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# BUTHIONINE SULFOXIMINE-MEDIATED DEPLETION OF GLUTATHIONE IN INTRACRANIAL HUMAN GLIOMA-DERIVED XENOGRAFTS*

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**Abstract**—D-54 MG, a human glioma-derived continuous cell line growing as subcutaneous or intracranial xenografts in athymic mice, was found to be sensitive to the effects of D,L-buthionine-(*SR*)-sulfoximine, a selective inhibitor of γ-glutamylcysteine synthetase. Intraperitoneal administration of one dose of buthionine sulfoximine (BSO, 5 mmol/kg) resulted in depletion of total intracellular glutathione to 57 and 47% of control 12 hr, and 73 and 23% of control 24 hr, after BSO in subcutaneous and intracranial xenografts respectively. Concurrent measurement of total glutathione in the contralateral (non-tumor-containing) cerebral hemisphere in mice bearing intracranial D-54 xenografts demonstrated insignificant depletion of glutathione. Multiple doses of BSO, at 12-hr intervals, resulted in further depletion to 27% (s.c.) and 16.5% (i.c.) of control 12 hr following the final dose of BSO. Quantitative analysis of BSO delivery to xenograft and contralteral brain tissue revealed transfer constants, $K_1$, of 15.8–24.1 × $10^{-3}$ and 2.4 × $10^{-3}$ ml·$g^{-1}$·$min^{-1}$ for xenograft and "normal" brain respectively. This highly selective depletion of glutathione in neoplastic tissue versus surrounding non-neoplastic host tissue may have therapeutic implications for the rational use of chemotherapeutic and radiotherapeutic intervention.

Glutathione (GSH), the major intracellular non-protein sulfhydryl, functions to minimize the cytotoxic effects of exogenous or endogenous electrophiles including chemotherapeutic alkylating agents, reactive oxygen species and radiation-induced free radicals [1–4]. Cellular GSH levels (typically 0.5 to 10 mM) reflect a homeostasis between *de novo* synthesis, catalyzed by γ-glutamylcysteine synthetase and GSH synthetase, and GSH utilization, a process that includes the reaction of GSH with electrophiles and the irreversible transport of GSH out of cells. In tissues or cells with significant rates of GSH utilization, GSH levels fall following exposure to buthionine sulfoxamine (BSO), a selective inhibitor of γ-glutamylcysteine synthetase [5–7]. Previous studies indicate that the central nervous system (CNS) is relatively refractory to the effects of BSO [6].

The effect of BSO on tumors within the CNS has not been examined previously. Earlier studies suggest, however, that the blood–brain barrier is compromised by tumor growth [8] and that the rate of GSH turnover is often increased in neoplastic relative to normal tissues [9]; either effect might allow selective BSO-mediated depletion of GSH in CNS neoplasms. In the present studies, we have examined the effects of BSO on a human glioma-derived cell line, D-54 MG, growing a subcutaneous or intracranial xenografts in athymic nude mice. We found that BSO markedly depleted GSH in the xenografts without altering the GSH concentration in the contralateral "normal" brain. This selective GSH depletion and potential "sensitization" of neoplastic cells may facilitate the use of alkylating agents, external beam irradiation, and monoclonal antibody-targeted irradiation in the treatment of CNS neoplasms.

## MATERIALS AND METHODS

*Animals*. Male or female athymic BALB/c mice (nu/nu genotype, 6 weeks old) were maintained as previously described [10].

*Xenograft establishment*. D-54 MG, the Duke University subline A-172 established by Giard *et al.* [11], and previously characterized in our laboratory [12], was used in all experiments. Subcutaneous and intracranial xenografts were grown as previously described [13].

*BSO regimen*. D,L-Buthionone-(*SR*)-sulfoximine (Sigma Chemical Co., St. Louis, MO) dissolved in 0.9% NaCl solution (44.4 mg/ml) was given by intraperitoneal injection at doses of 5 mmol/kg or

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20 mmol/kg. L-[$^{35}$S]Buthionine-*SR*-sulfoximine was synthesized from L-[$^{35}$S]methionine (New England Nuclear) [7].

*Tissue preparation and glutathione assay.* At specific time points after BSO administration, animals were killed by cervical dislocation; animals bearing intracranial tumors were treated 30 min before sacrifice with Evans blue (i.v.) to assist tumor dissection [14]. Tumor, liver, and samples of normal brain from the contralateral hemisphere were removed, placed on ice, and then quickly homogenized (Brinkmann Polytron) in cold 10% 5-sulfosalicyclic acid (1:5 w/v in ml, or 1.0 ml for intracranial samples). Homogenates were centrifuged at 4000 rpm for 5 min at 4°, and the pellets were assayed for protein [15].

The total glutathione* content of the supernatant fractions was determined by the method of Tietze [16] using solutions and procedures described by Griffith [17]. GSH-containing sample (5 μl) was added last to initiate the reaction, and the $A_{412}$ was monitored at room temperature for 1–3 min with a Gilford Response spectrophotometer. Quantitation was based on the assay of each sample with and without added internal standard (0.5 nmol Sigma GSH). GSH levels are expressed per milligram protein and per gram sample wet weight. Control GSH levels are based on tissue taken from untreated animals; tumors were matched for size (subcutaneous) or time after implantation (intracranial). Statistical analysis was by Wilcoxon rank sum test.

*Octanol/water partition coefficient.* The octanol/water partition coefficient of [$^{35}$S]BSO was measured as previously described [18].

[$^{35}$S]*BSO quantitation.* Animals with D-54 MG intracranial xenografts were prepared as previously described [19], with modifications necessitated by the small animal size. Unilateral arterial and venous catheters (PE-10) were inserted under halothane–nitrous oxide–oxygen anesthesia (1.5–2:30:70; by vol.), which was maintained throughout the experiment. Body temperature was maintained at 34–37° with heat lamps. Arterial blood pressure was not monitored. Arterial blood samples for measurements of radioactivity were obtained by allowing 10–20 μl to drop directly into a tared scintillation vial. At the conclusion of the experiment arterial blood was collected for measurement of hematocrit and blood gases. Previous studies in our laboratory indicate that the vascular volume of D-54 MG xenografts is 0.017 ml/g, similar to that of normal brain. Based on a whole blood GSH concentration of 0.69 mM [17], the contribution of brain or xenograft GSH derived from red blood cells is only 0.01 to 0.02 μmol/g, obviating the need for brain perfusion prior to measurement of GSH.

To initiate an experiment, 10 μCi of L-[$^{35}$S]buthionine-*SR*-sulfoximine (sp. act. = 910 μCi/mmol; radiochemical purity = 96% by amino acid analysis), dissolved in 0.1 ml of 0.9% saline, was injected as a bolus into the femoral vein. Arterial samples were collected at intervals, and 10 min after injection the animal was decapitated. The brain was removed and processed for quantitative autoradiography as previously described [19, 20]. The autoradiographic sections and histologic sections were digitized with a video-based digitizing system [19]. Whole tumor measurements and values from tumor-free brain were used for transport calculations. Tissue and plasma radioactivity values (the latter corrected by the arterial hematrocrit, 39.7 ± 1.8%) were used to calculate a unidirectional blood-to-tissue transfer constant, $K_1$ [19]. The apparent tissue [$^{35}$S]BSO content was corrected for the amount of radioactivity remaining in the vascular space at the end of the 10-min experiment.

* As assayed here, total glutathione is approximately GSH + 2 × GSSG. Since intracellular GSH ≫ GSSG, total glutathione = GSH, and "GSH" is used in this context throughout.

## RESULTS

*Subcutaneous xenograft sensitivity to BSO.* Control values for glutathione concentrations in subcutaneous xenografts and murine liver are presented in Table 1. Glutathione concentration was expressed as micromoles per gram tissue wet weight and nanomoles per milligram protein; the units of expression did not alter the magnitude of depletion.

Twelve hours after administration of one dose of BSO (5 mmol/kg), tumor glutathione concentration was 57.1% of control while liver glutathione concentration was 52.5% of control (P = 0.05 and 0.05) (Table 2). Twenty-four hours after drug administration, both tumor and liver glutathione levels had recovered to near control levels (72.8 and 85.2% of control respectively) (P = 0.200 and 0.05).

The effect of two doses of BSO (5 mmol/kg), given 12 hr apart, on the glutathione level at 24 hr is presented in Table 2. This regimen resulted in further depletion of glutathione in the xenograft to 26.7% of control (P = 0.05) while only depleting hepatic glutathione to 70.1% of control (P = 0.200).

*Intracranial xenograft sensitivity to BSO.* Control values for glutathione concentration in intracranial xenografts and brain from the contralateral hemisphere are presented in Table 1. There were no significant differences in glutathione concentration in the brain of tumor-bearing (contralateral hemi-

Table 1. Control glutathione concentrations

| Tissue | Glutathione concentration* | |
|---|---|---|
| | μmol/g tissue wet wt | nmol/mg protein |
| Liver | 6.34 ± 0.87 | 22.14 ± 3.00 |
| D-54 MG (s.c.)† | 0.68 ± 0.13 | 9.99 ± 2.76 |
| D-54 MG (i.c.) | 1.39 ± 0.21 | 13.94 ± 4.39 |
| Brain (cl)‡ | 1.68 ± 0.26 | 13.99 ± 4.48 |
| Brain (ntb) | 1.74 ± 0.08 | 13.38 ± 0.59 |

* Glutathione concentrations (mean ± SD) were based on 3–10 data points.

† Abbreviations: sc, subcutaneous; ic, intracerebral; cl, contralateral; and ntb, non-tumor-bearing.

‡ There were no statistical differences (P ≥ 0.05) between contralateral brain and brain from non-tumor-bearing mice in control and treated animals.

Table 2. BSO treatment regimens

| Tumor site | Regimens compared | Parameter measured | % Control [GSH]* Tumor | Brain |
|---|---|---|---|---|
| Subcutaneous | (A) 5 mmol/kg† | 12 hr GSH | 57.1 ± 3.0 | 52.5 ± 17.6 |
| | (B) 2nd dose at t = 12 hr | 24 hr GSH | 26.7 ± 2.4 | 70.1 ± 24.8‡ |
| | (C) no 2nd dose | 24 hr GSH | 72.8 ± 25.6 | 85.2 ± 4.6 |
| Intracranial | (A) 20 mmol/kg | 12 hr GSH | 43.3 ± 22.1 | 110.0 ± 20.5 |
| | (B) 5 mmol/kg | 12 hr GSH | 47.2 ± 17.7 | 104.3 ± 17.0 |
| Intracranial | (A) 2nd dose at t = 12 hr | 24 hr GSH | 22.2 ± 3.3 | 74.5 ± 10.0 |
| | (B) no 2nd dose | 24 hr GSH | 23.0 ± 4.1 | 93.5 ± 12.1 |
| Intracranial | (A) 3rd dose at t = 24 hr | 36 hr GSH | 16.5 ± 8.7 | 76.7 ± 17.5 |
| | (B) no 3rd dose | 36 hr GSH | 26.8 ± 15.0 | 92.6 ± 15.0 |

* Values are expressed as the mean percent control ± SD for three to six animals (over one to three experiments). See Table 1 for control values.

† Dose of BSO was 5 mmol/kg, unless noted otherwise.

‡ In subcutaneous experiments, [GSH] in liver was measured in place of brain in order to provide a positive control.

sphere) and non-tumor-bearing animals (P = 0.272).

The effect of one dose of BSO (5 mmol/kg) is presented in Fig. 1. Glutathione concentrations in the xenograft 12 and 24 hr after BSO administration were 47.2 and 23.0% of control respectively (P = 0.017 and 0.024 respectively). Glutathione levels in contralateral cerebrum from tumor-bearing mice at 12 and 24 hr after BSO administration were 104.3 and 93.5% control respectively (P = 0.722 and 0.215 respectively). There was no difference in effect of BSO in the contralateral hemisphere of tumor-bearing animals and brain taken from similarly treated non-tumor-bearing animals (P = 0.139).

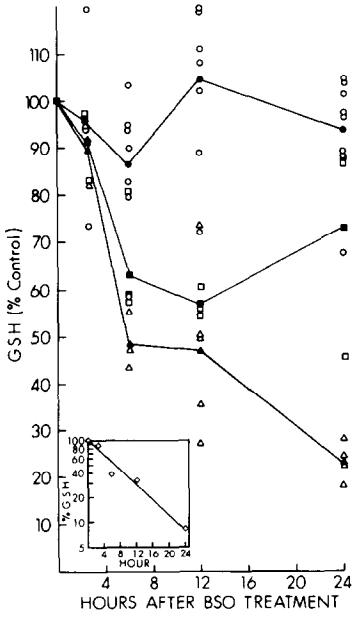


Fig. 1. Glutathione concentration in subcutaneous and intracranial D-54 MG xenografts and contralateral, "normal" murine brain following intraperitoneal administration of 5 mmol/kg D,L-BSO. Data represent individual samples (□, △, ○) and mean values (■, ▲, ●) for subcutaneous and intracranial xenografts, and contralateral brain respectively. Control values (μmol/g tissue wet weight) are listed in Table 1). Insert: Depletion of cytoplasmic glutathione in intracranial D-54 MG xenografts, corrected for a mitochondrial glutathione pool of 16%.

Attempts to maximize the depletion of glutathione in tumor cells while allowing selective rebound in normal tissues were examined; the data are presented in Table 2. Neither a larger dose (20 mmol/kg) nor a second dose at 12 hr enhanced the glutathione depletion in the xenograft; a second dose enhanced the depletion in contralateral brain, 74.5 versus 93.5% of control (P = 0.111). Three doses of BSO appeared to reduce the glutathione concentration in the xenograft further than two doses (16.5 versus 22.2% of control, P = 0.139), while not affecting contralateral normal brain (75 versus 77% of control, P = 0.437).

*Octanol/water partition coefficient.* The octanol/water partition coefficient of BSO was determined to be $4.34 \times 10^{-3} \pm 0.11 \times 10^{-3}$ (standard error) which indicates relatively high water solubility.

*[$^{35}$S]BSO quantitation.* The value of the transfer constant, $K_1$, for [$^{35}$S]BSO in tumor-free frontal cortex was $2.4 \times 10^{-3}$ ml·g$^{-1}$·min$^{-1}$. In whole tumor the value of $K_1$ ranged from 15.8 to $24.1 \times 10^{-3}$ ml·g$^{-1}$·min$^{-1}$. There was little regional variation in the value of $K_1$ throughout the D-54 MG tumors. The autoradiographs illustrate a marked increase in tumor radioactivity compared to normal brain (Fig. 2).

## DISCUSSION

The present studies indicate that a single dose of BSO depleted GSH in a human glioma-derived intracranial xenograft by 77% in 24 hr; the GSH concentration in the contralateral hemisphere was not affected significantly. The finding that larger doses of BSO or additional doses of BSO given at 12-hr intervals did not increase substantially the extent of depletion suggests that tumor GSH biosynthesis was quickly and totally inhibited by 5 mmol/kg BSO (i.p.) given once. The observation that maximal GSH depletion was only about 84% is reminiscent of earlier studies indicating that mito-

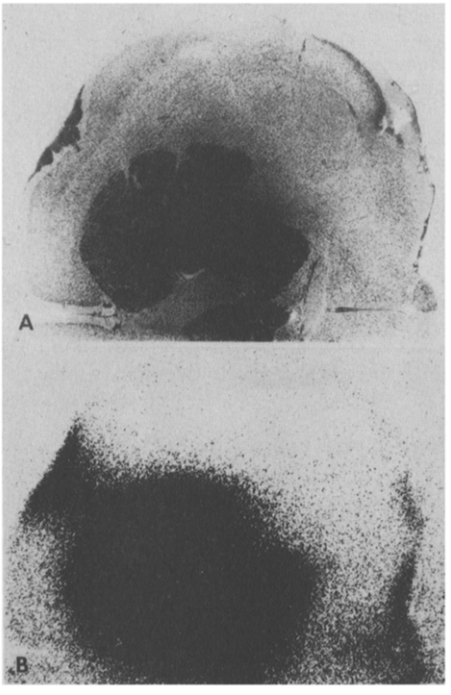


Fig. 2. (A) Histologic section of brain containing D-54 MG xenograft. The lobulated tumor mass is located periventricularly, is not necrotic, and is clearly demarcated from surrounding brain tissue. Smaller foci of tumor cells can be seen in the dorsal meninges, and in the third ventricle. Hematoxylin stain, X5. (B) Autoradiographic image of [$^{35}$S]BSO distribution in the same section. The highest concentrations of BSO are represented by the darkest part of the autoradiographic image, namely, in the D-54 MG xenograft. In contrast, relatively little BSO is seen in tumor-free brain.

chondrial GSH, typically accounting for 15–20% of GSH pool, is not susceptible to depletion by inhibitors of GSH synthesis [21]. Corrected for a mitochondrial GSH pool of 16%, the results indicate that cytoplasmic GSH was depleted from intracranial tumors with approximately first-order kinetics ($r$ = 0.98); the apparent $T_{\frac{1}{2}}$ for GSH turnover in intracranial tumors was about 6.5 hr (see insert, Fig. 1). In subcutaneous xenografts, GSH levels decreased more slowly following administration of BSO, and, in contrast to the intracranial xenografts, the GSH levels showed substantial recovery by 24 hr. Although the kinetics are complicated by GSH resynthesis, data at 2 and 6 hr indicate that the $T_{\frac{1}{2}}$ for GSH turnover was between 8 and 10 hr. The lower $T_{\frac{1}{2}}$ value for intracranial xenografts indicates a higher rate constant for GSH turnover. Since intracranial xenografts also maintained a higher level of GSH (Table 1), the results indicate that tumor GSH biosynthesis and utilization were both affected markedly by the tissue environment.

Since GSH is not depleted significantly in normal brain following BSO, the present studies do not establish the rate of GSH turnover. Earlier studies using intracisternally administered [$^{14}$C]glycine indicated a $T_{\frac{1}{2}}$ of 70 hr [22]. This value was calculated without accounting for [$^{14}$C]glycine recycling [22] or GSH synthetase-catalyzed glycine exchange [23] and must be considered only approximate. Nevertheless, it is apparent that GSH turnover is much more rapid in D-54 MG tumors than in normal brain.

Studies of [$^{35}$S]BSO accumulation in intracerebral D-54 MG and surrounding non-tumor-bearing brain suggest that differential BSO transport contributes to the impressive selectivity of tumor GSH depletion. The unidirectional blood-to-tissue transfer constant, $K_1$, of BSO across the tumor blood–brain barrier was 6- to 15-fold higher than that in normal brain; the restricted entry of BSO into normal brain is clearly evident in Fig. 2. Although this $K_1$ is characteristic of water-soluble compounds transported by passive diffusion, it is noted that BSO is an amino acid analogue and may enter the brain by carrier-mediated transport. Further studies determining $K_1$ and $k_2$ (the tissue-to-blood efflux constant) at multiple time points are expected to characterize the mechanism of BSO transport more fully. Preliminary results indicate that tissue-to-blood BSO transport is slow in the intracranial tumors, and it is probable that this factor contributes to the long-lasting inhibition mediated by BSO in those tumors. In contrast, BSO appeared to be cleared from the subcutaneous tumors relatively rapidly with consequent resynthesis of the GSH pool (Fig. 1).

Previous studies indicate that isolated tumor cells are sensitized by GSH depletion to the cytotoxic effects of ionizing radiation [2, 24, 25], reactive oxygen species [26], sesquiterpene lactones [26], and several clinically important alkylating agents [27, 28]; in many cases, tumor cell killing is increased by substantial factors (e.g. 3- to 30-fold). Few studies have extended these *in vitro* results to intact animals, but the results reported to date are generally encouraging. Ozols *et al.* [27] have shown, for example, that a human ovarian adenocarcinoma xenograft in nude mice is sensitized to melphalan by BSO-mediated GSH depletion, and Clement *et al.* have shown in normal mice that a subcutaneous $B_{16}$ melanoma is sensitized to $^{137}$Cs gamma radiation by GSH depletion [see Ref. 4]. In general, it is anticipated that the therapeutic success of *in vivo* GSH-depletion strategies will depend on both the extent and selectivity of tumor GSH depletion. Lee *et al.* [29] have demonstrated the marked diversity in the response of neoplastic and normal tissues to BSO, observing "significant variations" in the rate and degree of GSH depletion and recovery. Accordingly, therapeutic exploitation of BSO will require awareness of the GSH kinetics of the target tumor and normal tissues likely to demonstrate therapy-limiting toxicity. Nevertheless, a therapeutic "window" maximizing antitumor activity and minimizing normal tissue toxicity may be discernible. Furthermore, localized therapeutic intervention, such as with radiotherapy, may result in increased anti-tumor activity without concomitant increases in toxicity, particularly if the surrounding normal tissues do not demonstrate significant depletion of GSH. The present studies demonstrate that BSO-mediated inhibition of GSH synthesis allows an intracranial tumor to be depleted of GSH without significantly affecting normal brain GSH levels; both selective transport of BSO into the tumor and a

relatively rapid rate of tumor GSH turnover contribute to the selectivity of depletion.

Future studies will address the role of GSH depletion in selectively enhancing chemotherapeutic and radiotherapeutic activity against human glioma and medulloblastoma xenografts in athymic nude mice.

## REFERENCES


1. Calcutt G and Connors TA, Tumour sulphydryl levels and sensitivity to the nitrogen mustard merophan. *Biochem Pharmacol* **12**: 839–845, 1963.
2. Dethmers JK and Meister A, Glutathione export by human lymphoid cells: Depletion of glutathione by inhibition of synthesis decreased export and increases sensitivity to irradiation. *Proc Natl Acad Sci USA* **78**: 7492–7496, 1981.
3. Meister A, Metabolism and transport of glutathione and other γ-glutamyl compounds. In: *Functions of Glutathione: Biochemical, Physiological, Toxicological, and Clinical Aspects* (Eds. Larson A, Orrenius S, Holmgren A and Mamervik B), pp. 1–21. Raven Press, New York, 1983.
4. Griffith OW, Glutathione and cell survival. In: *Cellular Regulation and Malignant Growth* (Ed. Ebashi S), pp. 292–300. Japan Scientific Societies Press, Springer, Tokyo, 1985.
5. Griffith OW and Meister A, Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). *J Biol Chem* **254**: 7558–7560, 1979.
6. Griffith OW and Meister A, Glutathione: Interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci USA* **76**: 5606–5610, 1979.
7. Griffith OW, Mechanism of action, metabolism and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. *J Biol Chem* **257**: 13704–13712, 1982.
8. Groothuis DR and Blasberg RG, Rational brain tumor chemotherapy: The interaction of drug and tumor. *Neurol Clin* **3**: 801–816, 1985.
9. Minchinton AI, Rojas A, Smith KA, Soranson JA, Shrieve DC, Jones NR and Bremer JC, Glutathione depletion in tissues after administration of buthionine sulfoximine. *Int J Radiat Oncol Biol Phys* **10**: 1261–1264, 1984.
10. Schold SC Jr, Bullard DE, Bigner SH, Jones TR and Bigner DD, Growth, morphology, and serial transplantation of anaplastic human gliomas in athymic mice. *J Neurooncol* **1**: 5–14, 1983.
11. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H and Parks WP, In vitro cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst.* **51**: 1417–1423, 1973.
12. Bigner DD, Bigner SH, Ponten J, Westermark B, Mahaley MS Jr, Ruoslahti E, Herschman H, Eng LF and Wikstrand CJ, Heterogeneity of genotypic and phenotypic characteristics of fifteen permanent cell lines derived from human gliomas. *J Neuropathol Exp Neurol* **40**: 201–229, 1981.
13. Bullard DE, Schold SC Jr, Bigner SH and Bigner DD, Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines. *J Neuropathol Exp Neurol* **40**: 410–427, 1981.
14. Saris SC, Bigner SH and Bigner DD, Intracerebral transplantation of a human glioma line in immunosuppressed rats. *J Neurosurg* **60**: 582–588, 1984.
15. Bradford M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
16. Tietze F, Enzyme method for quantitative determination of nanogram amounts of total and oxidized glutathione. *Anal Biochem* **27**: 502–522, 1969.
17. Griffith OW, Determination of glutathione and glutathione-disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**: 207–212, 1980.
18. Blasberg RG, Patlak CS and Fenstermacher JD, Letter to the editor. *J Cereb Blood Flow Metab* **3**: 401, 1983.
19. Vriesendorp FF, Pegram C, Bigner DD and Groothuis DR, Concurrent measurements of blood flow and transcapillary transport in xenotransplanted human gliomas in immunesuppressed rats. *J Natl Cancer Inst* **79**: 123–130, 1987.
20. Blasberg RG, Groothuis DR and Molnar P, Application of quantitative autoradiographic measurements in experimental brain tumors. *Semin Neurol* **1**: 203–221, 1981.
21. Griffith OW and Meister A, Origin and turnover of mitochondrial glutathione. *Proc Natl Acad Sci USA* **82**: 4668–4672, 1985.
22. Douglas GW and Mortensen RA, The rate of metabolism of brain and liver glutathione in the rat studied with $C^{14}$-glycine. *J Biol Chem* **222**: 581–589, 1956.
23. Griffith OW, Glutathione turnover in human erythrocytes. *J Biol Chem* **256**: 4900–4904, 1981.
24. Clark EP, Epp ER, Biaglow JE, Morse-Gaudio M and Zachgo E, Glutathione depletion, radiosensitization, and misonidazole potentiation in hypoxic Chinese hamster ovary cells by buthionine sulfoximine. *Radiat Res* **98**: 370–380, 1984.
25. Shrieve DC, Denekamp J and Minchinton AI, Effects of glutathione depletion by buthionine sulfoximine on radiosensitization by oxygen and misonidazole *in vitro*. *Radiat Res* **102**: 283–294, 1985.
26. Arrick BA and Nathan CF, Glutathione metabolism as a determinant of therapeutic efficacy: A review. *Cancer Res* **44**: 4224–4232, 1984.
27. Ozols RF, Louie KG, Plowman J, Behrens BC, Fine RL, Dykes D and Hamilton TC, Enhanced melphalan cytoxicity in human ovarian cancer *in vitro* and in tumor-bearing nude mice by buthionine sulfoximine depletion of glutathione. *Biochem Pharmacol* **36**: 147–153, 1987.
28. Kramer RA, Greene K, Ahmad S and Vistica DT, Chemosensitization of L-phenylalanine mustard by the thiol-modulating agent buthionine sulfoximine. *Cancer Res* **47**: 1593–1597, 1987.
29. Lee FYF, Allalunis-Turner MJ and Siemann DW, Depletion of tumor versus normal tissue glutathione by buthionine sulfoximine. *Br J Cancer* **50**: 33–38, 1987.