L-Buthionine Sulfoximine Potentiates the Antitumor Effect of 4-Hydroperoxycyclophosphamide When Administered Locally in a Rat Glioma Model

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OBJECTIVE: L-buthionine sulfoximine (BSO) inhibits glutathione synthesis and may modulate tumor resistance to some alkylating agents, but it has not been proven effective in the treatment of intracranial neoplasms. To evaluate this drug for the treatment of brain tumors, we studied the use of BSO for potentiating the antineoplastic effect of 4-hydroxyperoxycyclophosphamide (4-HC) in the rat 9L glioma model.

METHODS: The survival of male Fischer 344 rats with intracranial 9L gliomas was measured after implantation of controlled-release polymers containing one of the following: no drug, BSO, 4-HC, or both BSO and 4-HC. The efficacy of intracranial 4-HC treatment was assessed with and without serial systemic intraperitoneal BSO injections. Tissue glutathione levels were measured in the brains, tumors, and livers of animals treated with intraperitoneal injections or local delivery of BSO.

RESULTS: The median survival of animals treated with intracranial polymers containing 4-HC was 2.3 times greater than that of controls. This survival benefit was doubled by local delivery of BSO. In contrast, systemic BSO therapy did not improve survival time. In animals that were treated systemically, both liver and tumor glutathione levels were significantly lower than they were in control animals. In the locally treated animals, glutathione levels were reduced in the brain tumor but not in the liver.

CONCLUSION: These results demonstrate that local but not systemic delivery of BSO enhances the antineoplastic effect of 4-HC in this rat 9L glioma model. In addition, because local delivery of BSO within the brain did not deplete glutathione levels systemically, this method of treatment may be safer than systemic administration of BSO. (Neurosurgery 48:392–400, 2001)

Key words: Biodegradable polymer, Buthionine sulfoximine, Drug delivery, Glutathione, 4-Hydroxyperoxycyclophosphamide

Selection of drug-resistant tumor cells is an important cause of chemotherapeutic failure with repetitive dosing regimens (59). A variety of mechanisms, including the induction of enzyme systems for drug detoxification, mediate this cellular resistance (22). The glutathione *S*-transferase (GST) enzyme system, which is known to participate in the detoxification of xenobiotics, also seems to play an important role in the inactivation of alkylating agents by catalyzing their reaction with glutathione to produce stable, nontoxic conjugates (21). Increases in cellular GST activity (8, 9, 18, 26, 48, 55) or glutathione content (1, 26, 42, 46, 53, 63, 64) are associated with resistance to alkylating agents in a variety of human and animal malignancies. L-buthionine-(*S,R*)-sulfoximine (BSO), a potent inhibitor of glutathione synthesis (33), depletes intracellular levels of glutathione in vitro and in vivo, modulates drug resistance, and augments the cytotoxicity of alkylating agents against many tumor lines (7, 20, 25, 29, 31, 34, 40, 49–52, 56, 57, 61, 67). Intraperitoneal (i.p.) administration of BSO effectively depletes cytoplasmic glutathione in subcutaneous and intracranial human glioma-derived xenografts (58);

however, this drug has not yet been developed as an effective chemosensitizer for the treatment of malignant gliomas.

Although aggressive management consisting of surgery, radiotherapy, and chemotherapy has yielded modest improvements in clinical outcomes for patients with malignant gliomas (10, 11, 39), their prognosis remains poor. Treatment failures usually result from local tumor recurrence (35), and drug delivery to intracranial lesions is complicated by the blood-brain barrier (65) and abnormal tumor microvasculature (36, 37). We have developed a method for local, interstitial drug delivery using implantable, biodegradable polymers. These polymers have been studied most extensively for carmustine (BCNU) delivery to malignant gliomas (13, 14); however, this approach permits us to investigate alternative chemotherapeutic agents that otherwise might not be available for brain tumor patients. One such agent is 4-hydroxyperoxycyclophosphamide (4-HC), an active metabolite of cyclophosphamide that penetrates the blood-brain barrier poorly but is effective against an intracranial rat glioma when delivered locally in biodegradable polymers (16, 38). The glutathione/GST enzyme system seems to be an important mechanism in mediating cellular resistance to cyclophosphamide and its active metabolites (43, 53, 54, 67, 69). Therefore, we chose to investigate whether glutathione depletion with BSO could be used to potentiate the efficacy of locally administered 4-HC in a rat glioma model. In these experiments, we also sought to determine an effective route of BSO administration.

MATERIALS AND METHODS

Experimental design

Glutathione depletion study

Craniotomies for implantation of intracranial 9L gliosarcoma were performed on 26 rats. Ten days later, the 19 surviving rats were randomly assigned to one of four treatment groups: 1) the systemic control group (n = 3), which received i.p. injections of sterile saline; 2) the systemic BSO group (n = 5), which received i.p. injections of BSO in sterile saline; 3) the intracranial control group (n = 5), which received an empty ethylene-vinyl acetate (EVAc) polymer implant (40% vinyl acetate by weight); and 4) the intracranial BSO group (n = 6), which received an EVAc polymer loaded 20% with BSO by weight. This polymer device was selected to optimize local BSO delivery. Three days after therapy was initiated, all animals were killed by cervical dislocation. Tumor and liver specimens were quickly removed, placed in 1 ml of 3% perchloric acid on ice, and sonicated. The mixture was then centrifuged at 7400 $\times$ *g* for 10 minutes at 4°C. Pellets were assayed for protein content (12). Supernatant fractions were analyzed for total glutathione content using the method of Tietze as modified by Griffith (32). This colorimetric assay was performed in a cuvette. The reaction was initiated at the time of sample addition and was monitored for 1 to 3 minutes at 41.2 nm on a Gilford Response spectrophotometer (Gilford Institute Laboratories, Oberlin, OH). Glutathione levels were calculated as nanograms per microgram of protein for each of the tissue samples and as a percentage of control values. This study permits comparison of relative levels of glutathione depletion in the livers and brain tumors of animals treated systemically with those of animals treated locally with BSO.

Systemic BSO efficacy studies

Craniotomies for intracranial implantation of 9L gliosarcoma were performed on 52 rats. One animal did not survive the procedure. The remaining 51 animals were randomized to one of four treatment groups on the second postoperative day: 1) the control group (n = 13), which received i.p. injections of sterile saline and intracranial implantation of a blank fatty acid dimer-sebacic acid (FAD:SA) polymer; 2) the systemic BSO group (n = 13), which received i.p. administration of BSO and intracranial blank FAD:SA; 3) the intracranial 4-HC group (n = 12), which received i.p. administration of saline and intracranial 20% 4-HC in FAD:SA; and 4) the combination therapy group (n = 13), which received i.p. administration of BSO and intracranial 20% 4-HC in FAD:SA. The i.p. injections were administered on Days 2 to 5, and the polymers were implanted on Day 5. The animals were checked daily. Their brains were removed at the time of death for histological analysis.

The in vivo release of 4-HC from 20% loaded FAD:SA polymers is known to reach its peak from 5 to 20 days after implantation (17). Therefore, the study described above was repeated with the same experimental design, except that systemic therapy was administered to coincide with the peak in local delivery of 4-HC. In this second experiment, the i.p. injections were administered on Days 10 to 13, and each treatment group comprised 12 animals, except for the combination therapy group, which comprised 13 rats.

Intracranial BSO efficacy study

Craniotomies for intracranial implantation of 9L gliosarcoma were performed on 40 rats. On postoperative Day 5, these animals were randomly assigned to four treatment groups and underwent reoperations for polymer implantation: 1) the control group (n = 10), which received empty (blank) FAD:SA; 2) the BSO group (n = 10), which received 10% BSO in FAD:SA; 3) the 4-HC group (n = 10), which received 20% 4-HC in FAD:SA; and 4) the combination therapy group (n = 10), which received 20% 4-HC and 10% BSO co-loaded in the same FAD:SA polymer. The animals were checked on a daily basis. Their brains were removed at the time of death for histological analysis.

Polymer preparation

EVAc was obtained from DuPont, Inc. (Wilmington, DE). This polymer was washed extensively in absolute ethyl alco-

hol with daily total volume changes to extract inflammatory impurities as described previously (66). The polymers were then dried in a vacuum desiccator for 4 to 5 days. EVAc, with or without BSO (Sigma Chemical Co., St. Louis, MO) was then dissolved in methylene chloride to yield a 10% solution of EVAc (weight/volume). This solution was then poured into cylindrical glass molds at −70°C. Once solidified, the polymers were transferred to glass plates at −30°C and allowed to dry for 4 days. The polymers were then placed in a vacuum desiccator to dry for an additional 4 days. This process yielded cylindrical polymers without BSO (empty EVAc) or with 20% BSO by weight. These cylinders were then cut into 1-mm slices, yielding 7- to 10-mg polymer discs.

The biodegradable FAD:SA polymer, which is derived from naturally occurring oleic acid and sebacic acid, was synthesized according to the method of Domb and Maniar (24). These polymers were prepared by melt-mixing 4-HC and/or BSO into the melted polymer at 65°C for 10 seconds and casting the uniform mixture into a film at a thickness of 1 mm. With a 2-mm bore, the film was then cut into discs at room temperature. With this technique, FAD:SA polymers without drug (blank controls) or with 20% 4-HC, 10% BSO, or a combination of 20% 4-HC and 10% BSO (by weight) were prepared. These polymers weighed an average of 10 mg and contained a total of 2 mg of 4-HC, 1 mg of BSO, and 2 mg of 4-HC with 1 mg of BSO, respectively.

Animals

A total of 170 male Fischer 344 rats weighing 200 to 250 g were obtained from Harlan Bioproducts for Science (Indianapolis, IN). All rats were housed in standard facilities with not more than five rats per cage and were given free access to water and ProLab RMH 1000 formula (Agway, Inc., Syracuse, NY).

Anesthesia

All animals were anesthetized by i.p. injections of 3 to 4 ml/kg of a stock solution of normal saline containing ketamine hydrochloride (25 mg/ml), xylazine (2.5 mg/ml), and ethyl alcohol (14.25%).

Tumor line

The 9L gliosarcoma was obtained from the Brain Tumor Research Center, University of California-San Francisco (San Francisco, CA). It was maintained as a solid subcutaneous mass in the flanks of male Fisher 344 rats. The tumor in carrier rats was passaged every 3 to 4 weeks. Solid 9L gliosarcoma masses were removed in sterile fashion from the flanks of anesthetized carrier rats. The tumor was then cut into fragments measuring approximately $1 \times 1 \times 1$ mm^3. The fragments were kept on ice under sterile conditions in a covered petri dish with 0.9% saline until implantation in the brains of experimental animals or the flanks of new carrier rats.

Tumor implantation

The scalps of anesthetized rats were shaved and disinfected with 70% ethyl alcohol and 10% povidone-iodine solution (Betadine; Purdue Frederick Co., Norwalk, CT). A midline incision was made to expose the sagittal, coronal, and lambdoid sutures. Using microsurgical technique, a 3-mm burr hole was drilled in the left parietal region, centered 3 mm lateral to the sagittal suture and 5 mm caudal to the coronal suture. The dura was then incised. A corticotomy and local resection of underlying white matter were performed with gentle suction, which exposed the dorsal brainstem. The resulting surgical defect was irrigated with sterile 0.9% saline until clear. Once the bleeding was controlled, a $1- \times 1- \times 1$-mm^3 tumor fragment was placed in the cortical defect over the brainstem. The wound was closed with surgical clips. After the rats awakened from anesthesia, they were returned to the animal housing facility. The few animals that died before initiation of therapy were not included in the data analysis.

Polymer implantation

Animals were randomly assigned to treatment groups for efficacy and glutathione depletion studies on the 5th and 10th postoperative days, respectively. Each rat was anesthetized, the scalp was disinfected, and the surgical clips were removed. The skin incision was reopened to expose the burr hole and irrigated with sterile 0.9% saline. A polymer disc appropriate for the randomly assigned treatment was inserted through the burr hole into the tumor bed. The wound was irrigated again and closed with surgical clips. After the animals awakened from anesthesia, they were returned to the housing facility.

Systemic BSO administration

In animals assigned to systemic treatment groups, BSO was administered in a sterile 0.9% sodium chloride solution (40 mg/ml) by i.p. injection at 12-hour intervals for a total of seven doses. Each animal received a total of 5 mmol/kg per day; this dose has been demonstrated to deplete glutathione levels effectively in human glioma-derived intracranial xenografts (58). Equivalent volumes of sterile sodium chloride were administered to control animals in the systemic therapy experiments.

Statistical analysis

The statistical outcome measured in the three efficacy studies was length of survival, without regard to the cause of death, from the time of tumor implantation. Event times were censored for rats that were still alive 200 days after tumor implantation. To analyze the effects of 4-HC combined with BSO, the proportional hazards model (23) was used. Hazard ratios and significance levels for the effects of 4-HC, BSO, and both agents were calculated and compared with controls. For the glutathione depletion study, results expressed in nanograms per microgram of protein were compared with controls using the *t* test. All reported *P* values are two-sided.

RESULTS

Glutathione depletion study

After 3 days of therapy, systemic administration of BSO resulted in approximately an 84% reduction of glutathione levels within intracranial 9L gliosarcoma tumors compared with levels measured in systemically treated control rats ($P = 0.04$). Similarly, glutathione levels measured in tumors treated with polymers containing 20% BSO (by weight) were reduced by approximately 89% as compared with levels measured in locally treated control animals ($P < 0.001$). Therefore, both systemic and intracranial administration of BSO were effective in depleting glutathione levels within intracranial brain tumors (*Table 1*).

Glutathione levels measured within the livers of animals treated with systemic injections of BSO were 65% lower than levels in systemic controls ($P = 0.01$). In contrast, intracranial administration of BSO by polymer implantation resulted in liver glutathione levels that were only 22% lower than those measured in intracranial control animals. This reduction was not statistically significant. Therefore, systemic BSO administration causes a significant depletion of glutathione level in the liver, whereas intracranial delivery of BSO does not (*Table 1*).

Systemic BSO efficacy studies

Systemic administration of BSO, alone or in combination with 4-HC, conferred no survival benefit for animals with intracranial 9L glioma. The delivery of 4-HC by polymer implantation prolonged survival in all experiments (*Figs. 1–3*); however, the addition of systemic BSO did not provide this survival benefit. These observations were true for i.p. injection of BSO on Days 2 to 5 after tumor implantation (*Fig. 1*) as well as on Days 10 to 13 after tumor implantation (*Fig. 2*).

Intracranial BSO efficacy study

Intracranial implantation of polymers releasing BSO alone (median survival, 13 d) did not improve survival of rats with intracranial 9L gliosarcoma, as compared with control animals (median survival, 13.5 d) treated with empty polymers. The median survival of animals treated with polymers containing 20% 4-HC alone (31 d) was 2.3 times greater than that of controls (hazard ratio, 0.046; $P < 0.001$). For rats treated with polymers containing both 20% and 10% 4-HC, however, median survival was 4.6 times greater than that of controls (median survival, 61.5 d; hazard ratio, 0.033; $P < 0.001$), and twice that of rats treated with 4-HC therapy alone (*Fig. 3*). The addition of intracranial BSO therapy to 4-HC reduced the relative risk by almost 40% (combined BSO with 4-HC hazard ratio, 0.620). There was no change in the percentage of long-term survivors, which was defined as animals surviving beyond 120 days.

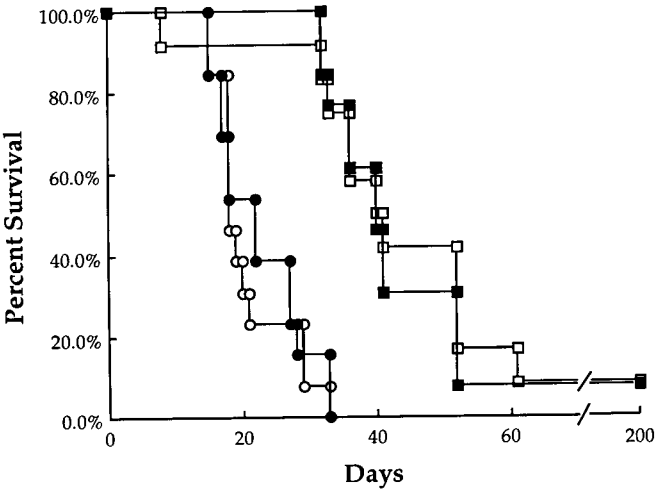

FIGURE 1. Survival curves for animals with intracranial 9L gliosarcomas treated with early i.p. administration of BSO (●; n = 13), intracranial implantation of polymers containing 20% 4-HC by weight (□; n = 12), or the combination of i.p. BSO and intracranial polymers containing 4-HC (■; n = 13) compared with controls treated with placebo (○; n = 13). BSO was administered on Days 2 to 5, and polymers were implanted on Day 5 after tumor implantation.

Histological examination

For each animal, one coronal section through the largest diameter of the lesion was prepared and stained with hematoxylin and eosin. These histological sections confirmed the presence of large masses of spindle-shaped cells in control animals. In animals treated with BSO or 4-HC alone, focal areas of necrosis were noted within otherwise viable tumors (*Fig. 4*). In long-term survivors treated with 4-HC or with the combination of 4-HC and BSO, no viable tumors were found. The surgical sites were fibrotic, exhibiting old organizing necrotic tissue and infiltrating macrophages. No mass effect was present (*Fig. 5*).

TABLE 1. Tissue Glutathione Levels

Treatment Group	No. of Rats	Glutathione (ng/μg protein)[a]	
		Brain Tumor	Liver
Systemic control	3	9.04 ± 3.37	9.51 ± 1.20
Systemic L-buthionine sulfoximine	5	1.42 ± 1.14	3.33 ± 1.18
Intracranial control	5	10.49 ± 1.63	11.48 ± 1.26
Intracranial L-buthionine sulfoximine	6	1.13 ± 0.55	8.90 ± 0.88

[a] **Glutathione values are presented as mean ± standard error.**

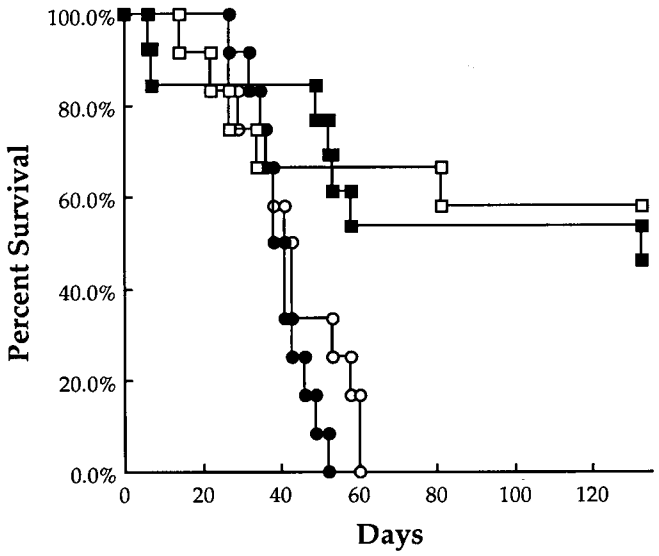

FIGURE 2. Survival curves for animals with intracranial 9L gliosarcomas treated with late i.p. administration of BSO (●; n = 12), intracranial implantation of polymers containing 20% 4-HC by weight (□; n = 12), or the combination of i.p. BSO and intracranial polymers containing 4-HC (■; n = 13) compared with controls treated with placebo (○; n = 12). BSO was administered on Days 10 to 13, and polymers were implanted on Day 5 after tumor implantation.

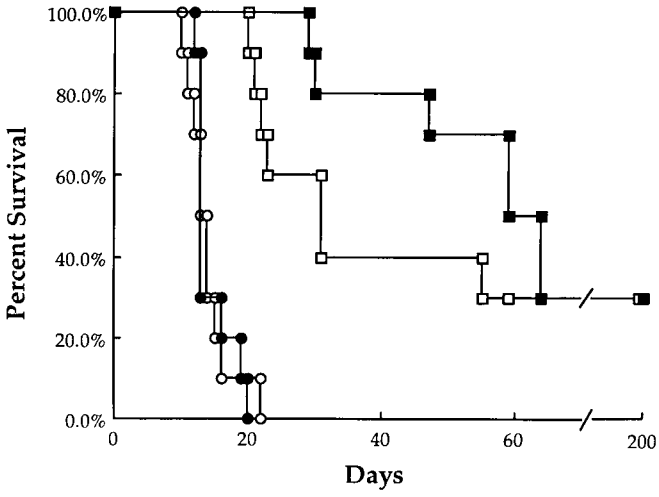

FIGURE 3. Survival curves for animals with intracranial 9L gliosarcomas treated 5 days after tumor implantation with intratumoral polymers containing (by weight) 10% BSO (●), 20% 4-HC (□), or both 10% BSO and 20% 4-HC (■) compared with control animals (○) treated with empty polymers. Each treatment group comprised 10 animals.

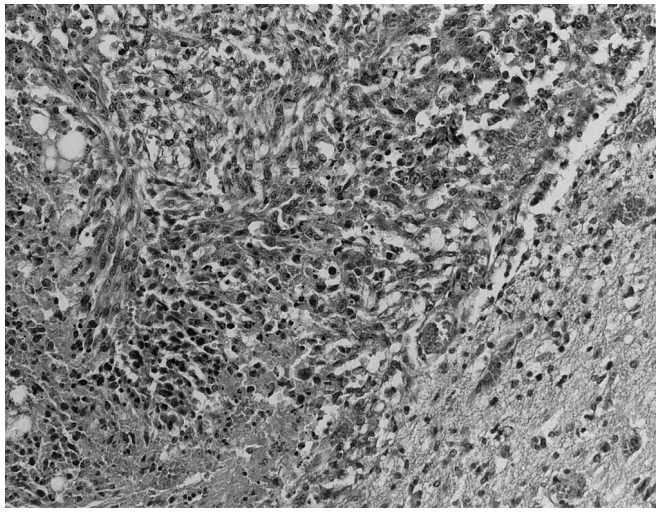

FIGURE 4. Photomicrograph of a tumor in an animal treated with 4-HC alone. The tumor is a spindle cell neoplasm identical to those in control animals, with the exception of a small focus of necrosis observed at the top of the photomicrograph. This lesion is representative of findings in animals that died from intracranial tumors despite therapy with 4-HC or BSO (hematoxylin and eosin; original magnification, ×200).

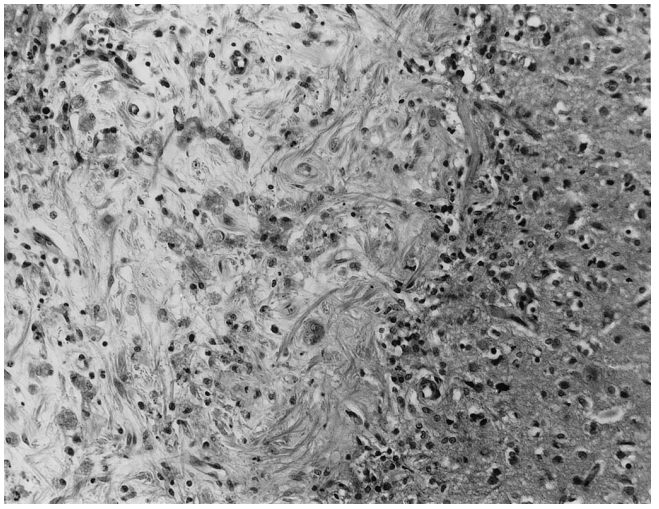

FIGURE 5. Photomicrograph of the brain of an animal treated with 4-HC and BSO. A circular mass of fibrovascular tissue is revealed in the region of tumor implantation. No residual neoplasm was observed. These findings are representative of all long-term survivors treated with 4-HC alone or a combination of 4-HC and BSO (hematoxylin and eosin; original magnification, ×200).

DISCUSSION

These experiments demonstrate the technical feasibility and therapeutic efficacy of inhibiting glutathione synthesis as an adjunct to interstitial delivery of 4-HC for treatment of 9L gliosarcoma in rats. Increased levels of glutathione, although variable among tumor specimens (6, 30), have been found to correlate with resistance to alkylating agents and increased GST expression (5). GST, an enzyme that catalyzes the conjugation reactions of glutathione, may exist in several different isoforms. Of these, the GST-π isoform is associated most often

with resistance in human brain tumor specimens (5), and it also has been found to be a significant predictor of survival in patients with malignant astrocytomas (4). In the rodent 9L gliosarcoma model, however, the overexpression of GST-μ is associated with resistance to alkylating agents (60), which illustrates the difficulty of correlating GST isoform expression among different models. To avoid that dilemma in this study, we investigated only the expression of glutathione and the effects of BSO, a potent inhibitor of glutathione synthesis, on tumor sensitivity to interstitially delivered 4-HC.

Both systemic (i.p.) and local (intracranial) delivery of BSO effectively reduce glutathione levels in the 9L gliosarcoma model. This finding is consistent with the observation that systemically delivered BSO penetrates the blood-brain barrier (27). These data also demonstrate that the polymers release biologically active BSO in quantities that result in clinically significant activity within these brain tumors. In contrast to systemic therapy, however, intracranial implantation of polymers containing BSO does not deplete glutathione levels in the liver. Systemic inhibition of glutathione synthesis with BSO has been demonstrated to impair diaphragm function (47) and to potentiate a variety of xenobiotics, exacerbating toxic effects such as chloroform hepatotoxicity (68), nitrosourea-induced nephrotoxicity and hepatotoxicity (41), ozone-induced pulmonary fibrosis (62), and the toxic effects of cyclophosphamide on cardiac and skeletal muscles (28, 44) and regenerating bone marrow (19). Therefore, increased safety may be an important advantage of local therapy.

Although i.p. administration of BSO effectively depletes tumor glutathione levels, neither of our systemic BSO efficacy studies demonstrated potentiation of the survival benefit achieved with local 4-HC therapy alone. One possible explanation for this treatment failure is that the optimal timing of BSO administration with respect to the 4-HC therapy course has not yet been determined. In this study, early (Days 2–5) and late (Days 10–13) treatment with i.p. administration of BSO were equally ineffective. Another potential explanation is that a 3-day course of i.p. BSO therapy may be inadequate. Finally, pharmacokinetic studies suggest that continuous administration of BSO may be preferable to intermittent bolus administration (45).

The continuous release of BSO from a polymeric matrix is another advantage of the local delivery strategy over intermittent systemic administration. The addition of BSO to biodegradable polymers containing 4-HC, which permits concurrent and continual drug release, has doubled the median survival achieved with 4-HC alone. Continuous BSO dosing has been demonstrated to provide improved efficacy (3, 45), which may be related to several factors, including rapid in vivo drug clearance and the various cytoprotective roles of glutathione. In addition to direct inactivation of the reactive intermediates of alkylating agents, glutathione may decrease cytotoxicity by preventing deoxyribonucleic acid (DNA) cross-linking, by conjugation with DNA-alkylating agent monoadducts, and by direct repair of cross-linked DNA (2, 42). Because the production of DNA cross-links by alkylating agents may require up to 12 hours to complete (15), continuous BSO suppression of glutathione may prevent glutathione quenching of cross-links formed during this period. Intermittent BSO dosing, however, may allow intermittent elevations of glutathione, which would negate the cytotoxic effects of coadministered alkylating agents (2). Future in vivo studies with BSO should investigate the role of continuous systemic dosing combined with administration of 4-HC; such studies might also explore the potential roles of BSO before treatment and in prolonged treatment.

In contrast to systemic administration, local delivery of BSO provided a simple and effective method of potentiating the benefit of interstitial 4-HC therapy. This strategy is best suited for tumors with high levels of glutathione; however, it may also be effective in tumors with low levels of glutathione by suppressing induction of de novo glutathione synthesis that may lead to the development of resistance. This effective and clinically feasible application of BSO for adjuvant brain tumor therapy warrants further investigation in combination with other alkylating agents and radiotherapy.

The populations of cells in malignant gliomas are heterogeneous with respect to drug sensitivity and response to modulators of drug resistance (6). The glutathione/GST system is one of several known mechanisms of drug resistance to alkylating agents such as cyclophosphamide. Therefore, it is important to study local delivery of agents that modulate other mechanisms of drug resistance, such as the *O*-6-alkyltransferase and aldehyde dehydrogenase enzyme systems (21, 29), for the treatment of gliomas.

In the future, it may be possible to customize malignant glioma treatment by submitting biopsy specimens to a battery of in vitro tests and characterizing the biological properties of individual lesions. Information such as drug sensitivity and mechanisms of drug resistance in each tumor will then form the basis for rational selection of adjuvant therapy. In this manner, intraoperative chemotherapy, biological response modifiers, and modulators of drug resistance delivered with biodegradable polymers may be used to supplement postoperative adjuvant chemotherapy and radiotherapy (7, 16).

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DISCLOSURE

HB is a consultant to Guilford Pharmaceuticals, Inc., and Aventis Pharmaceuticals, Inc. The Johns Hopkins University and HB own Guilford stock, the sale of which is subject to certain restrictions under university policy. The terms of this arrangement are managed by the university in accordance with its conflict of interest policies.

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COMMENTS

The authors present an investigative report from the Johns Hopkins Polymer Laboratory and address a potential resistance mechanism to alkylating agents. They report that intratumoral release of buthionine sulfoximine (BSO) potentiates the effects of intratumoral 4-hydroxyperoxycyclophosphamide (4-HC), resulting in a survival rate 4.6 times greater than that of control animals and providing a twofold increase in survival as compared with 4-HC alone. It is interesting that systemic and intratumoral BSO deplete glutathione levels within the tumor to the same degree; however, systemically administered BSO with or without 4-HC does not demonstrate a survival benefit. This distinction is explained in the article to some degree, and it may indeed indicate that continuous administration of BSO is more effective than occasional administration of the same agent. The most important problem the authors address is systemic glutathione depletion, which can cause severe toxicity in the liver. Local administration seems to mitigate this problem with minimal liver depletion of glutathione as described.

Continuous dosing of BSO is critical for activating mechanisms beyond conjugation of alkylating agent intermediates; BSO also prevents deoxyribonucleic acid cross-linking via quenching of monoadducts. This benefit would not occur with an intermittent dosing schedule. Two other points deserve emphasis: glutathione is variably expressed in brain tumors, and glutathione *S*-transferase isoforms may be variably expressed in brain tumors leading to different degrees of drug conjugation. Future studies should analyze whether this strategy would be effective in treating tumors with low glutathione levels and provide a better understanding of the glutathione *S*-transferase isoform expression profile in brain tumors. This excellent study further emphasizes the multifactorial nature of brain tumor drug resistance. In combination with other regimens targeted at overcoming drug resistance, strategies such as these are required to achieve long-term tumor growth suppression.

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Resistance to chemotherapy with alkylating agents can be modulated by using a variety of agents. BSO is an example of such an agent that can inhibit glutathione synthesis and prevent the inactivation of the alkylating agent. In the treatment of malignant gliomas, BSO has not demonstrated effectiveness when administered systemically. In this article, locally delivered BSO used in conjunction with 4-HC seems to double the effectiveness of 4-HC alone.

This study suggests that locally delivered glutathione synthesis inhibitors can enhance the effectiveness of alkylating agents, at least in a rat glioma model. As the authors note, local delivery offers a greater chance of successful inhibition and decreases the potential toxicity of BSO.

The authors preview the coming era of glioma treatment, when multiple mechanisms can be exploited to maximize therapeutic efficacy. This could result in reduced reliance on extremely stressful, cytotoxic, systemically administered therapy by combining the local delivery route with a multiple-mechanism approach.

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The strategy of adding an agent to reverse drug resistance during glioma treatment is novel. As the authors demonstrate, it seems to increase effectiveness. This is an innovative use of agents for diffusion therapy. My concern is that the 9L tumor model is not an accurate representation of the human condition; consequently, the data must be interpreted with caution. Although 20% loaded fatty acid dimer-sebacic acid polymers provide a relatively high concentration of the drug, their ability to penetrate the brain at cytotoxic levels is questionable.

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Sipos et al. carefully and extensively studied the effects of BSO, a potent inhibitor of glutathione synthesis, on tumor sensitivity to 4-HC using the rat 9L glioma model. The authors demonstrated that intratumoral administration of BSO conjugated in polymers significantly decreased tumor glutathione levels and enhanced the efficacy of locally administered 4-HC; the result was increased survival of tumor-bearing animals.

One of the approaches to potentiating the effect of chemotherapy is modulation of drug resistance. Many attractive chemosensitizers were studied; however, none have proven effective in the treatment of patients with malignant glioma. This study demonstrates beautifully that local administration of BSO significantly decreases tumor levels of glutathione *S*-transferase, which play an important role in inactivating alkylating agents without causing systemic effects. Before this interesting finding is applied to the treatment of patients with glioma, further studies are necessary to address questions such as drug distribution in the tumor tissue, adequate methods of administering drugs locally and repeatedly to patients, suitable alkylating agents to be used in combination, and optimal timing and route of administration.

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