Intraarterial Administration of Melphalan for Treatment of Intracranial Human Glioma Xenografts in Athymic Rats¹

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ABSTRACT

Malignant gliomas will affect 15,000-17,000 Americans each year and carry a dismal prognosis. Adjuvant chemotherapy is hampered by inadequate drug delivery, systemic toxicity, and a markedly variable biological sensitivity. Intraarterial (i.a.) therapy may enhance selectivity by improving tumor drug delivery and reducing systemic toxicity. Using melphalan given i.a., we studied the therapy of intracranial human glioma xenografts in male athymic nude rats (mean weight, 300 g) which were inoculated intracerebrally with D-54 MG and D-456 MG. On Days 6 and 7 (D-54 MG) or Days 9 and 10 (D-456 MG), rats randomized by body weight and treated with single-dose melphalan given i.a. at 0.5 or 0.75 mg produced significantly higher median survival (D-54 MG, Days 33 and 32; D-456 MG, Days 52 and 54, respectively) compared with i.a. saline (D-54 MG, Day 14, P < 0.001; D-456 MG, Day 24, P = 0.000) or melphalan given i.v. at 0.75 mg and 0.9 mg (D-54 MG only; Day 23, P < 0.001; Day 26, P < 0.001, respectively) and at 0.5 and 0.75 mg (D-456 MG only; Day 26 for both doses, P = 0.00). Although a dose-dependent increase in median survival (D-54 MG, 0.25 mg, Day 18; 0.5 mg, Day 28; 0.75 mg, Day 32.5) was observed with i.a. administered melphalan, no significant difference was apparent between 0.5 and 0.75 mg in either tumor model (D-54 MG, P = 0.15; D-456 MG, P = 0.37). Toxicity studies in nontumor-bearing athymic rats yielded a maximum tolerated dose of 0.8 mg for i.a. administered melphalan. This dosage was superior in spite of different xenograft permeabilities (apparent mean blood-to-tissue transport [K] values for α-aminoisobutyric acid, 5.8 for D-54 MG and 1.3 for D-456 MG). Pharmacokinetic experiments demonstrated a significant first pass advantage for i.a. (versus i.v.) melphalan. The short plasma half-life, marked anti-glioma activity, and lack of requirement for metabolic activation indicate that i.a. melphalan holds considerable promise for human glioma therapy.

INTRODUCTION

Malignant gliomas compose 65% of all primary brain tumors, most of which are of high grade at diagnosis. Despite aggressive multimodality therapy, median survival of patients with glioblastomas remains at 11 months and, for anaplastic astrocytomas, at 26 months, with less than 10% of these patients alive beyond twice the median survival period (1, 2). Radical surgery and radiotherapy have not resulted in significant improvements in prognosis (3, 4). Conventional chemotherapy involving mainly the nitrosoureas has been hampered by problems of relative tumor insensitivity, inadequate delivery, and systemic toxicity (5, 6). Chemotherapeutic agents have historically been chosen based on their ability to cross the BBB (7). However, a degree of intrinsic antiglioma activity of the drug in spite of suboptimal physicochemical characteristics (11).

Intraarterial therapy has long been considered a potential method to improve selective therapy of gliomas. Although early trials with this approach were disappointing (12), theoretical studies using computer modeling predicted 200–500% higher drug levels in the brain with i.a. versus i.v. administration and reduced systemic toxicity due to both the decrease in drug dose that would be required and the effect of “first pass” metabolism (13, 14). The relatively localized growth pattern of gliomas and the proximity of the two major trunk vessels that supply blood to the brain, the carotid or the vertebral arteries, make i.a. delivery more advantageous than i.v. therapy (15). Subsequent preclinical trials in immunosuppressed and athymic rats demonstrated the therapeutic advantage for both 1,3-bis(2-chloroethyl)-1-nitrosourea (16) and 4-hydroperoxycyclophosphamide (17), although clinical use of 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin (18) failed to retain the therapeutic advantage for patients, probably due to the high local toxic effects of these agents.

Melphalan, a bifunctional alkylating agent incorporating a phenylalanine group, was initially synthesized to improve chemotherapy of melanomas (19). Although melphalan has not been effective in this regard, it has shown significant activity against a wide variety of tumors including ovarian carcinoma, medulloblastomas, neuroblastomas, and pineoblastomas (20–23). The drug’s relatively high protein binding, marked ionization at physiological pH (24), low lipophilicity (log P of −1.70; Ref. 25), and substantial cumulative hematopoietic toxicity after systemic administration (26) suggested that repetitive systemic administration of melphalan was not optimal for chemotherapy of brain tumors. These drawbacks are offset by the short in vivo half-life, the significant intrinsic activity against high-grade gliomas growing s.c. in athymic mice, and evidence for the active transport of melphalan across the BBB (27, 28) and into cells (29–31) via specific amino acid carrier systems.

We have conducted a series of experiments designed to test the efficacy of i.a. melphalan in the treatment of human glioma xenografts implanted i.c. in athymic rats. The therapeutic efficacy of melphalan against two different malignant glioma xenografts was examined, and a 4-fold difference in vascular or tissue permeability between was observed. Pharmacokinetic studies showed a 2–3-fold higher level of melphalan in the tumor when equal quantities were delivered either i.a. or i.v. We suggest, based on these results, that i.a. melphalan be initiated into clinical trials in patients with high-grade gliomas.

MATERIALS AND METHODS

Tumor and Animal Models

D-54 MG, the Duke subtype of the malignant glioma A172 initially established by Giard et al. (32), and D-456 MG (childhood glioblastoma...
GLIOMA THERAPY WITH INTRAARTERIAL MELPHALAN

Multiforme (Ref. 6) were used for all experiments (32). These tumors, grown as s.c. xenografts in athymic mice, are known to have doubling times of 2.5 and 5.0 days in these animals. Tumors removed from mice were finely minced and homogenized; necrotic debris was removed and passed through a tissue sieve into Richter’s zinc option medium (Ref. 33; GIBCO, Grand Island, NY). The resulting cell suspension was then passed through a 25-gauge needle, centrifuged at 500 × g for 3 min, mixed with an equal volume of methyl cellulose, and loaded into a 500-ml Hamilton syringe. Male nude athymic rats weighing approximately 300 g, bred and cared for at the Duke Cancer Center Isolation Facility as described previously (34), were inoculated in the right caudate nucleus with 10 ml of this mixture using a Kopf stereotactic instrument (David Kopf Instruments, Tujunga, CA) with coordinates 1 mm anterior and 2 mm lateral to the bregma. At this point, a hole was drilled through the skull using a 19-gauge needle. The needle was advanced downward 5 mm below the dura, and injections were given. The skull holes were sealed with bone wax, and the skin was closed with surgical staples.

Intraarterial Injections

Rats anesthetized with an appropriate dose of a 6:5 ketamine:xylazine mixture were placed on their dorsal surface. Their limbs were restrained, and suitable measures were taken to ensure an open airway. A 3-cm longitudinal midline incision was then made extending between the mentum and the sternal notch. The right sternomastoid and paratracheal muscles were retracted laterally and medially, respectively, exposing the mylohyoid muscle and the hyoid bone. The course and branches of the right carotid arterial system were then exposed by dividing the mylohyoid muscle and removing the lateral third of the hyoid bone. The superior thyroid and occipital branches of the external carotid were cauterized, and the external carotid was tied distally. Deep dissection of the internal carotid artery was followed by cauterization of its pterygopalatine branch, thus creating a continuous conduit from the external to the internal carotid. The external carotid was then catheterized distally proximally using a PE-10 polyethylene catheter, into which the injections were given. The external carotid was then cauterized, and the skin was closed with surgical clips.

I.v. Injections

Rats anasthetized as described above were inoculated i.v. through a right femoral venous catheter.

Drugs

Melphalan (50 mg/vial) was obtained from the Burroughs Wellcome Co. (Research Triangle Park, NC). The contents of one 50-mg vial were dissolved in 25 ml Dulbecco’s PBS at pH 7.4 to obtain a stock solution at 2 mg/ml. Further dilutions were made to yield dose solutions of 1.5, 1.0, and 0.5 mg/ml. Doses were then individually sterile filtered, aliquoted into vials, and snap frozen in liquid nitrogen. Vials were rewarmed in a water bath at 37°C just before injections were given.

Therapy Experiments

Rats were randomized by weight into groups of 10 at 5 (D-54 MG) or 9 (D-456 MG) days after tumor implantation. These animals were treated with saline given i.a. or i.v. and single-dose melphalan given i.a. at 0.25, 0.5, or 0.75 mg or i.v. at 0.5 or 0.75 mg, respectively. Animals were weighed on alternate days. A complete autopsy was performed at death for all animals.

Toxicity Experiments

Nontumor-bearing athymic rats randomized by weight into groups of 10 were given 0.25, 0.5, 0.75, and 1.0 mg of single-dose i.a. melphalan. After drug administration, animals were weighed daily. A complete autopsy was performed at the time of death for all animals.

Histopathological Examination

All animals were checked for mortality twice a day, and autopsies were performed on animals that died. No animals underwent autopsy more than 12 h after death. At autopsy, the brain and the right eye were removed and grossly examined, and representative sections were submitted for histopathological examination. Representative sections of the brain included coronal tissue blocks taken at the levels of the nucleus caudatus putamen, the dentate gyrus, and the cerebellum. A horizontal section of the right eye was also obtained. Sections 5-μm thick were cut, deparaffinized, and stained with hematoxylin and eosin-Luxol fast blue as described previously (17). Slides were then examined by an examiner blinded to the treatment groups.

Statistical Analysis

The Wilcoxon rank sum test was used to compare group median survival. A P < 0.05 was considered to be statistically significant.

Pharmacokinetic Experiments

Rats implanted i.c. as described previously with D-54 MG were randomized on Day 12 after tumor implantation into groups of three. Melphalan (0.75 mg) was administered either i.a. or i.v., and each group was decapitated at 1, 5, or 15 min thereafter. The brain was rapidly removed, and the tumor was dissected out. Tumor and ipsilateral and contralateral brain were snap frozen in liquid nitrogen and submitted for melphalan levels. Tumor dissection was facilitated by the administration of 2% Evans blue dye (0.5 ml in 1% phosphate buffer) 20 min before decapitation. In addition, a cardiac puncture was performed at the time of decapitation to obtain a blood sample for plasma melphalan levels at each time point.

A Waters isocratic high pressure liquid chromatography system with a 3.9 × 150 mM Novapak C18 column was used to assay for melphalan at a wavelength of 254 nm and flow rate of 1.0 ml/min. The mobile phase consisted of 20% acetonitrile and 80% 0.025 mM sodium monobasic phosphate buffer with 30 mM ammonium acetate. The injection volume was 40 μl, and the run time was 10 min. A standard curve was prepared in plasma and had a range of 0.0–10 μg/ml, a sensitivity of 0.5 μg/ml, and an r² of 0.99945. The low, medium, and high quality control samples were well within acceptable tolerance levels.

Plasma samples were processed by adding 11 μl 60% perchoric acid to 250 μl plasma, then vortexing and centrifuging the mixture for 2 min on a microcentrifuge. The supernatant was filtered through a 0.45-μm filter and injected onto the column. Tissue samples were homogenized, and for each 0.1 g tissue, 0.4 ml of 10% perchoric acid was added. Subsequent analysis was performed in a manner similar to that of the plasma samples.

Permeability Studies

Tumor Implantation and Animal Preparation. Rats were implanted in the right caudate nucleus with D-456 MG as described previously (35). Blood-to-tissue transfer constants were determined at two time points: 2 weeks (the approximate period of drug administration in therapy experiments with this tumor model) and 3 weeks after tumor implantation. Groups of five rats were used for each time and were anesthetized i.p. with 200 μl of a 64.8-mg/ml solution of sodium pentobarbital. The right femoral artery and vein were exposed, and PE-50 polyethylene catheters were inserted into these vessels. The venous catheter was injected with 100 units heparin and 0.5 ml of a 1% solution of Evans blue dye in phosphate buffer. Rats were then immobilized in a plaster cast and allowed to recover.

Experimental Procedures. Blood-to-tissue transfer constants were measured using 14C-labeled AIB obtained from American Radiochemical Corp. (St. Louis, MO). A bolus of 50 mg C14-labeled AIB in 0.5 ml of a 0.9% NaCl solution was injected into the femoral venous catheter. Timed arterial blood samples were collected at 15, 30, 45, 60, 120, 180, 300, and 600 s after isotope injection. Ten min after isotope administration, rats were decapitated. The brain was rapidly removed, and the tumor was dissected away. Tumor and ipsilateral and contralateral brain were collected in separate vials. An arterial blood sample was taken at the end of the experiment to ensure that hematological parameters and blood gas levels were in the physiological range. Body temperature was monitored continuously through the experiment using rectal probes. To each blood and tissue sample were added 2 ml of TS-2 tissue solubilizer (0.5 N solution; Research Products International Corp., Mt. Prospect, IL) and 18 ml of liquid scintillant composed of 1916 ml toluene (Mallinckrodt Specialty Chemicals Co., Paris, KY), 4 ml glacial acetic acid (J. T. Baker Inc., Phillipsburg, NJ), and 80 ml of concentrated liquid scintillator.
(PPO-POPOP; Amersham Corporation, Arlington Heights, IL); the resulting mixture was counted in a beta scintillation counter.

Calculations

\[ K = \frac{C_i(T)}{\int C_p(t) dt} \]  

where \( K \) is the blood-to-tissue transfer constant, \( C_i \) is the measured radioactivity in the tumor sample at the end of the experiment (\( T \)), and \( C_p \) is the measured radioactivity of the isotope in the plasma at various times (\( t \)). Although this equation does not account for that fraction of AIB remaining within the tumor vascular compartment, intravascular AIB is known to distribute mainly in plasma and is small in decapitated rats (36). Given the range of \( K \) values reported in this study and previous estimates of the comparatively small effect that intravascular correction has on reported \( K \) values (37), the results here are expressed as an apparent \( K \) calculated from the equation above.

RESULTS

Dose Escalation/Toxicity Experiments. To determine the maximum tolerated i.a. doses of melphalan, dose escalation toxicity experiments were performed with groups of 10 nontumor-bearing rats receiving 0.25, 0.5, 0.75, and 1.0 mg melphalan i.a., respectively. Daily monitoring of group weight revealed a direct relationship between group weight loss and the dose of melphalan administered (Fig. 1). No fatality was observed in rats receiving 0.75 mg melphalan or less, although a mean group weight loss of up to 25% of pretreatment weight was observed with the rats given 0.75 mg melphalan. Two deaths were recorded in rats given 1.0 mg melphalan i.a., an observation that resulted in 0.75 mg melphalan given i.a. being fixed as the peak dose for subsequent therapy experiments.

Of the 10 nontumor-bearing rats receiving 1.0 mg melphalan i.a., diffuse intraparenchymal cerebral hemorrhage was observed in 2 animals, and focal hemorrhage was noted in an additional 7. Hemispheric leukomalacia was observed in both animals that died. Ocular complications were seen in three animals in this group, including the two rats that showed acute conjunctivitis, one of which demonstrated optic nerve demyelination (Table 1).

Of the 10 rats receiving 0.75 mg melphalan i.a., focal cerebral hemorrhage was observed in 2. No other pathological lesions were seen in the remaining animals. Microscopic examination of the eye revealed only acute conjunctivitis in one rat and mild chronic conjunctivitis in another, neither of which had cerebral lesions (Table 1).

Of the 10 rats receiving 0.5 mg melphalan i.a., none evidenced cerebral pathology. The eyes, however, revealed chronic conjunctivitis in 3 of the 10 rats. The histopathological examination in rats given 0.25 mg revealed small focal hemorrhages in four, of which three also had mild chronic inflammatory infiltrates in their conjunctivae. The 10 saline controls showed no abnormalities except for severe chronic conjunctivitis in one animal (Table 1).

Initial Therapy Experiments. Initial therapy experiments were designed with groups of 10 rats bearing D-54 MG tumors and receiving 0.9 mg melphalan i.a. or i.v., 0.5 mg melphalan i.a., or saline i.a. At 0.9 mg melphalan given i.a., serious toxicity was observed with death occurring in 7 of 10 animals within 2 days after treatment (Fig. 2). However, rats treated with 0.5 mg i.a. melphalan exhibited significantly improved survival (Day 32) compared with rats given 0.9 mg (Day 23, \( P < 0.001 \)) melphalan i.v. and the saline control groups (Day 14.5, \( P < 0.001 \); Fig. 2).

Histopathological examination of the brains of animals receiving 0.9 mg melphalan i.a. revealed hemispheric leukomalacia in 7 of 10 (Fig. 3), and demyelination only in 1 of 10. Ocular microscopic examination revealed acute or chronic conjunctivitis in 5 of 10 rats. No significant pathological changes were observed in the saline control groups. Of the seven rats given 0.5 mg melphalan i.a., five revealed histopathological lesions confined to the tumor-bearing hemisphere, with focal cerebral necrosis observed in one rat.
GLIOMA THERAPY WITH INTRAARTERIAL MELPHALAN

Fig. 3. Brain section of a tumor-bearing rat treated with 0.9 mg melphalan given i.a. A, severe unilateral cerebral encephalomalacia (arrowheads). X10. B, further magnification of affected hemisphere. X12. Similar changes were seen in 7 of 10 rats treated at this dosage. H&E, Luxol fast blue stains.

peritumoral hemorrhage in two other rats, and focal demyelinating changes in two rats, one of which also had a focus of meningeal fibrosis. Evidence of acute conjunctivitis was observed in one of the seven animals.

Treatment Experiments. Upon determination of maximum tolerated i.a. doses, a dose escalation therapy experiment was undertaken, with groups of 10 rats receiving 0.25, 0.5, and 0.75 mg melphalan i.a. We observed a dose-dependent increase in longevity, with animals given 0.5 and 0.75 mg melphalan surviving longest (Days 29.5 and 32.5, respectively), although there was no significant difference between these two doses ($P = 0.15$; Fig. 4). Group survival in rats treated with i.a. melphalan at either 0.5 or 0.75 mg was significantly better than either saline given i.a. (Day 15, $P < 0.001$ for both groups) or melphalan given i.v. at 0.75 mg (Day 18.5, $P < 0.001$ and $P = 0.001$, respectively), although i.a. administered melphalan at 0.25 mg failed to show significantly improved survival over i.v. administered melphalan at 0.75 mg (Day 18, $P = 0.4$; Fig. 4). The superiority of treatment using the i.a. approach was confirmed in a subsequent experiment in which groups of eight rats were treated with i.a. or i.v. administered melphalan at 0.75 or 0.5 mg. Survival was significantly improved in the rats treated with melphalan given i.a. (Days 31.5 and 32.5 at 0.5 and 0.75 mg melphalan given i.a. versus Day 17 at 0.5 and 0.75 mg melphalan given i.v.; $P = 0.00$ for each i.a. group versus either i.v. group). Again, there was no significant difference between 0.5 and 0.75 mg melphalan given i.a. ($P = 0.439$; Fig. 5).

To extend these observations further, similar treatment experiments were performed using a second tumor line, D-456 MG (Fig. 6). Here, survival was compared in rats given 0.5 and 0.75 mg melphalan, either i.a. or i.v. Once again, i.a. administered melphalan resulted in significantly improved survival as compared with both i.a. administered saline (Days 54 and 52 at 0.75 and 0.5 mg i.a. melphalan versus Day 23 i.a. saline, $P = 0.00$ for both groups) and i.v. administered melphalan at either dose (Day 26 for both doses, $P = 0.00$), although no difference was observed between the 0.5- and 0.75-mg doses ($P = 0.324$; Fig. 6).

Permeability Studies. To obtain a quantitative correlate of the improvement in survival seen with the i.a. route, we decided to compare the relative permeability of the two intracerebral tumor models used in these experiments. Detailed estimates of blood-to-tissue transfer constants were already available for D-54 MG (38). We
GLIOMA THERAPY WITH INTRAARTERIAL MELPHALAN

Fig. 6. Treatment of i.c. D-456 MG with melphalan given i.a. and i.v. in athmic rats (n = 10 in all groups, except the group given 0.5 mg melphalan i.v., where n = 9).

Table 2 Apparent K values for AIB in i.c. D-456 MG (expressed as ml/100 g/min)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time point 1: day 10-11 postimplantation</th>
<th>Time point 2: day 17-18 postimplantation</th>
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<tbody>
<tr>
<td></td>
<td>Tumor</td>
<td>Ipsilateral brain</td>
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<tr>
<td>1</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.47</td>
<td>0.07</td>
</tr>
<tr>
<td>3</td>
<td>0.21</td>
<td>0.09</td>
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<tr>
<td>4</td>
<td>0.2</td>
<td>0.15</td>
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<tr>
<td>5</td>
<td>0.125</td>
<td>0.08</td>
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</table>

undertook experiments with D-456 MG to determine whether there existed a significant difference with this tumor. The apparent K values for AIB in D-456 MG are shown in Table 2. Permeability data were obtained at two time points after tumor implantation. The first of these time points was approximately at the date of treatment in therapy studies and, therefore, is perhaps a reflection of tumor permeability to drug at the time of treatment. K values were, on the average, lower at the first time point. More significantly, K values for D-456 MG were, on the average, four to five times lower compared with D-54 MG (mean K, 1.27 for D-456 MG versus mean K, 6.1 for D-54 MG), a probable indication that the superiority of i.a. administered melphalan is independent of tumor permeability characteristics.

Pharmacokinetic Studies. i.a and i.v. administration of melphalan resulted in similar plasma distribution profiles (Fig. 7). However, tumor levels in rats treated with i.v. melphalan were below the limits of detection in all of the animals at every time point. Intraarterial melphalan, however, resulted in a mean tumor melphalan level of 3236 ng/g at 1 min, rising up to a mean of 6300 ng/g at 15 min after drug administration (Fig. 8). The limit of detection in the assay method used was 2000 ng/g melphalan/sample. Furthermore, the levels of melphalan in the ipsilateral hemispheres of rats treated both i.a. and i.v. were below the level of detection in the assay method used. No melphalan was detected in the contralateral hemisphere in any of the animals treated either i.a. or i.v.

DISCUSSION

Glioma therapy has seen little improvement in spite of intensive exploration of various treatment modalities. Despite ongoing technological advancements enabling access to areas of the brain that were previously considered remote, surgical approaches, still the primary intervention of choice (39), are hampered by incomplete tumor removal and consequent recurrence, which is generally fatal (7). Adjuvant chemotherapeutic approaches, directed at tumor remaining after surgery, are impaired by the relative resistance of gliomas to conventional drugs, the diffuse nature of tumor growth, and the heterogeneous cell population within each tumor resulting in varying drug sensitivities (5, 6, 40, 41). Furthermore, the variable blood supply and spatial differences in the permeability of the tumor result not only in therapeutic agents that are inhomogeneously distributed, but also in chemotherapeutic levels in areas of the tumor that are potentially inadequate for beneficial effects (42).

The selection of appropriate drugs has been guided by two objectives: to enable the tumor to access sufficient amounts of drug across the BBB and to achieve therapeutic drug levels in the tumor without a concomitant unacceptable rise in systemic toxicity. Agents that are highly lipid soluble were thought to have the best potential in gaining access across the BBB and, therefore, to tumor. Nitrosoureas, the drugs most used for glioma chemotherapy, however, resulted in high local toxicity to the brain as a result of their lipophilicity. The selection of lipophilic drugs alone is questionable in view of more than one factor. Morphological studies showed nonuniform and fenestrated endothelia within the tumors (8). Measurements of tumor blood flow and transfer constants in a series of rat brain tumor models showed significant heterogeneity in the permeability and blood flow both between and within individual tumors (9). This observation
extended to patients undergoing contrast-enhanced computed tomography and to a quantitative analysis of the rate of transcapillary transport of meglumine iohalumate (10). These observations suggest that the choice of agents should not rest primarily on permeability considerations; rather, drugs with suboptimal physicochemical properties, but significant intrinsic antigloma activity, should undergo preclinical and clinical evaluation (11). Several preclinical trials with the nonlipophilic alkylating agents melphalan and 4-hydroperoxycyclophosphamide have indicated that these drugs can potentially provide significant benefit for in vivo therapy of gliomas (43).

The second objective, to provide therapeutically significant levels of drug within the tumors without substantial systemic effects, was thought could be achieved by using the i.a. route of administration. Theoretical studies using computer modeling predicted a 2–5-fold increase in drug uptake in the brain with i.a. administration as compared with i.v. delivery. This i.a. advantage was due to both the smaller drug doses required and the effect of first pass metabolism. Although numerous clinical trials with the conventional nitrosoureas failed to show significant benefit, recent preclinical studies with 4-hydroperoxycyclophosphamide (17) showed that other alkylating agents may be better choices for i.a. therapy. Arterial delivery was not without its problems, however. Irregular distribution of drug within tumor and significant local toxicity in areas supplied by the artery of inoculation, due to both high local drug concentrations and incomplete mixing of the drug with the blood stream, were some of the problems (44). Superselective delivery may help dispense the drug as close to the tumor as possible, avoiding release of the drug in trunk vessels (15), and diastole pulse-phased infusions may permit better mixing of the drug to homogenize distribution (44).

The bifunctional classical alkylating agent melphalan was initially synthesized with a phenylalanine group to enable specific transport across the cell membrane (19). Greig et al. (27) and later Cornford et al. (28), showed that melphalan is transported both across the BBB and into intracerebral tumor in a facilitated manner. Melphalan exhibits significant activity against a wide spectrum of tumors including ovarian carcinoma, medulloblastoma, neuroblastoma, and pineoblastoma (20–23). The drawback of therapy for central nervous system tumors using repetitive dosing systemic melphalan is that drug dosage is limited because of high toxicity, particularly hematopoietic toxicity. Nevertheless, an attempt to treat leptomeningeal tumor with intracerebral melphalan using two different tumor models showed that, with the compartmental approach, local toxicity was the dose-limiting factor, and that melphalan was therapeutically effective at doses well below drug levels that produced local toxicity (45). These findings indicate that a local approach to melphalan delivery may be effective in avoiding the systemic toxicity of melphalan. The short half-life of the drug (26) and previous demonstration of the ability of melphalan to be transported into cells and across the BBB were additional factors indicating the potential benefit of i.a. melphalan in glioma chemotherapy.

The experiments reported here were designed to examine the efficacy of arterial melphalan in the treatment of tumor located i.c. In the current state of therapy experiments, we observed a survival advantage in excess of 100% when melphalan was given i.a. at a 65% maximum tolerated dose as compared with melphalan given i.v. at a 100% maximum tolerated dose. In multiple experiments, a statistically significant increase in survival was apparent with melphalan at 0.5 and 0.75 mg, although no difference was observed between these two groups. In addition, 20% of rats treated with 0.5 mg melphalan given i.a. showed long-term survival, with one rat surviving for more than 170 days after tumor implantation.

The local toxic effects in both the intrathecal and i.a. therapeutic approaches tested in this preclinical model seem to reflect similar pathophysiological events (45). Single-dose intrathecal melphalan resulted in intraparenchymal necrosis, hemorrhage, peripheral demyelination, and spinal cord edema, the frequency and degree of toxicity increasing with the dose administered. i.a. melphalan also essentially resulted in a similar toxicity profile, with diffuse hemorrhagic cerebral lesions and hemispheric and focal necroses being the major lesions observed and the frequency and severity of toxic changes becoming higher with increasing dose. Ocular toxic changes in rats may reflect the supply distribution of the internal carotid artery in this animal. The surgical approach used in these experiments delivers the drug into the internal carotid below the level of origin of the ophthalmic artery, a technical constraint that may be overcome in humans by performing drug injections above the level of origin of the ophthalmic artery.

D-54 MG is originally derived from an anaplastic astrocytoma. Previous detailed measurements of blood-to-tissue transfer of AIB (a reflection of tumor permeability) demonstrated mean unidirectional blood-to-tissue transfer constants (K values) in the range of 4.0–5.5, indicating that D-54 MG was perhaps the most permeable tumor model to date (38). The high permeability of this model may result in its “leakiness” as to molecules, thereby allowing, in these experiments, a large amount of melphalan into the tumor. Considering this possibility, we performed preliminary studies of tumor permeability with D-456 MG, a tumor that was originally derived from a childhood glioblastoma multiforme and that has a slower growth period in the intracerebral location (in vivo doubling time approximately 5.0 days for D-456 MG versus 2.5 days for D-54 MG). Although preliminary, the K values for AIB with D-456 MG in our experiments were, on the average, about 1.20, at least four times less permeable than D-54 MG. Furthermore, we measured the transfer constants at two different time points in D-456 MG, and observed a 4–5-fold increase in the K values at 21 days compared with 14 days after tumor implantation. This increase in K values with increasing tumor size has previously been observed in RG-2 gliomas, where an increase in mean tumor size showed a corresponding steep rise in permeability (35). From the therapeutic standpoint, however, we demonstrated similar increases in survival with similar doses of melphalan in two tumor lines with completely different permeability profiles, indicating that permeability per se may not be a major limitation in the efficacy of arterial melphalan. Although the data presented here provide a strong indication that D-456 MG is a less permeable tumor than D-54 MG, quantitative autoradiographic experiments for determination of the unidirectional transfer constants are likely to provide more accurate information in addition to uncovering any spatial differences in permeability, a phenomenon that has often been observed in other experimental glioma models (35).

The pharmacokinetic data provide further support for the therapeutic efficacy of i.a. melphalan. Although plasma profiles of i.a. and i.v. melphalan were essentially similar, tumor levels with i.a. melphalan rose from about 1.5-fold at 1 min to about 5-fold higher levels at 15 min after drug delivery as compared with i.v. melphalan. Although the differences in drug levels may predict a higher difference in treatment than the 2-fold increase in median survival that we observed, several reasons, including heterogeneous drug distribution in tumor microenvironment, different sensitivities of tumor cell subpopulations, and relatively high rates of tumor growth, may be responsible for this difference. The extremely low levels of melphalan in the ipsilateral hemisphere may reflect a selective enhanced uptake of the drug into the tumor cells. The complete lack of melphalan in the contralateral hemisphere serves to illustrate further not only the localized nature of drug delivery that is possible with i.a. therapy, but also the potential of superselective delivery in humans to circumvent ocular toxic effects.

3808
We have demonstrated a statistically significant improvement in therapeutic results for athymic rats bearing i.c. human glioma xenografts by giving melphalan i.a. The improved benefit is retained in two tumor models: one an anaplastic astrocytoma and the other a glioblastoma multiforme, each tumor showing a different permeability profile. Furthermore, pharmacokinetic studies have demonstrated the statistically significant increases in tumor drug uptake as compared to i.v. delivery. The high antiglioma activity, short half-life, and lipophilicity of melphalan in our animal model suggest that i.a. melphalan may bear statistically significant clinical potential for the treatment of malignant brain tumors. We suggest, based on these results, that i.a. melphalan therapy be initiated into Phase I trials for patients having high-grade gliomas.

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Intraarterial Administration of Melphalan for Treatment of Intracranial Human Glioma Xenografts in Athymic Rats

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