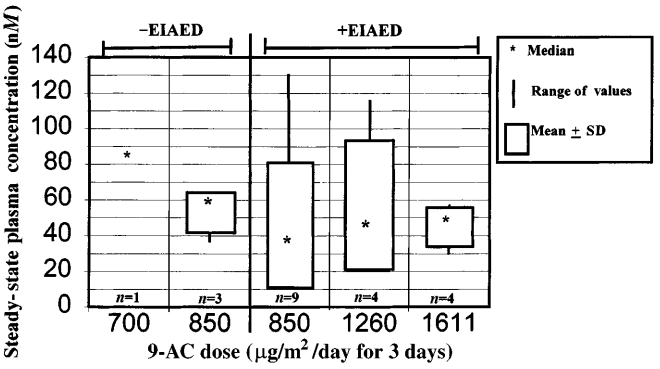

Fig. 3 Steady-state plasma concentrations of 9-AC observed with cycle one of 9-AC in patients with recurrent high grade astrocytomas in relation to dose and anticonvulsant status (*EIAED* enzyme-inducing antiepileptic drugs)

Pharmacologic analyses

Total 9-AC clearance was calculated in patients receiving and not receiving EIAED using the average of four samples collected on days 2 and 3 to estimate the Css. The mean clearance was 38 ml/min per m^2 for patients not receiving EIAED and 64 ml/min per m^2 in patients receiving EIAED. We assumed these patients had achieved steady-state concentrations based on the recently reported elimination half-lives of 4.5 h for the lactone and 8.4 h for 9-AC [39]. This was further evaluated by comparing average 9-AC levels from day 2 and day 3 of the 72 h infusion. Twelve patients had concentrations that rose by 30% or more on day 3, 5 patients had 9-AC levels that fell by 30% or more, and 31 patients had levels that did not vary by more than 30%. Two sample sets were discarded because measured concentrations were very high, suggesting that the samples probably were drawn from the site at which the drug was infusing. The average 9-AC clearances in patients receiving and not receiving EIAED at the 850 $\mu g/m^2$ per day dose level were not significantly different.

This requires further investigation given the relatively small number of patients studied at this dose level and the large variance in values which have also been noted in previous pharmacokinetic studies of this agent [39].

Discussion

This report describes results from two studies designed as conventional phase II studies to evaluate the efficacy of 9-AC in high grade astrocytomas. However, once 14 patients with newly diagnosed glioblastoma multiforme and 15 patients with recurrent high grade astrocytomas were treated, it was evident from the lack of observed myelosuppression that these studies needed to be redesigned. The MTD of 850 $\mu g/m^2$ per 24 h for 3 days, which had been established in phase I studies in patients with systemic malignancies, was too low for patients with brain tumors receiving concomitant EIAED. Using the CRM, the dose of 9-AC was escalated and an MTD of 1776 $\mu g/m^2$ per 24 h for 3 days was established for newly diagnosed patients and 1611 $\mu g/m^2$ per 24 h for 3 days for patients with recurrent disease. These doses are 209% and 180% of the MTD for patients with systemic malignancies.

These results are remarkably similar to those obtained by the NABTT CNS Consortium in their recently completed study of paclitaxel in a nearly identical patient population [11]. The MTD of paclitaxel in patients who were not taking EIAED was 140 mg/m^2 as a continuous intravenous infusion over 96 h, which is the recommended MTD for this infusion schedule in patients with systemic malignancies. However, the MTD in patients taking EIAED was 200 mg/m^2.

9-AC is an analogue of the topoisomerase I inhibitor, camptothecin, which is an alkaloid extracted from the Chinese plant *Camptotheca acuminata* [42]. Early studies of camptothecin demonstrated that it inhibits RNA and DNA synthesis in a variety of animal and human tumor cell lines in vitro and in vivo [13]. In the 1970s, phase I and II trials were conducted using the camptothecin salt. These demonstrated minimal antitumor activity and significant bladder toxicity. However, there was renewed interest in camptothecin and its analogues once it became clear that the inhibition of DNA topoisomerases plays a role in cytotoxicity [4, 7, 19, 21, 22, 30, 32].

Several camptothecin analogues have been synthesized, including CPT-11, topotecan, and 9-AC [27]. 9-AC has demonstrated high antitumor activity against breast and lung tumors, melanoma xenografts, hepatic metastases from primary colon cancer, and intraperitoneally implanted leukemia and sarcoma [25, 28]. In animals, 9-AC appears to be rapidly absorbed following subcutaneous and oral dosing and is widely distributed throughout the body [8, 9, 36]. In mice, about half of the administered 9-AC dose is excreted in the urine and feces [34, 35]. Under physiological conditions the lactone moiety of 9-AC undergoes a rapid and reversible pH-dependent conversion to a carboxylated open-ring form which does not inhibit topoisomerase I activity [10, 40]. At equilibrium at pH 7.4, the open-ring form predominates. No metabolites of 9-AC have yet been identified [37, 38].

Several phase I dose-finding studies have been completed with 9-AC. In one, 9-AC was administered as a 72-h continuous infusion every 3 weeks, with a starting dose of 5 $\mu g/m^2$ per h [31]. Of the 30 patients who entered this study, all but one had received prior treatment. The major toxicity observed was hematologic, with 13/30 patients having grade III or IV leukopenia (five of which were dose limiting). Eight of the 30 patients had grade III or IV thrombocytopenia. Other toxicities included fever with neutropenia, nausea/vomiting, and mucositis. Alopecia was reported in the majority of patients. The MTD on an every-3-week regimen, without growth factor support, was 45 $\mu g/m^2$ per h. The dose-limiting toxicity was leukopenia. There was also significant thrombocytopenia at the MTD. Minor responses were reported in patients with colon, lung and gastric adenocarcinoma.

In another phase I study the dosing schedule was every 2 weeks [6]. Of 44 patients entered, most if not all were previously treated. Based on the first part of the study which did not include G-CSF, the recommended dose was 35 $\mu g/m^2$ per h every 2 weeks. Granulocytopenia was the dose-limiting toxicity. Alopecia and nausea/vomiting were observed frequently at doses of 35 $\mu g/m^2$ per h and higher. Mucositis and diarrhea were also reported, but were not common. When the protocol was amended to include G-CSF administration, doses as high as 74 $\mu g/m^2$ per h were administered. However, both patients treated at this dose had grade IV thrombocytopenia and neutropenia. Several patients also had decreases in hemoglobin requiring transfusions. Three minor responses were observed in this study, one each in ovarian, colorectal and non-small-cell lung cancer.

Based upon the above phase I data, a number of phase II trials of 9-AC were initiated. Febrile neutropenia and grade IV thrombocytopenia were consistently noted in patients who received a dose of 59 $\mu g/m^2$ per h for 72 h with G-CSF. As a result, the NCI issued updated guidelines on 9-AC dosing for ongoing phase II trials with G-CSF of 1100 $\mu g/m^2$ per 24 h (45 $\mu g/m^2$ per h) with dose reductions to 900, 700 and 600 $\mu g/m^2$ per 24 h (37.5, 29.1, 25 $\mu g/m^2$ per h) as needed. Without the use of prophylactic G-CSF, a starting dose of 850 mg/m^2 per day for 3 days (approximately 35 $\mu g/m^2$ per h) administered as a continuous infusion every 2 weeks was recommended. This schedule and CTEP's suggested dose modifications formed the basis for the clinical protocols reported here.

There are preliminary pharmacologic data from a National Naval Medical Center phase I study which used an every-2-weeks schedule in patients with solid tumors [7, 27]. Following a 72-h continuous infusion, steady-state concentrations were achieved within 48–72 h. Plasma lactone concentrations declined in a biphasic fashion with an apparent mean terminal elimination

half-life of 9 h. Estimates based on total drug showed a half-life of 5–6 h. This may reflect faster systemic clearance of the open-ring carboxylate form. Over a dose range of 5–59 μg/m^2 per h, C_m values of lactone were found to be relatively proportional. Lactone levels following the 50 or 59 μg/m^2 per h infusion (doses near the MTD) were in the 6–8 n*M* (2.1–2.9 ng/ml) range. Systemic clearance of 9-AC lactone appeared dose-independent, and over the dose range of 5–59 μg/m^2 had a mean of 26 l/h per m^2. The carboxylate to lactone (C/L) ratio in three patients receiving 47 μg/m^2 per h infusion varied between 7 and 13. The most recent pharmacodynamic studies of 9-AC demonstrate a sigmoidal Emax correlation between myelosuppression and 9-AC lactone levels. In the initial phase I study (every 3 weeks), the Css was approximately 50 n*M* at the 35, 45 and 60 μg/m^2 per h doses and drug elimination was similar to that found in the other study.

No available data on 9-AC suggest that activation of the hepatic P450 system by EIAED would significantly affect the pharmacology of this agent. In addition, the agents defined by these protocols as +EIAED have a wide range of P450 activation. Thus, the reason our patients tolerated significantly higher doses 9-AC doses has yet to be determined. Regardless of the mechanisms, 9-AC could easily have been dismissed as "another inactive agent" in high grade astrocytomas were it not for careful attention to the toxicities noted at the initial dose and correlative pharmacologic studies. Similarly, paclitaxel could have been deemed ineffective at the 140 mg/m^2 dose without having received an adequate trial at the true MTD.

These observations have significant implications for patients with brain tumors. Typically doses of new agents used in phase II brain tumor studies are taken directly from phase I studies conducted in patients who are not taking EIAED. EIAED-induced alterations in the metabolism of chemotherapy drugs as different as paclitaxel and 9-AC suggest that other drugs may have been deemed ineffective without having been studied at their true MTD. Unfortunately, a review of the literature reveals that EIAED use and the extent of myelosuppression, a reasonable surrogate for adequate dosing for many agents, are poorly documented in published reports of phase II studies in brain tumors [29]. Furthermore, the results of many negative trials remain unpublished. As a result, some agents which were previously reported as ineffective in patients with brain tumors may need to be reevaluated at higher doses.

The doses for some antineoplastic drugs which are currently administered to patients with brain tumors may also need to be reviewed. For example, dibromodulcitol, procarbazine and 5-fluorouracil have some reported efficacy in high grade astrocytomas, but are also metabolized by the hepatic microsomal system [1, 2, 5, 20, 23, 24]. These agents are often reported to be "well tolerated" in patients with primary brain tumors, but may be prescribed at subtherapeutic doses in patients on EIAED. Other antineoplastic agents used in patients with brain tumors also have some degree of hepatic metabolism, such as the nitrosoureas and hydroxyurea. Further studies are needed to determine whether the pharmacology of these agents is affected by EIAED. The complexity of these drug interactions is highlighted by the observation that chemotherapeutic agents also affect the levels and efficacy of several commonly used EIAED [17]. Careful pharmacologic studies and dose escalation plans appear critical in the evaluation of new agents in patients with brain tumors.

These findings also have implications for patients with neoplasms that originate outside of the central nervous system. Patients with systemic cancers also receive EIAED for seizure disorders, brain metastases, and neuropathic pain. In addition, other drugs can affect the hepatic microsomal system thereby altering blood levels of antineoplastic agents. These drug interactions could be responsible for the wide variations in toxicity and efficacy seen in patients receiving the same dose of chemotherapy. Further research in needed to better understand the clinical importance and the precise mechanisms involved in these drug interactions.

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