Phase I Study of Continuous-Infusion L-S,R-Buthionine Sulfoximine With Intravenous Melphalan

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Background: Increased intracellular glutathione has long been associated with tumor cell resistance to various cytotoxic agents. An inhibitor of glutathione biosynthesis, L-S,R-buthionine sulfoximine (BSO), has been shown to enhance the cytotoxicity of chemotherapeutic agents in vitro and in vivo. We performed a phase I study of BSO administered with the anticancer drug melphalan to determine the combination’s safety/tolerability and to determine clinically whether BSO produced the desired biochemical end point of glutathione depletion (<10% of pretreatment value). Methods: Twenty-one patients with advanced cancers received an initial 30-minute infusion of BSO totaling 3.0 g/m² and immediately received a continuous infusion of BSO on one of the following schedules: 1) 0.75 g/m² per hour for 24 hours (four patients); 2) the same dose rate for 48 hours (four patients); 3) the same dose rate for 72 hours (10 patients); or 4) 1.5 g/m² per hour for 48 hours (three patients). During week 1, the patients received BSO alone; during weeks 2 or 3, they received BSO plus melphalan (15 mg/m²); thereafter, the patients received BSO plus melphalan every 4 weeks. Glutathione concentrations in peripheral blood lymphocytes were determined for all patients; in 10 patients on three of the administration schedules, these measurements were made in multiple sections from tumor biopsy specimens taken before, during, and after continuous-infusion BSO. Results: Continuous-infusion BSO alone produced minimal toxic effects, although BSO plus melphalan produced occasional severe myelosuppression (grade 4) and frequent low-grade nausea/vomiting (grade 1–2). This treatment also produced consistent, profound glutathione depletion (<10% of pretreatment value). The degree of glutathione depletion in peripheral lymphocytes was considerably less than that observed in tumor sections. Conclusions: Continuous-infusion BSO is relatively nontoxic and results in depletion of tumor glutathione. [J Natl Cancer Inst 1997;89:1789–96]

Tumor cell resistance to antineoplastic therapies is a major obstacle to the successful treatment of almost all cancers. Therefore, much emphasis has been placed on identifying cellular mechanisms of resistance to antineoplastics and potential methods of overcoming (modulating) these resistance mechanisms. Not surprisingly, tumor cell drug resistance—either clinical or in vitro—has been associated with a variety of cellular mechanisms.

Glutathione is a ubiquitous tripeptide that has a prominent role in many cellular functions including detoxification of xenobiotics (1). Researchers, for years, have observed that elevations in intracellular glutathione have been associated with resistance to irradiation or chemotherapy and, correspondingly, that diminution of intracellular glutathione is associated with sensitization to irradiation or chemotherapy (2–7). Recently, two important observations have been made pertaining to drug resistance associated with elevated glutathione. Drug resistance has been correlated with elevations in both messenger RNA levels (8,9) and the enzymatic activity of γ-glutamylcysteine synthetase (GCS) (8), the rate-limiting biosynthetic step in glutathione production (10); and the transfection of complementary DNAs for the heavy and light subunit of GCS into tumor cells resulted in increased glutathione levels and GCS activity as well as resistance to melphalan (L-PAM) (11). Unlike many other proposed mechanisms of drug resistance, elevated glutathione may act through different pathways to limit the effectiveness of multiple types of chemotherapeutic agents. Researchers have observed glutathione to protect tumor cells by detoxifying chemotherapeutic drugs via conjugation reactions catalyzed by glutathione S-transferases or such independent reactions as the dechlorination of L-PAM (4) or the quenching of drug–DNA adducts (12,13). Glutathione also contributes to drug resistance through the GS-X pump or multidrug resistance-related proteins, producing decreased intracellular drug accumulation (14,15).

L-S,R-Buthionine sulfoximine (BSO) is a specific inhibitor of GCS developed by Griffith and Meister (16). It has been shown to reverse drug resistance in tumor cells that overexpress glutathione (17–20). In cells that do not overexpress glutathione, BSO increases the sensitivity to many chemotherapeutic agents when intracellular glutathione is decreased, usually to less than 10% of pretreatment levels (15,21). Therefore, we (22) and others (23) undertook phase I studies of BSO to evaluate whether modulation of glutathione could be clinically meaningful. Our initial phase I study of BSO plus L-PAM employed a schedule of repeated short infusions (six to 10 doses every 12 hours) of BSO, with L-PAM given after the last or second-to-last BSO dose. That schedule proved to be well tolerated and safe, but pharmacodynamic data from that trial as well as preclinical data strongly suggested that continuous exposure to BSO would produce better reduction in tumor glutathione levels.
We subsequently performed an initial phase I study of continuous-infusion BSO with intravenous L-PAM to determine its safety/tolerability, but more importantly to determine whether clinical administration of BSO could produce the desired biochemical end point of tumor glutathione depletion to less than 10% of pretreatment levels.

**Patients and Methods**

**Patient Selection**

Individuals with advanced cancer for whom no standard effective therapy was available and who gave informed consent according to institutional and Food and Drug Administration guidelines were eligible. Provided the following criteria were met: adequate bone marrow (white blood cell [WBC] count ≥4000/μL and platelet count ≥100,000/μL), normal renal function (serum creatinine concentration ≤1.5 mg/dL in women or ≤1.8 mg/dL in men or creatinine clearance ≥60 mL/minute) and adequate hepatic function (aspartate aminotransferase less than four times normal, bilirubin level ≤1.5 mg/dL) and metabolic function (calcium level <11.0 mg/dL). After enrolling the initial six patients, all further patients were required to have tumors that could be biopsied easily, which was defined as every level being greater than or equal to 3000 cells/μL and the platelet count was greater than or equal to 200,000 cells/μL. Those exhibiting unacceptable nonhematologic toxicity received a 50% decrease in BSO dose. Hematologic and nonhematologic toxicity were monitored weekly.

**Drug Administration and Dose Escalation**

All patients were treated as inpatients in the National Institutes of Health-sponsored General Clinical Research Center facility at the University of Wisconsin Hospital. BSO was administered as an initial 30-minute infusion (a fixed dose of 3.0 g/m²) followed immediately by a continuous infusion lasting 24–72 hours. During week 1 of course 1 only, all patients initially received BSO but not L-PAM. Patients receiving dose levels 1a (a 24-hour continuous infusion of BSO, 0.75 g/m² per hour), 1b (48 hours, 0.75 g/m² per hour), and 2a (48 hours, 1.5 g/m² per hour) were re-treated in week 2, course 1 with BSO as described, followed by intravenous L-PAM (15 mg/m²) at the end of the continuous infusion. Patients receiving dose level 1c (a 72-hour continuous infusion of BSO, 0.75 g/m² per hour) were re-treated in week 3 with L-PAM administered at hour 48.5 (24 hours prior to completion of the 72-hour infusion). All further treatment in the study consisted of BSO plus L-PAM as described, every 4 weeks.

If necessary, all courses were delayed until the patient’s WBC count was greater than or equal to 3000 cells/μL and the platelet count was greater than or equal to 100,000 cells/μL or until there was complete resolution of all preceding course toxicity. No antiemetics were given as part of the BSO continuous infusion, but antiemetics (intravenous granisetron or ondansetron plus dexamethasone) were given prior to L-PAM as part of the regimen.

The starting schedule for BSO administration was based on the pharmacodynamic results of our first BSO phase I trial (22), which suggested that an initial rapid increase in the intracellular concentration of L-PAM dose and those exhibiting unacceptable nonhematologic toxicity received a 50% decrease in BSO dose. Hematologic and nonhematologic toxicity were monitored weekly.

**Analysis of Glutathione**

Peripheral mononuclear cells (PBLs) and the cellular constituents of malignant fluid collections were collected and analyzed as follows. Whole blood samples were drawn into heparinized tubes at some or all of the following time points relative to the start of the BSO infusions: BSO duration of 24.5 hours—0, 10, 24.5, 34, and 48.5 hours; BSO duration of 48.5 hours—0, 10, 24.5, 30, 48.5, 58, and 72.5 hours; and BSO duration of 72.5 hours—0, 24.5, 48.5, 72.5, and 96.5 hours. For all patients, BSO administration started promptly at 9 AM and infusions end at 9:30 AM on the appropriate day, due to concerns over circadian variation observed in tissue glutathione levels in animals (24). The PBLs were obtained and assayed as described previously (22) by use of the method of Tietze (25) as modified by Bump et al. (26). Values are expressed as nanograms of glutathione per 10⁶ PBLs. Tumor fluid was also collected and analyzed as described previously (22).

**Analysis of Serum BSO Levels**

BSO was supplied by the Division of Cancer Treatment, National Cancer Institute (DCT/NCI) in 1-g vials (100 mg/mL) that contained 10 mL sterile water for injection. BSO was lyophilized from this sterile water, reconstituted with sterile water, and filtered through a 0.45-μm filter, diluted with normal saline to a concentration of less than or equal to 2 mg/mL, and administered as a 20-minute infusion. L-PAM and BSO were administered through a central venous catheter.

**Analysis of Serum BSO Activity**

PBLs for measuring GCS activity were obtained, handled, and assayed as described previously (22), except that, rather than resuspending them in H₂O, the PBLs were resuspended in 10 mM Tris–HCl (pH 7.5) containing 25 mM MgCl₂. As before, GCS activity was quantified by use of a modification of a high-performance liquid chromatography technique described by Nandi et al. (27). Protein concentration was determined as described by Bradford (28) with the use of serum albumin as a standard.

**Analysis of Serum Albumin Levels**

BSO was supplied by the Division of Cancer Treatment, Diagnosis, and Centers, NCI, Bethesda, MD. Patients with unacceptable toxicity received a 33% decrease in L-PAM dose (and those exhibiting unacceptable nonhematologic toxicity received a 50% decrease in BSO dose). Hematologic and nonhematologic toxicity were monitored weekly.

**Frequency and Duration of Treatment**

If there was evidence of clinical improvement, patients were eligible to continue receiving treatment after course 1. Courses could be repeated as frequently as every 28 days.

**Analysis of Glutathione**

Peripheral mononuclear cells (PBLs) and the cellular constituents of malignant fluid collections were collected and analyzed as follows. Whole blood samples were drawn into heparinized tubes at some or all of the following time points relative to the start of the BSO infusions: BSO duration of 24.5 hours—0, 10, 24.5, 34, and 48.5 hours; BSO duration of 48.5 hours—0, 10, 24.5, 30, 48.5, 58, and 72.5 hours; and BSO duration of 72.5 hours—0, 24.5, 48.5, 72.5, and 96.5 hours. For all patients, BSO administration started promptly at 9 AM and infusions end at 9:30 AM on the appropriate day, due to concerns over circadian variation observed in tissue glutathione levels in animals (24). The PBLs were obtained and assayed as described previously (22) by use of the method of Tietze (25) as modified by Bump et al. (26). Values are expressed as nanograms of glutathione per 10⁶ PBLs. Tumor fluid was also collected and analyzed as described previously (22).

**Tumor Glutathione Determination**

Tumor glutathione determination was undertaken as follows. During week 1 of either course 1 or course 2, excisional biopsies of subcutaneous, cutaneous, or otherwise accessible mucosal tumors were performed immediately prior to the start of BSO infusion and at time points during or immediately after completion of the infusion. The biopsy specimens (usually 1–2 cm in diameter) were immediately sectioned into pieces greater than 100 mg (tumor only, by gross inspection) and placed individually into tared containers. The samples were flash-frozen in liquid nitrogen and stored at −80°C until assayed. Prior to thawing, 1 mL of 20 mM sulfosalicylic acid was added to each sample, and samples underwent homogenization in a Polytron three times, each for 30 seconds. The supernatant was assayed for glutathione as described previously.

**Analysis of GCS Activity**

PBLs for measuring GCS activity were obtained, handled, and assayed as described previously (22), except that, rather than resuspending them in H₂O, the PBLs were resuspended in 10 mM Tris–HCl (pH 7.5) containing 25 mM MgCl₂. As before, GCS activity was quantified by use of a modification of a high-performance liquid chromatography technique described by Nandi et al. (27). Protein concentration was determined as described by Bradford (28) with the use of serum albumin as a standard.

**Analysis of Serum BSO Levels**

Patient sera were collected either specifically for BSO assay or for GCS activity assay and stored at −80°C. BSO (L,R) and L,S isomers levels were assayed as described previously (22). Briefly, methanol extracts of the plasma were prepared and treated with o-phthalaldehyde prior to chromatography. An internal standard (L-α-amino-3-hydroxybutyric acid) was employed. Individual R and S isomers were separated and individually quantitated (against purified L,R-BSO and L,S-BSO provided by Owen Griffith, Medical College of Wisconsin, Milwaukee). Plasma standard curves are linear from 50 to 5000 μM total.
BSO, with the limits of quantitation approximately 10 μM for each isomer. The within-day coefficient of variability (c.v.) was less than 3% over this dose range, and the between-day c.v. was 3%–7%. Recovery from plasma was near 100%.

Results

A total of 43 courses were administered to 21 patients (one patient and course were unevaluable, as shown in Table 1). The majority of patients had a history of prior chemotherapy and had advanced ovarian cancer or melanoma.

Toxicity

L-S,R-BSO, given as an initial loading infusion followed by a continuous infusion of 24–72 hours’ duration, resulted in minimal toxicity. The predominant toxicity was grade 1 to 2 nausea with occasional vomiting, which occurred in approximately one half of the patients. When it did occur, it readily responded to phenothiazines or butyrophenones—except for one patient whose grade 2 nausea did not improve until a serotonin antagonist was administered. One patient had halitosis and vivid/troubling nightmares on some nights during BSO administration and for a few days after BSO. No other neurotoxicity, hepatotoxicity, or nephrotoxicity was observed or mentioned by subjects.

The combination of continuous-infusion BSO and L-PAM resulted in toxicity similar to that observed in our prior phase I study of BSO/L-PAM. Most of the patients had myelosuppression and nausea/vomiting consistent with the toxicity of L-PAM (Table 2). The myelosuppression was predominately granulocytopenia and thrombocytopenia and was occasionally severe in patients who were pretreated heavily. These symptoms were also reversible and of limited duration. Only one patient, a heavily pretreated patient with ovarian cancer with prolonged (>14 days) thrombocytopenia, required a delay in treatment because of persistent myelosuppression. Unacceptable myelosuppression (grade 4 granulocytopenia or grade 3–4 thrombocytopenia or >7 days’ duration) also occurred in one other heavily pretreated patient with ovarian cancer. The nausea/vomiting observed in these patients was consistent with that expected from L-PAM given with scheduled antiemetics, usually being mild and of limited duration. Three episodes of greater than or equal to grade 3 nausea/vomiting were observed.

Other observed toxic effects were four courses in which patients experienced grade 1–2 diarrhea and three courses in which patients experienced grade 1–2 mucositis/stomatitis. Two patients died from progressive cancer during the study; one patient died on day 27 of course 2 (level 2a) as a result of progressive melanoma and the other patient developed evidence of rapidly progressive colon cancer and died shortly after receiving BSO alone (level 1c).

Clinical Activity

Eight patients included in the study had received previous treatment for ovarian cancer (two prior treatment regimens, two patients; three prior regimens, three patients; and four prior regimens, three patients). These patients had all been exposed previously to platinum-based drugs, alkylating agents, and taxanes and had not responded to the immediately prior regimen (exception—one patient had recurred after responding to high-dose paclitaxel [Taxol] [300 mg/m2]). Beneficial changes occurred in three of these eight patients. A patient at level 1a with stage IV disease developed malignant obstructive jaundice immediately after receiving BSO plus L-PAM. The jaundice and hepatic insufficiency resolved during course 1 follow-up. At 3 weeks after receiving BSO plus L-PAM, the patient showed resolution of obstructive jaundice; significant shrinkage of all disease (some lesions had decreased by >50%), especially in the liver; and a decrease in CA 125 from greater than 10000 to 5600. This patient unfortunately had also experienced significant, prolonged myelosuppression requiring a 6-week delay in further treatment and a reduction in L-PAM dosage from 15 to 7.5 mg/m2 (course 2). The patient showed progressive disease after receiving course 2 and was removed from the study. A patient at level 1c had a greater than 50% decrease in CA 125 (from a prestudy value of >1000) for four courses without objective tumor response and was removed from the study after five courses having stable disease. Another patient at level 1c, who had been requiring weekly paracenteses for malignant ascites, did not require paracentesis after receiving BSO plus L-PAM and had successively decreasing CA 125 values while in the study. L-PAM was reduced to 10 mg/m2 due to unacceptable myelosuppression during courses 2 and 3. This patient was removed from the study after three courses with stable disease, but with reaccumulating ascites. A patient with metastatic breast cancer who had received multiple treatments (a high-dose regimen of cyclophosphamide and mitoxantrone, paclitaxel, etoposide, and continuous-infusion fluorouracil/leucovorin) in the prior 12 months was treated at level 1c. After course 1, the patient was noted to have a greater than 50% reduction in extensive subcuticular disease. A computed tomography scan after course 2 showed a less than 50% reduction in liver metastases. The patient was removed from the study after course 3 because of clinical deterioration and disease progression. A patient with metastatic melanoma who had a complete resolution of disease lasting 18 months after treatment with the initial phase I trial of BSO plus L-PAM was re-treated on this trial at level 1b. He received six courses of BSO plus L-PAM, with no evidence of disease response or progression. He was removed from the study with stable disease.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.*</th>
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<tbody>
<tr>
<td>Patients</td>
<td>21 (13 females)</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>54 (27–79)</td>
</tr>
<tr>
<td>Prior chemotherapy exposure</td>
<td>20</td>
</tr>
<tr>
<td>Primary tumor types or sites</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>8</td>
</tr>
<tr>
<td>Melanoma</td>
<td>7</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
</tr>
<tr>
<td>Colon</td>
<td>2</td>
</tr>
</tbody>
</table>

No. of subjects/No. of courses per level†

| Level 1a: four subjects/seven courses |
| Level 1b: four subjects/10 courses |
| Level 1c: 10 subjects/15 courses |
| Level 2a: three subjects/seven courses |

*Unless otherwise specified, values = number of patients.
†Does not include one unevaluable course and three courses in which the dose was reduced.
Glutathione Depletion

We examined glutathione levels in PBLs as a surrogate for levels in tumor tissue. PBL glutathione concentrations at baseline (mean ± standard deviation [SD], n = 21, 479 ± 121 ng glutathione/10^6 PBLs) were very similar to data published previously (22,29). Continuous-infusion BSO resulted in a gradual decrease in PBL glutathione concentrations over the course of the infusion, with the lowest concentrations occurring at the end of the infusions and persisting for approximately 24 hours after infusion. This was true of levels 1a (24-hour continuous infusion), 1b (48-hour continuous infusion), and 2a (48-hour continuous infusion) (data not shown) as well as 1c (72-hour continuous infusion) (Fig. 1). At their lowest, glutathione concentrations for level 1c were approximately 100 ng glutathione/10^6 PBLs or 30%–40% of control. Lowest-point (nadir) values for levels 1a, 1b, and 2a were slightly higher. As observed in our prior phase I trial (22), the percent depletion from baseline was uniform, regardless of baseline glutathione concentration. Interestingly, the week 2 or 3 baseline PBL glutathione values were consistently lower than week 1 (Fig. 1). Level 1c baseline PBL glutathione values from week 1 differed significantly from those of week 3 (mean ± SD, 430 ± 139 versus 278 ± 78; P = .009, by paired t test, n = 9). This persistent, significant depletion in PBL glutathione values was also evident from week 1 to week 2 (P = .002, n = 11). Mononuclear cells in the ascitic fluid of a patient with malignant ascites who was treated at level 1b were examined for glutathione during continuous-infusion BSO and found to have serial glutathione values very similar to circulating PBLs.

Tumor Glutathione Depletion

Tumor glutathione values were evaluated in 10 patients (1b = one patient, 2a = two, and 1c = seven) using tumor samples obtained by excisional biopsies. Analysis of multiple sections of each tumor showed a variability in baseline glutathione values for most patients’ tumors. Tumor glutathione values during or immediately after continuous-infusion BSO showed extensive depletion of glutathione, with occasional variability depending...
on the degree of depletion. All of the patients’ tumors that were examined revealed marked depletions in glutathione values, especially at hours 48 and 72. After 48 to 72 hours of continuous-infusion BSO, tumor glutathione levels were often undetectable and were consistently measured at or below 10% of control levels. The following are examples of absolute values for tumor glutathione from individual patients (mean ± SD, nanogram glutathione/mg tissue): subcuticular metastatic melanoma (level 1c) pretreatment, 313 ± 133; hour 24.5, 37 ± 37; and hour 48.5, 18 ± 17; subcuticular metastatic breast carcinoma (level 1c) pretreatment, 49 ± 2; hour 48.5, 1 ± 0.3; and vaginal metastases from ovarian carcinoma (level 2a) pretreatment, 266 ± 38 and hour 48.5, 4 ± 4. Fig. 2 is based on calculating the mean glutathione value of the separate sections for each time point. Relative to the mean baseline value of glutathione, percentage of control was calculated for each time point. Fig. 2 shows the compiled mean ± standard error (SE) (% of control) for each time point: levels 1b and 1c—hour 24, 32% ± 21%; hour 48, 14% ± 7%; hour 72, 1.3% ± 0.4%; and level 2a—hour 48, 5% ± 3%.

GCS Activity

Evaluation of PBL GCS activity was done for the majority of patients at levels 1a, 1b, and 2a and for the initial patients at level 1c. The pretreatment activity of GCS (1012 ± 212 pmol/minute per mg protein) was similar to our prior phase I data per mg protein): week 1—1012 and week 2—982; \( P = .75 \), by paired \( t \) test, \( n = 13 \).

BSO Pharmacokinetics

Extensive blood sampling to determine the pharmacokinetics of BSO was done on patients at levels 1a, 1b, and 2a, with limited sampling performed on level-1c patients. All patients received the same initial 30-minute BSO infusion of 3.0 g/m². The peak BSO concentration was at the end of infusion (1530 ± 480 \( \mu M \), mean ± SD; \( n = 19 \)). On completion of the initial 30-minute infusion, we immediately started the continuous-infusion BSO at either level 1 (0.75 g/m² per hour) or level 2 (1.5 g/m² per hour). Steady-state concentrations of BSO were reached after 6–8 hours and did not significantly differ from week 1 to weeks 2 or 3 (data not shown). The steady-state concentrations of \( L,S,R \)-BSO were approximately 500 \( \mu M \) at level 1 and 1000 \( \mu M \) at level 2 (Table 3). A significant difference in steady-state concentration was observed between the \( L,S \) and \( L,R \) isomers at both dose levels (level 1—\( L,S 230 ± 126 \mu M, L,R 295 ± 151 \mu M, P < .0001 \), by paired \( t \) test, \( n = 16 \) and level 2—\( L,S 540 ± 374 \mu M, L,R 725 ± 427 \mu M, P < .0001, n = 3 \)). The area under the concentration × time curve (AUC) increased with either prolonged duration of infusion or increased BSO dose. It appeared that the steady-state concentration and AUC both increased linearly with the dose or duration of infusion, but given our limited datasets, this must be interpreted cautiously. Total-body clearance of \( L,S,R \)-BSO (\( n = 19, 123 ± 45 \text{ mL/minute per m}^2 \) did not vary with dose (\( n = 11, P = .23 \)) or repeated infusions (\( n = 11, P = .66 \)). Clearance of the two isomers did differ, as has been noted previously (22), with \( L,R \) clearing faster than \( L,S \) (\( L,R 153 ± 58 \text{ mL/minute per m}^2, L,S 102 ± 36; P < .0001 \)). However, elimination of the two isomers was biexponential, with an observed \( t_{1/2} \) (half-life) \( \alpha \) of 37 minutes (harmonic mean, \( n = 19 \)) and a mean \( t_{1/2} \) \( \beta \) of 4.3 hours. Neither the \( t_{1/2} \) \( \alpha \) or \( t_{1/2} \) \( \beta \) differed significantly between the \( S \) or \( R \) isomer.

No pharmacodynamic relationships could be found between \( L,S,R \)-BSO AUC and glutathione depletion in tumor tissue or PBLs.

Discussion

The main end points of phase I studies are the related goals of determining the maximum tolerated dose/schedule and finding a recommended phase II dose/schedule. As more and more potential anticancer agents are being tested on the basis of the clinical potential of their specific biochemical effect, rather than for their general anticancer properties, the connection between the above goals may be lost. A good example of this is BSO, which is being evaluated as an adjunct to chemotherapy solely for its ability to deplete intracellular glutathione. While BSO could potentially have effects in vivo independent of its action on glutathione, the clinical evaluation of BSO as a modulator of chemotherapy represents the clinical evaluation of glutathione depletion as a modulator of cytotoxicity. Thus, the initial clinical evaluation of BSO has to be twofold: study of the usual phase I goals (e.g., assessment of toxicity and pharmacokinetics) plus determination of the clinical feasibility of achieving the desired biochemical end point.

A common feature of the many in vitro evaluations of glutathione depletion to reverse drug resistance or enhance chemotherapy cytotoxicity, regardless of the mechanism of depletion involved, was the extent of the glutathione depletion, usually to...
less than 10% of pretreatment values (3–6). Therefore, we sought to duplicate in vivo this extensive tumor glutathione depletion.

Our initial trial of BSO plus L-PAM evaluated the glutathione content of PBLs only, to avoid potential problems (associated with significant variability) in interpreting glutathione data from random tumor biopsies (30,31). In planning the continuous BSO trial, it was decided to seek tumor glutathione data under these conditions: 1) whenever possible, from complete excisional biopsies for each time point; 2) using separate, excised lesions that were of the same metastatic type and general location, due to observed glutathione differences between different types of metastases (32); and 3) separately assaying multiple sections of gross tumor for glutathione to lessen the problem of intratumoral glutathione variability.

The tumor glutathione data that we obtained clearly revealed extensive glutathione depletion related to continuous-infusion BSO. Importantly, we reached the desired biochemical end point of consistent tumor glutathione depletion to less than 10% of pretreatment levels without significant BSO-related toxicity. Extensive tumor glutathione depletion was observed consistently following 48 or 72 hours of continuous-infusion BSO (dose level 1 or 2), and less so after 24 hours. Whether the inconsistent degree of glutathione depletion at hour 24 is accurate or a reflection of limited data is unclear. Performing glutathione assays on multiple sections from individual lesions also confirmed tumor glutathione heterogeneity in glutathione concentrations. The heterogeneity observed had minimal impact on our data due to the extensive depletion of glutathione observed; many tumor sections had undetectable levels of glutathione. However, given the heterogeneity, in the few tumors exhibiting less extensive depletion of glutathione, but an overall average depletion to 5%–10% of pretreatment levels, single samples could have concentrations of from 1% to 50% of pretreatment.

The PBL glutathione data were similar to our prior experience (22). All patients’ PBLs had a gradual decrease in glutathione, with the nadir occurring at the end of the 24–72-hour infusion. Whether prolonging the continuous-infusion BSO would lead to even further PBL glutathione depletion is uncertain. Regression analysis predicted further PBL glutathione depletion with repeated BSO dosing during our prior BSO trial, but extended dosing did not produce further glutathione reduction (22). The nadir PBL glutathione values were slightly lower with this schedule compared with the repeated bolus administration. However, these values were not reflective of the degree of tumor glutathione depletion. The value of measuring PBL glutathione is its predictive value as a surrogate for tumor concentrations. Based on these data, PBL glutathione level is not an effective surrogate for tumor glutathione measurement under conditions involving BSO exposure. There are many potential explanations for this; the most likely would involve differences in glutathione turnover between tissue types. The GCS enzyme activity in PBLs showed consistent inhibition throughout continuous-infusion BSO, unlike the data from the bolus BSO schedule in which PBL GCS activity recovered to pretreatment levels after each dose (22). Similarly, it is debatable how reflective PBL GCS activity is of tumor cell GCS activity. Furthermore, it is interesting to note that after BSO administration is terminated, PBL glutathione fails to recover to pretreatment levels over 1–2 weeks while PBL GCS activity rapidly recovers to pretreatment levels after BSO. These data imply that PBLs maintain their usual glutathione equilibrium (production/consumption) for prolonged periods of time after continuous-infusion BSO exposure, despite decreased intracellular glutathione concentrations. Yao et al. (33) observed increased expression of GCS (heavy subunit) messenger RNA above baseline in PBLs from selected patients undergoing treatment with bolus BSO in a time course that mirrored the fall in PBL glutathione. Our data imply that, under conditions of continuous BSO exposure, PBL glutathione depletion does not lead to enhanced glutathione biosynthesis. The data of Yao et al., however, imply that PBL depletion results in at least some feedback signaling for enhanced biosynthesis. There are many potential explanations for these results, and further investigation of glutathione regulation will need to be performed in tumor cells as well.

On the basis of preclinical data and prior data (22,23) the pharmacokinetics of BSO were as expected with biexponential elimination and differential clearance between l,R and l,S stereoisomers. Steady-state concentrations of l,S,R-BSO of 0.5–1.0 mM for more than 24 hours were obtained without significant toxicity or signs of drug accumulation. These steady-state concentrations, which are well above the concentrations used in vitro to enhance chemotherapy’s cytotoxicity, are reached within 6–8 hours after initiating BSO infusion. Due to enhanced clearance, steady-state levels of l-R-BSO (inactive stereoisomer) are significantly lower than those for the active isomer, l,S-BSO.

### Table 3. BSO pharmacokinetic parameters*

<table>
<thead>
<tr>
<th>Pharmacokinetic</th>
<th>Level 1a (n = 4) (0.75 g/m² per h × 24)</th>
<th>Level 1b (n = 4) (0.75 g/m² per h × 48)</th>
<th>Level 1c (n = 8) (0.75 g/m² per h × 72)</th>
<th>Level 2a (n = 3) (1.5 g/m² per h × 48)</th>
<th>Overall mean (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₜ₀ Week 1 (µM)</td>
<td>650 ± 500 (S isomer %)</td>
<td>530 ± 130 (S isomer %)</td>
<td>465 ± 189 (S isomer %)</td>
<td>1260 ± 800 (S isomer %)</td>
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</tr>
<tr>
<td>AUC (mM × h)</td>
<td>18.5 ± 1.2</td>
<td>28.2 ± 0.6</td>
<td>31.3 ± 8.4</td>
<td>61.8 ± 37.0</td>
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<tr>
<td>Cₜ₀ mL/min per m²</td>
<td>108 ± 47</td>
<td>111 ± 21</td>
<td>136 ± 45</td>
<td>112 ± 50</td>
<td>123 ± 45</td>
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<td>t½, min</td>
<td>37†</td>
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*BSO = L,S,R-buthionine sulfoximine; Cₜ₀ = plasma steady-state levels, AUC = area under the concentration × time curve; Clₜ₀ = total-body clearance. Values in columns = mean ± standard deviation.
†Harmonic mean.
The reason for the difference in clearance is unknown at present. No pharmacodynamic relationships between BSO and glutathione depletion were observed. This is probably due to the extreme degree of tumor glutathione depletion observed and the relatively small range of BSO exposure (a twofold AUC change).

Our initial phase I trial of BSO plus L-PAM, which used a paired course design, revealed two important facts about the use of repeated bolus administrations of BSO with L-PAM. Bolus administration of BSO and L-PAM produced significantly more myelosuppression than L-PAM alone. The increased toxicity could not be automatically attributed to glutathione depletion alone because a bolus of BSO significantly reduced the clearance and volume of distribution of L-PAM, with a correspondingly higher L-PAM AUC. If the increased toxicity and changes in L-PAM pharmacokinetics were related to glutathione depletion, then continuous-infusion BSO should be expected to produce the same effects. If the observed effects were linked to an influence of BSO on L-PAM independent of glutathione, then continuous-infusion BSO may not produce the same effects. Bolus administration of BSO is characterized by very short-lived, high concentrations of the agent, while continuous-infusion BSO is a constant, relatively low concentration exposure. It would be helpful to answer these issues and others (will a lower steady-state concentration of BSO produce significant tumor glutathione depletion? is the initial 30-minute bolus infusion necessary and can the BSO infusion be shortened?) with further study, including a paired course design.

Our data clearly show that continuous-infusion BSO produces the desired biochemical end point in a consistent manner. This is the most important of many reasons why a continuous-infusion BSO schedule seems preferable to a repeated-bolus BSO schedule, even if a bolus schedule were observed to consistently deplete tumor glutathione levels to less than 10% of control levels. Continuous-infusion BSO could easily be administered on an outpatient basis. The pharmacokinetic profile of BSO and observations on the intracellular half-life of L-S-BSO (34), in relation to maintaining constant inhibition of GCS, strongly favor a continuous-infusion approach.

Current NCI-sponsored phase I/II trials of BSO plus L-PAM at many institutions are using the above schedule (L-S,R-BSO 3.0 g/m² over 30 minutes followed by 0.75 g/m² per hour × 72 hours and 15 mg/m² intravenous L-PAM given at hour 48 of continuous infusion). The clinical results from these trials will be important in determining the future clinical usefulness of L-S,R-BSO or the active isomer L-S-BSO (Oncotec, Inc., Irvine, CA). However, it is important to remember that BSO enhances the cytotoxicity of multiple anticancer agents and a modulator is not only as good as the agent it is modulating. Thus, its evaluation as a modulator should include trials in combination with other cytotoxic agents, such as carboplatin, which have broader clinical applicability.

References

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Notes

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