Phase II and Pharmacokinetic Study of Aziridinylbenzoquinone [2,5-Diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone, Diaziquone, NSC 182986] in High-Grade Gliomas

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ABSTRACT

2,5-Diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone (AZQ; Diaziquone, NSC 182986) is a rationally designed antitumor drug possessing sufficient lipid solubility to allow penetration into the central nervous system. Thirty-one patients with high-grade glioma and progressive disease following radiation, with or without previous chemotherapy, have been treated with 144 cycles of drug, consisting of 20 mg/sq m given as an i.v. infusion on Days 1 and 8 of a 28-day cycle. Responses were measured by serial computer tomography scanning. Of the 28 evaluable patients, 6 (21%) had limited improvement (10 to 40% reduction in tumor size) on computer tomography scan, 10 (36%) had disease stabilization, and 12 (43%) had progressive disease. The drug was well tolerated clinically, with little acute toxicity. The major toxicity was myelosuppression, which appeared cumulative, using this dose regimen. AZQ was measurable in both tumor tissue and tumor cyst fluid in patients on therapy. Plasma samples taken during the period of infusion confirm that 50% or more of the total AZQ exposure occurs during the infusion period. AZQ behaves as intended by design and demonstrates activity in this poor-prognosis group of patients.

INTRODUCTION

AZQ2 is a rationally designed antitumor agent with sufficient lipid solubility and low ionization properties to allow penetration into the central nervous system, while retaining adequate aqueous solubility for drug formulation (3, 6, 8, 16). In preclinical testing, AZQ demonstrated broad murine antitumor activity, inhibiting growth of B16 melanoma, colon 26, colon 28, CD8F mammary, L1210, and P388 tumors, as well as human mammary and colon tumor xenografts (3). Of interest was the finding that AZQ caused significant increases in life span and some cures in mice with ependymoblastoma, and intracranially implanted L1210 and P388 tumors (3).

The mechanism of action of AZQ remains uncertain. The structure suggests alkylating potential, and compounds of this class are known to cross-link DNA and inhibit DNA synthesis with little effect on RNA or protein synthesis (1,9). More recently, Gutierrez et al. (14) have shown that AZQ is capable of forming free radical species, and either this free radical or the Superoxide anion has been shown to be important to cytotoxicity.

Previous pharmacokinetic studies in both monkeys (13) and humans (4, 18, 19) have confirmed that AZQ demonstrates excellent cerebrospinal fluid penetration. In addition, Phase I studies with this drug suggested activity in glioblastoma (19). This Phase II study further defines (a) clinical activity in high-grade glioma, (b) pharmacokinetics with measurements of drug levels during infusion, and (c) drug distribution with measurement of levels in brain tumor and tumor cyst fluid.

MATERIALS AND METHODS

Clinical Information. Thirty-one patients, 21 men and 10 women, ranging in age from 22 to 72 years (mean age, 44 years), were entered onto study. All patients had pathological confirmation of grade 3 or 4 glioma, and 11 had undergone subtotal tumor resection. All patients had prior cranial irradiation, 3 with concurrent administration of bromodeoxyuridine (a radiosensitizing agent). In all, 17 patients had been previously treated with chemotherapy, predominantly nitrosoureas. Written, informed consent was obtained from all patients.

Prior to beginning therapy, all patients underwent a complete physical examination with special attention to neurological signs. Pretreatment evaluation also included complete blood count with white cell differential and platelet count, prothrombin and partial thromboplastin times, and urinalysis. A brain CT scan was performed before treatment, and then every 3 months unless otherwise clinically indicated. Complete blood counts were performed weekly while the patient was on study, and serum chemistries were repeated on Day 1 of each cycle. All patients had a normal pretreatment hemogram with WBC greater than 4,000/cu mm, platelet count greater than 100,000/cu mm, as well as adequate renal and hepatic function, as determined by serum creatinine less than 2.0 mg/100 ml, and serum glutamic-oxaloacetic transaminase less than 100 units/100 ml.

Drug Formulation and Dosage. AZQ was provided in 10-ml vials containing 10 mg of drug. This was dissolved in 0.5 ml of N,N-dimethylacetamide and 9.5 ml of 0.01 M phosphate buffer, pH 6.5, to obtain a final concentration of 1 mg/ml. The prescribed dose (20 mg/sq m) was further diluted in 150 ml 0.9% NaCl solution, and administered i.v. on Days 1 and 8 of a 28-day cycle. Dose modifications were made according to WBC and platelet counts at nadir or on Day 1, whichever required the greater reduction, as follows: 75% initial dose for WBC nadir of 1,500 to 1,999/cu mm and platelet nadir of 75,000 to 99,999/cu mm; 50% for WBC nadir of 1,000 to 1,499/cu mm and platelet nadir of 50,000 to 74,999/cu mm, or Day 1 WBC of 2,000 to 2,999/cu mm and platelet count of 75,000 to 99,999/cu mm; 25% for WBC nadir of <1,000/cu mm and platelet nadir of <50,000/cu mm, or Day 1 WBC of 1,500 to 1,999 and platelet count of 50,000 to 74,999. Treatment was delayed one week if Day 1 WBC was <1,500 or platelet count was <50,000.

Pharmacological Studies. AZQ levels were measured in 2 patients' plasma, tumor cyst fluid, and tumor biopsy specimens, using a modifi-
cation of a previously described HPLC assay (15). In 5 additional patients, plasma levels were measured during constant drug infusion via external pump (I-Med Model 927 infusion pump; I-Med Corporation, San Diego, Calif.) with infusion times of 35 to 78 min. Samples of venous blood were drawn into 10-ml heparinized tubes prior to initiation of drug infusion, and at predetermined times during and after the completion of treatment. Blood samples were iced until centrifugation for 10 min at 2000 rpm in a Servall RC2 centrifuge (DuPont Instruments, Wilmington, Del.). The plasma was then decanted and stored at -20° until analysis. Tumor cyst fluid and brain tissue samples were immediately frozen in dry ice and stored at -20° until analysis.

AZQ in plasma was isolated and concentrated as previously described (15, 19), except for plasma samples where very low drug levels were expected. In these instances, two 1.0-ml aliquots of the same plasma sample were extracted simultaneously. The CHCl3 extracts were then combined before evaporation to dryness under N2. Resuspension of the residue in 0.5 ml 25% CH3CN-H2O resulted in a 4-fold concentration of AZQ. This procedure increased the limit of quantitation of the assay to 3 ng/ml, although even smaller amounts of AZQ could be detected. Tumor cyst fluid was extracted and handled in the same manner as plasma. Frozen brain tumor tissue was weighed and transferred to an all-glass-15-ml capacity Tenbroeck Tissue grinder and allowed to thaw. The tissue grinder was placed in an ice water bath, and 1.0 ml of 0.1 M phosphate buffer (pH 6.5) and 5 μl of 4.9 x 10^{-5} M 2,5-diamino-3,6-dichloro-1,4-benzaquinoine internal standard were added. The brain tissue was homogenized for 2 min and the homogenate was transferred to a 15-ml glass conical centrifuge tube. The tissue grinder was further rinsed with 0.5 ml phosphate buffer (pH 6.5), followed by 5.0 ml chloroform; both rinses were added to the homogenate, and sample preparation continued as for plasma.

The speed of the HPLC analysis was improved by use of a RCM-100 radial compression module (Waters Associates, Milford, Mass.) in conjunction with previously described instrumentation (15). A 5-μm Radial-Pak C18 Bondapak 8 x 100 mm; Waters Associates) was isocratically eluted with 25% CH3CN-H2O at 2.0 ml/min. The AZQ peak occurred reproducibly at 4.65 min and had a capacity factor (k') of 4.52. A standard curve for AZQ in plasma was prepared for each patient by the addition of known amounts of AZQ to that patient’s preinfusion plasma. Calculation of the standard curve as well as AZQ concentration in unknown samples was carried out as previously described (15). Correlation coefficients for the standard curves were greater than 0.998. The plasma standard curve was also used for tumor cyst fluid and brain tumor tissue.

Kinetic Calculations. The postinfusion Coad versus time data were fit to the bioexponential function representing a 2-compartment open model by an iterative nonlinear least-squares regression through the MLAB computer program (17). Based on observed assay characteristics, each data point was weighted by 1/Coad^2. Pharmacokinetic parameters were then calculated using standard equations incorporating infusion time, as outlined by Gibaldi and Perrier (12). Concentrations during the infusion were simulated from postinfusion data alone.

The AUC was determined by the linear trapezoidal rule from Time zero (beginning of infusion) until the last measured sample after the infusion. The remainder of the AUC was extrapolated to infinity by adding the term: 1.44 x Coad x t1/2d, where Coad is the last measured plasma concentration.

RESULTS

Responses. Thirty-one patients were treated with AZQ during this study. Three patients were lost to follow-up and cannot be considered evaluable. The remaining patients received a total of 142 cycles of therapy.

Responses were measured with serial CT scanning at 3-month intervals unless otherwise clinically indicated. Of the 28 evaluable patients, 6 (21%) had limited improvement (10 to 40% tumor reduction) on scan, 10 (36%) had stabilization of previously progressive disease, and 12 (43%) had progressive disease. Of interest, the patient in whom AZQ was detectable in tumor tissue at surgery had a good response to AZQ, and she remains alive with continued response 17 months after the treatment was initiated.

The characteristics of each group of patients are shown in Table 1. Responding patients tended to have less prior treatment and better initial Karnofsky performance status. Two of the responding patients have died at 4 and 5 months following the commencement of treatment. The remaining 4 patients (2 with grade 3 and 2 with grade 4 astrocytoma) remain alive at 8, 16, 17, and 22 months. All patients with progressive disease have expired, with a median survival of 4.0 months.

Toxicity. AZQ was well tolerated clinically. There were no drug-related deaths. Two patients did require hospitalization and i.v. antibiotics for fever and neutropenia. Only 3 patients experienced mild nausea, and 2 patients complained of diarrhea following AZQ treatment. The major dose-limiting toxicity using this schedule was myelosuppression, predominantly neutropenia. Twenty-nine % (8 of 28) of patients required dose modification of Cycle 2, and dose modification became increasingly necessary with further treatment cycles as follows: Cycle 3, 54% (7 of 13); Cycle 4, 54% (7 of 13); and Cycle 5, 67% (6 of 9).

Pharmacokinetics. Table 2 lists the concentrations of AZQ measured in tumor tissue and cyst fluid, together with the corresponding plasma AZQ levels for all samples examined from 2 patients given a 20-mg/sq m dose of the drug intraoperatively. Drug concentrations in tumor cyst fluid were found to approximate the corresponding plasma levels.

Chart 1 shows the Coad versus time curve for the one patient in whom measurable levels of AZQ were found in brain tumor tissue. The levels of AZQ in tumor cyst fluid and peripheral tumor tissue are indicated at the appropriate times.

The plasma decay curves were similar to those reported previously (4, 19, 21), with a rapid redistribution phase, followed by a slower elimination phase. Table 3 summarizes the pharmacokinetic parameters for the 5 patients receiving drug as a
constant infusion. It can be seen that in all but one case the majority of the total plasma AUC occurred during the infusion. AZQ total body clearance averaged 467 ± 57 (S.D.) ml/min/sq m, with less variance than in previous studies which did not sample during the infusion period (4, 19, 21). The apparent volume of distribution, equivalent to 28% of body space, was also in agreement with previous reports. The observed mean elimination half-life for the terminal phase (t1/2ß) of 30 ± 3 min also fell within the range of previous reports.

DISCUSSION

The currently available options for chemotherapy of high-grade malignant glioma are limited. In a cooperative study of 358 patients, the Brain Tumor Study Group was unable to demonstrate any significant improvement in survival for patients treated with nitrosoureas and radiotherapy or radiation alone, and recommended a continued search for effective chemotherapeutic regimens (22).

AZQ is a new synthetic antitumor agent which was designed at the National Cancer Institute by considering structure-activity relationships in aziridinylbenzoquinones, and by balancing aqueous and lipid solubility to allow both formulation and central nervous system penetration (6, 8, 9, 16). Because of its broad spectrum of activity in preclinical antitumor screening, AZQ has been entered into clinical trial. In a Phase I study of AZQ, Schilsky et al. (19) defined the maximal tolerated dose of AZQ on a Day 1 and Day 8 schedule, confirmed the presence of drug in cerebrospinal fluid of patients on therapy, and demonstrated drug activity in 2 patients with malignant glioma and deteriorating neurological status. This information has led to a number of Phase II trials of AZQ in brain tumor patients, with some encouraging preliminary results (2, 5, 7, 10, 11).

This study further confirms significant antitumor activity in patients with grades 3 or 4 glioma. Although 6 patients responded to AZQ with reduction of tumor size on CT scan, and 10 had stabilization of progressive disease, when these patients are stratified by clinical status it is obvious that they had better pretreatment performance status, and less prior therapy than patients with progressive disease. Only patients with documented regression of tumor on CT scan demonstrated clinical improvement (4 of 6) (Table 1).

The drug is well tolerated with negligible acute toxicity. Using a Day 1 and Day 8 administration schedule, dose-limiting toxicity was myelosuppression which appeared cumulative; dose modification became increasingly necessary with subsequent treatment cycles. As in our Phase I study (19), AZQ lowered hemoglobin as well as WBC and platelet counts. Again, these patients did not evidence hemolysis or blood loss, and anemia appeared to be secondary to suppression of RBC production. It will be informative to see whether similar toxicities are observed using alternate dosing regimens.

This study also offers further evidence that unchanged AZQ penetrates central nervous system tumors (Table 2). Previous work has shown that the drug crosses the blood-brain barrier to achieve considerable levels within cerebrospinal fluid of both monkeys and humans (4, 13), as well as normal brain tissue in mice (20) and humans (18). AZQ concentrations in tumor cyst fluid approximated corresponding plasma levels (Table 2), with the level from Patient 2 being in the range of maximum concentrations observed within the cerebrospinal fluid. Although AZQ was detectable in only 1 of 5 brain tumor specimens, very high drug concentrations would have had to be present because of the small amounts of tissue available for analysis. Further, the limit of detection cannot be expected to be as low as it is for brain tumor levels of AZQ.

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<th>Amount (g)</th>
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<th>AZQ plasma (ng/ml)</th>
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Table 2

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Table 2

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<th>CLss* (ml/min/sq m)</th>
<th>Vss (ml/kg)</th>
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* CLss, apparent total body clearance; Vss, apparent volume of distribution at steady state; t1/2ss, redistribution phase half-life; t1/2ø, plasma elimination half-life.
physiological fluids, since a relatively lipophilic drug must be extracted from a lipophilic matrix. In the case of Patient 1, where a high concentration of AZQ was found in the peripheral tumor, the central tumor specimen was too small for adequate analysis, as well as being almost completely necrosed.

The terminal half-lives for plasma elimination of AZQ determined for patients in this study were in the same range as those reported for several previous clinical trials (4, 19, 21). Measurable levels of drug (>3 ng/ml) were not found in plasma samples taken 6 or more hr after the end of the infusion, even when the more extensive low-level work-up procedure was used. No evidence for a third phase of plasma elimination was demonstrable.

Plasma protein binding may restrict drug distribution and account for a volume of distribution of only 28% of body space. Indeed, measurement of total and free AZQ levels in patient specimens demonstrates that 75 to 90% of AZQ is protein bound.

The results of the present study verify previous estimates that a large fraction of the AUC (>50%) occurs during the infusion. There is reasonable agreement between theoretic predictions and actual measurements. The solid curve shown in Chart 2 is based solely upon data from the postinfusion period, permitting validation of the infusion estimates. If all data points obtained both during and after the infusion were to be included in the modeling, an improved fit might be achieved, but it would not have been possible to evaluate the infusion points independently. Unfortunately, many clinical pharmacokinetic studies of intermediate infusion times, including our previous AZQ studies (4, 19), focus upon the postinfusion period, and fail to study drug levels during the infusion. Although it is possible to estimate how much of the AUC occurs during infusion, these corrections are model dependent, and may be seriously flawed when the estimated portion exceeds the measured portion. Since AUC is often related to a volume of distribution of only 28% of body space, Indeed, measurement of total and free AZQ levels in patient specimens demonstrates that 75 to 90% of AZQ is protein bound.

AZQ is a new, active agent in the treatment of high-grade glioma. It is well tolerated with less acute toxicity than nitrosoureas. Further clinical trials directly comparing AZQ to nitrosoureas in comparable groups of patients are now in progress.

ACKNOWLEDGMENTS

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REFERENCES

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