Pharmacokinetics of Buthionine Sulfoximine (NSC 326231) and Its Effect on Melphalan-induced Toxicity in Mice


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ABSTRACT

Intravenous doses of buthionine sulfoximine (BSO, NSC 326231), an inhibitor of glutathione synthesis, were eliminated rapidly from mouse plasma in a biexponential manner. The initial phase of the plasma concentration versus time curve had a half-life of 4.9 min and accounted for 94% of the total area under the curve. The half-life of the terminal phase of the curve was 36.7 min and the area accounted for only 6% of the total area under the curve. Plasma clearance of BSO was 28.1 ml/min/kg and the steady state volume of distribution was 280 ml/kg. The oral bioavailability of BSO, based on plasma BSO levels, was extremely low. However, comparable glutathione depletion was apparent after i.v. and p.o. doses of BSO, suggesting a rapid tissue uptake and/or metabolism of BSO. Therefore, due to the rapid elimination of BSO from mouse plasma, plasma drug levels do not directly correlate with BSO-induced tissue glutathione depletion. Administration of multiple i.v. doses of BSO to male and female mice resulted in a marked 88% depletion of liver glutathione at doses of 400–1600 mg/kg/dose. Toxicity of i.v. administered BSO was limited to a transient depression of peripheral WBC levels in female mice given six doses of 1600 mg/kg. Multiple i.v. doses of BSO of up to 800 mg/kg/dose (every 4 h for a total of six doses) did not alter the toxicity of i.v. administered melphalan. However, multiple doses of 1600 mg/kg/dose of BSO did potentiate histopathological evidence of melphalan-induced bone marrow toxicity in 30% of the mice and, additionally, the combination of BSO and melphalan produced renal tubular necrosis in 80% of the male mice. The potentiation of melphalan-induced toxicity did not appear to be related to GSH depletion, since a quantitatively similar amount of GSH depletion occurred at lower doses of BSO without any increase in melanolytic toxicity.

INTRODUCTION

Treatment of certain types of neoplasms with chemotherapeutic agents is frequently hindered by the development of resistance of tumor cells to the antineoplastic agents. There are a number of specific biochemical alterations, such as decreased drug uptake, increased drug efflux, and increased drug metabolism, which occur in resistant tumor cells and explain the development of cellular resistance to some types of cytotoxic chemotherapeutic agents (1, 2). Recently, evidence has been collected which suggests that in some tumors clinical resistance may be correlated to the ability of tumor cells to detoxify the cytotoxic moiety of the antineoplastic agent (3).

Intracellular GSH provides one of the primary mechanisms by which cells detoxify activated molecules, including various chemotherapeutic agents. GSH, a ubiquitously distributed intracellular sulfhydryl tripeptide, normally protects cells from damage produced by toxic agents either by binding directly to the activated molecule or by providing reducing equivalents (4, 5). Ozols et al. (6–8) have developed a human ovarian tumor model system which consists of cell lines derived from tumors that are clinically resistant to alkylating agents and from chemotherapy-sensitive tumors. Intracellular GSH is highest in the cell lines derived from clinically resistant tumors, while GSH levels are comparatively much lower in cell lines derived from tumors that are clinically sensitive to cytotoxic chemotherapeutic agents (9, 10). The same GSH pattern was also described in murine L1210 leukemia cell lines (11–13) and in human medulloblastoma cell lines (14); cell lines that are resistant to alkylating agents have much higher levels of intracellular GSH than cell lines which were sensitive to cytotoxic chemotherapeutic agents.

The correlation of tumor cell glutathione to the relative sensitivity of cells to cytotoxic chemotherapeutic agents can be exploited therapeutically (4, 15, 16). In theory, depletion of intracellular glutathione in tumor cells should allow subsequently administered chemotherapy to be more efficacious. BSO is a selective inhibitor of glutathione biosynthesis (15, 17) which produces a dose-dependent depletion of intracellular glutathione in vitro and in vivo in both tumor tissue and normal tissue (18). In tumor-bearing mice, BSO pretreatment increased the efficacy of several chemotherapeutic agents, such as mitomycin C (19), melphalan (13, 20–22), cisplatin (23), bleomycin (23), and cyclophosphamide (19, 24, 25). In most animal tumor models, BSO administered as a single agent at doses which produced GSH depletion did not alter tumor growth in vitro (21, 26–28) or in tumor-bearing mice (22, 24, 31) and, additionally, BSO alone did not alter tumor-associated lethality (13, 20, 30). However, exposure of tumor cells to high concentrations of BSO for extended periods inhibits protein and DNA synthesis (29) and tumor cell survival in clonogenic assays (30).

The ability of BSO pretreatment to potentiate the efficacy of cancer chemotherapeutic agents is dependent on the cytotoxic agent and the tumor model system. BSO given prior to the administration of melphalan or cyclophosphamide markedly enhanced the efficacy of the cytotoxic agent, as measured by clonogenic assays (13, 19, 23, 31), and there is an increase in life span of mice treated with BSO and melphalan, compared with mice treated with melphalan alone, in animal models for human ovarian (20) and medulloblastoma (22) tumors. BSO pretreatment, however, does not potentiate the efficacy of all chemotherapeutic agents in all types of tumor models. In some tumor-bearing murine models, BSO pretreatment does not alter the efficacy of various chemotherapeutic agents (32) or, even though the cytotoxicity of the chemotherapeutic agent is increased by BSO pretreatment, there is no increase in animal survival (13). These apparent discrepancies may be due to differences in the kinetics of tumor cell growth, BSO pretreatment schedule, and/or the inherent sensitivity of the tumor cells to the particular chemotherapeutic agent.

Because BSO pretreatment has been shown preclinically to potentiate the efficacy of some cytotoxic chemotherapeutic agents, the National Cancer Institute is developing BSO as an adjuvant to cancer chemotherapy. The present studies were...
designed to describe the pharmacokinetics of BSO and to evaluate the toxicity of BSO and its effect on the toxicity of melphalan in BALB/c × DBA/2 F1, (hereafter called CD2F1) mice, in preparation for clinical trials of this combination chemotherapy.

MATERIALS AND METHODS

Animals. CD2F1 mice were supplied by Charles River Laboratories, Inc., through the Animal Genetics and Production Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Mice were maintained on a 12-h light, 12-h dark cycle and had free access to food and water.

Chemicals. L-Buthionine(S,R)sulfoximine (NSC 326231) and melphalan (NSC 8806) were clinical formulations supplied by the Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. All other chemicals were obtained from commercial sources.

Pharmacokinetic and Bioavailability Studies. BSO or vehicle (0.9% sodium chloride) was administered either p.o. by gavage or i.v. in the tail vein. Blood samples were obtained by cardiac puncture into a heparinized syringe. Plasma was isolated by centrifugation, removed, pooled, and either frozen at −20°C for plasma BSO determination or immediately processed for plasma GSH determination. Liver, lung, and kidney were removed, rinsed, weighed, frozen in liquid nitrogen, and stored at −70°C for tissue GSH determination. BSO plasma concentrations were analyzed by standard pharmacokinetic techniques (34). The area under the plasma concentration versus time curve and the first moment curve were determined by the linear trapezoidal rule.

Half-lives were determined from the best fit of a biexponential equation to the plasma concentration-time profile. The fit was determined with a nonlinear regression routine. Other parameters were calculated as indicated in Table 1.

Plasma BSO Analysis. Pooled plasma BSO was determined by the method described by Duff and Murrill (35). Briefly, plasma samples (0.5 ml) were placed in culture tubes with Teflon-lined caps and methanol (2 ml) was added to precipitate macromolecules. After centrifugation, a 1.0-ml aliquot of the supernatant was removed and added to 0.2 ml of the derivatizing reagent. The derivatizing reagent was prepared by dissolving 200 mg of o-phenthaldehyde in 5 ml of methanol, adding 0.2 ml of 2-mercaptoethanol, and diluting with 0.01 ml of sodium borate buffer (pH 9.5) to 50 ml. After derivatization, samples were filtered through an unwetted Sep-Pak C18 solid phase extraction cartridge and a 0.5-ml aliquot of the derivatized samples was spiked with 0.1 ml of butyrophene (1.3 mg/liter methanol) as the internal standard. Standard curves were prepared by spiking untreated mouse plasma samples with 0 to 100 mg BSO/ml plasma. Separation of BSO from other plasma constituents was performed by high pressure liquid chromatography on an Altex Ultrasphere C18 column using an isocratic gradient solvent system. Solution A (0.005 M tetrabutylammonium hydroxide adjusted to pH 7.4 with phosphoric acid) and solution B (100% methanol) were used for the mobile phase. The elution program was initiated with a solvent ratio of 51% solution A/49% solution B, which ran for 40 min. This was followed by a linear gradient for the next 10 min to reach a solvent ratio of 30% solution A/70% solution B. This ratio was held for 5 min before returning to initial conditions. Derivatized BSO was detected by fluorescence at excitation wavelength of 340 nm and emission wavelength of 491 nm; the internal standard was detected by UV absorbance at 254 nm.

Glutathione Analysis. Pooled plasma (0.5 ml) was mixed with cold 25% perchloric acid (0.125 ml) to precipitate proteins. The mixture was centrifuged and the supernatant was removed. An aliquot of the supernatant was buffered to pH 7.0 with 2 N KOH/0.3 M 2-(N-morpholino) ethanesulfonic acid, the samples were centrifuged, and supernatants were removed for GSH determinations. Frozen tissue samples were homogenized in 4 volumes of cold 5% perchloric acid. After centrifugation, supernatant was removed and diluted 10- to 20-fold with 0.1 M phosphate buffer (pH 7.4). Glutathione was determined by the method described by Akerboom and Sies (36), as modified by Smith and Boyd (37).

BSO Toxicity Studies. Male and female CD2F1 mice (6–8 weeks old) were treated with either 400, 800, or 1600 mg/kg BSO or VCTL i.v. every 4 h for a total of six doses. Liver glutathione was determined in one group of mice five BSO-treated male mice from each BSO dose group and five vehicle-treated male mice 1 h after the fifth dose of BSO or VCTL. The other groups of mice were observed daily for adverse clinical signs and mortality. Blood samples for clinical pathology determinations (consisting of hematology and clinical chemistry parameters) were taken on study days 2, 8, 15, 22, and 29. Hematology parameters measured were RBC count, hemoglobin, hematocrit, platelet count, reticulocyte count, total WBC, differential leukocyte count, and nucleated RBC count. Clinical chemistry parameters determined were blood urea nitrogen, serum alanine transaminase, serum aspartate transaminase, and total protein. Complete gross pathological and histopathological evaluations were done on animals necropsied on study days 2 and 29. Tissues [bone (femur), bone marrow, brain, colon, duodenum, gonads (ovaries, testes), heart, ileum, jejunum, kidney, liver, lung, lymph nodes, pancreas, spleen, stomach, and thymus] were fixed in buffered formalin and 5-μm paraffin sections were stained with hematoxylin and eosin.

In the BSO plus melphalan combination study, 400, 800, or 1600 mg/kg BSO or VCTL was administered i.v. every 4 h for a total of six doses. One h after the fifth dose of BSO, 5 mg/kg (15 mg/m2) melphalan was administered at 4 p.m. on study day 1. Before melphalan administration, five BSO-treated male mice from each dose group and five VCTL-treated male mice were used for liver glutathione determinations. Blood samples for clinical pathology determinations were taken on study days 2, 4, 6, 11, 17, and 30. Complete gross pathological and histopathological evaluations were done on mice necropsied on study days 2, 6, and 30.

Statistical Methods. Statistical analyses were performed by comparing control group data with treated using either paired t tests or one-way analysis of variance coupled with Duncan’s multiple range test.

RESULTS

The plasma concentration versus time curve (Fig. 1) after i.v. dosing revealed a biexponential disappearance for BSO. The half-life of the initial phase was 4.9 min, while the half-life of the terminal phase was 36.7 min (Table 1). The AUC of the initial phase accounted for approximately 94% of the total area under the curve, while the AUC of the terminal phase accounted for only 6% of the total AUC. The steady state volume of distribution (Vss) was 280 ml/kg and was severalfold smaller than the terminal volume (Vt). Since the elimination of BSO from plasma occurred during the initial phase, the Vss was felt to be the more appropriate term to represent the distribution

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Fig. 1. Plasma BSO elimination in male CD2F1 mice. A dose of 800 mg/kg BSO was administered i.v. Plasma from six mice was collected at the indicated times and the plasma from three mice was pooled for drug level analysis. Each line represents the BSO concentration obtained from the pooled plasma from three mice.

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volume of BSO. Plasma clearance of BSO in CD2F1 mice was 28.1 ml/min/kg. Interestingly, the two stereoisomers of BSO form a double peak in this chromatography system (Fig. 2). These peaks are approximately the same height in spiked plasma samples and in the plasma samples drawn soon after dosing (e.g., 2-min sampling time). However, the two stereoisomers disappeared from the plasma very differently. The stereoisomer which elutes first disappears more quickly than the second. Oral administration of BSO (800 mg/kg) did not result in measurable plasma drug levels except at 1 h after dosing (11 μg/ml).

Plasma and liver GSH levels after p.o. and i.v. dosing are shown in Fig. 3. Liver GSH depletion was similar to plasma GSH depletion after p.o. and i.v. BSO administration. Maximum depletion of 64–67% (33–36% of control values) was observed in liver GSH 2–4 h after i.v. and 2–8 h after p.o. BSO administration. Liver GSH levels returned to control values 8–12 h after i.v. or p.o. doses of BSO. Maximum plasma glutathione depletion was 78% (22% of control values) after both p.o. and i.v. administered BSO. Recovery of plasma GSH after i.v. administration of BSO was relatively rapid; levels were not different from control values 16 h after BSO. Kidney GSH levels were depleted by 78 and 77% (22 and 23% of control values) and lung GSH levels were depleted by 29 and 20% (71 and 80% of control values), respectively, 4 h after a single i.v. or p.o. dose of BSO (Table 2).

Clinically, it is proposed that multiple i.v. doses of BSO be administered to produce maximal GSH depletion before the administration of a cytotoxic chemotherapeutic agent, such as melphalan. Therefore, the toxicity of BSO was evaluated in CD2F1 mice following multiple i.v. doses to produce and maintain maximal GSH depletion. The data in Fig. 3 indicate that maximal plasma and liver GSH depletion occurs 4 h after a single i.v. 800 mg/kg dose of BSO. The administration of 400, 800, or 1600 mg/kg BSO i.v. every 4 h for a total of six doses resulted in an 85–89% reduction in liver GSH, measured 1 h after the fifth dose of BSO. Each of the three dose levels of BSO produced a quantitatively similar amount of liver GSH depletion; thus there is no apparent dose response. The only toxicity produced by multiple doses of BSO was a substantial 75% decrease in WBC 12 h following the last dose of BSO in female mice given 1600 mg/kg (Fig. 4). This WBC depression was not associated with histopathological evidence of bone marrow atrophy or aplasia and WBC values had returned to and remained in the control range beginning on study day 8.

There were no clinical signs of toxicity or changes in clinical chemistry parameters and no gross or histopathological lesions were detected after BSO was given by this dosing regimen.

Melphalan administered at a dose of 5 mg/kg (15 mg/m2) produced microscopic evidence of gastrointestinal toxicity on day 2 and a reversible depression of peripheral WBC values in male and female mice (Table 3; Fig. 5). Histopathologically, thymic atrophy was present in all melphalan-treated mice on day 6, but no bone marrow atrophy occurred after the administration of this dose of melphalan. Pretreatment of mice with up to 800 mg/kg BSO i.v. every 4 h for a total of five doses prior to melphalan administration and an additional BSO dose after melphalan administration did not potentiate melphalan-induced leukopenia (Fig. 5). In mice given doses of 1600 mg/kg BSO every 4 h for a total of six doses, BSO did not affect the WBC nadir but did appear to delay the recovery of WBC values after melphalan administration in both male and female mice. In this dose group, 30% of the mice had histopathological evidence of bone marrow atrophy accompanied by splenic myeloid tissue atrophy on day 6 (Table 3). In addition, melphalan administration to mice treated with multiple doses of 1600 mg/kg BSO produced a rise in blood urea nitrogen (peak levels, 47–117 mg/dl) and histopathological evidence of renal tubular necrosis on day 6 (Fig. 6). The tubular necrosis was characterized by loss of nuclear and cytoplasmic detail and was accompanied by regeneration/hyperplasia. The incidence of renal tubular necrosis was very high, occurring in 80% of the male

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**Table 1** Pharmacokinetic analysis of plasma BSO concentrations in CD2F1 mice after 800 mg/kg i.v. dose of BSO

<table>
<thead>
<tr>
<th>Parameters (units)</th>
<th>Calculation</th>
<th>Estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg/ml × min)</td>
<td>C*D</td>
<td>28,500</td>
</tr>
<tr>
<td>Ci (ml/min/kg)</td>
<td>D/AUC</td>
<td>28.1</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>4.89</td>
<td></td>
</tr>
<tr>
<td>t1/2w (min)</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>AUMC (μg/ml × min^2)</td>
<td>C*D</td>
<td>284,000</td>
</tr>
<tr>
<td>t1/2w</td>
<td>AUMC/AUC</td>
<td>9.96</td>
</tr>
<tr>
<td>Vm (ml/kg)</td>
<td>t1/2w/Ci</td>
<td>280</td>
</tr>
<tr>
<td>Vm (ml/kg)</td>
<td>Ci/(0.693/t1/2w)</td>
<td>1,490</td>
</tr>
</tbody>
</table>

* C, BSO plasma concentration; Ci, plasma clearance; D, dose (mg); t1/2, half-life of initial disposition phase; t1/2w, half-life of terminal disposition phase; AUMC, area under the first moment curve; t1/2w, mean residence time; Vm, apparent volume of distribution at steady state; Vm, apparent volume of distribution during the terminal phase.

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**Fig. 2.** Sample chromatogram of plasma BSO high pressure liquid chromatography analysis. A, Sample chromatogram of mouse plasma spiked with 20 μg of BSO; B, chromatogram of plasma BSO concentration 60 min after a single i.v. dose of 800 mg/kg BSO.

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**Fig. 3.** Effect of a single i.v. or p.o. dose of BSO on liver and plasma GSH. Male CD2F1 mice were treated with 800 mg/kg BSO and liver (left) and plasma (right) GSH levels were determined at the indicated times. Liver GSH values are the mean ± SE of six determinations. Plasma from three mice was pooled for GSH determination. Plasma values are the average of two determinations. * mean significantly (P < 0.05) different from the control values.
DISCUSSION

There is substantial clinical interest in evaluating the effect of BSO-induced tumor tissue GSH depletion on the efficacy of chemotherapeutic agents used in the treatment of cancer (1, 3). The present studies were designed to describe the pharmacokinetics of BSO in mouse plasma, correlate them with tissue GSH depletion, and evaluate the toxicity of multiple doses of BSO alone and in combination with melphalan, a potent bifunctional alkylating agent used to treat a variety of neoplastic diseases.

We have shown that BSO administered i.v. is cleared very rapidly from mouse plasma, whereas plasma BSO levels after p.o. administration of up to 800 mg/kg/dose of BSO given every 8 h for a total of 15 doses (5 days) did not induce any clinical signs of toxicity, hematological or clinical chemistry changes, or histopathological lesions (39). The present studies suggest that BSO administered i.v. at doses up to 800 mg/kg/dose every 4 h for a total of six doses also produced substantial GSH depletion with no measurable acute toxicity. The only evidence of toxicity was WBC depletion, which occurred when multiple i.v. doses of 1600 mg/kg BSO were administered to female CD2F1 mice (Fig. 4). This WBC depletion was mild and transient and was unaccompanied by histopathological evidence of bone marrow atrophy. Other investigators have reported that BSO alone did not produce any WBC depletion in mice (13, 33, 40) and in these studies there were no adverse effects on bone marrow stem cells (31, 33, 40). Therefore, BSO alone is not myelotoxic, except potentially at very high, multiple i.v. doses of drug.

Table 2 Comparison of liver, lung, and kidney glutathione levels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>GSH level</th>
<th>% of control</th>
<th>GSH level</th>
<th>% of control</th>
<th>GSH level</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>5.88 ± 0.86 (4)*</td>
<td>100</td>
<td>1.96 ± 0.42 (4)</td>
<td>33*</td>
<td>2.12 ± 0.56 (4)</td>
<td>36*</td>
</tr>
<tr>
<td>Lung</td>
<td>2.31 ± 0.17 (4)</td>
<td>100</td>
<td>1.63 ± 0.20 (4)</td>
<td>71*</td>
<td>1.84 ± 0.09 (4)</td>
<td>80*</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.03 ± 0.42 (4)</td>
<td>100</td>
<td>0.68 ± 0.08 (4)</td>
<td>22*</td>
<td>0.69 ± 0.02 (4)</td>
<td>23*</td>
</tr>
</tbody>
</table>

* Tissue samples were taken 4 h after 800 mg/kg i.v. or p.o. dose of BSO.

DISCUSSION

Fig. 4. Effect of multiple doses of BSO on WBC levels. Mice were treated with the indicated i.v. doses of BSO every 4 h for a total of six doses. Blood was obtained and WBC levels were determined in male (left) and female (right) CD2F1 mice. Values represent mean ± SE of five determinations. *, mean significantly (P < 0.05) different from control values.

BSO PHARMACOKINETICS AND TOXICITY

Table 3 Toxicity of BSO alone and in combination with melphalan

<table>
<thead>
<tr>
<th>Microscopic lesions</th>
<th>BSO 800, melphalan 0</th>
<th>BSO 800, melphalan 5</th>
<th>BSO 1600, melphalan 0</th>
<th>BSO 1600, melphalan 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>None</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Bone marrow atrophy</td>
<td>2/5</td>
<td>1/5</td>
<td>2/5</td>
<td>1/5</td>
</tr>
<tr>
<td>Renal necrosis</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Small intestinal necrosis</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Splenic myeloid atrophy</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Thymic atrophy</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

* Incidence of lesion occurrence as number of mice with lesion/total number of mice examined.

** Dose of BSO (mg/kg) administered i.v. every 4 h for a total of six doses.

† Dose of melphalan (mg/kg) administered i.v. 1 h after the fifth dose of BSO.
Combining BSO with cytotoxic chemotherapeutic agents presents a different set of toxicological questions. Theoretically, BSO-induced GSH depletion could potentiate known toxicities of the chemotherapeutic agent or the combination of BSO and the chemotherapeutic agent could produce new types of dose-limiting toxicities. Only a few studies have addressed the effect of BSO pretreatment and the resultant GSH depletion on the toxicity of antineoplastic agents. Methylcyclohexylchloroethylnitrosourea, a potent alkylating nitrosourea, has been shown to be nephrotoxic, but not hepatotoxic, when administered to Fischer 344 rats. BSO pretreatment potentiates the nephrotoxicity of methylcyclohexylchloroethylnitrosourea and, additionally, the combination produces hepatotoxicity in Fischer 344 rats (41). Conversely, Mayer et al. (42) provide evidence that BSO pretreatment decreases the nephrotoxicity of cisplatin, even though another study suggested that GSH depletion produced by administration of diethylmaleate, a compound that directly binds to GSH, potentiates the nephrotoxicity of cisplatin (43). Kramer et al. (13) suggested that BSO pretreatment did increase the lethality of melphalan but the potentiation of lethality was dependent on the route of BSO administration and the extent of GSH depletion. Additionally, much higher (10–40 mg/kg) i.p. doses of melphalan were used in these studies than were used in the present study. Therefore, it is clear that GSH depletion can markedly affect the toxicity of certain cancer chemotherapeutic agents in animal models.

Clinically, i.v. doses of melphalan produce a dose-limiting myelosuppression, characterized by both leukopenia and thrombocytopenia. High doses of melphalan produce dose-limiting gastrointestinal toxicity in cancer patients given bone marrow transplants to offset the myelosuppressive effects of the drug (44). Myelosuppression and gastrointestinal toxicity are produced in mice by doses of melphalan (15 mg/m²) which are comparable to the doses that produce similar toxicity in humans (20 mg/m²). The similarity in the pattern of toxicity at similar doses of melphalan suggests that CD2F, mice are an acceptable preclinical animal model to evaluate melphalan-induced toxicity.

BSO treatment at doses up to 800 mg/kg/dose (every 4 h for a total of six doses) did not have any effect on the myelosuppression produced by melphalan administration. However, pretreatment of mice with multiple doses of 1600 mg/kg/dose of BSO significantly altered the pattern of melphalan toxicity. Firstly, the myelotoxicity of melphalan was potentiated by BSO treatment, as evidenced by histologically identifiable bone marrow atrophy accompanied by splenic myeloid tissue atrophy, even though there appeared to be no significant change in the peripheral WBC nadir. The recovery of WBC counts to control levels was delayed in mice treated at the highest dose level of BSO and melphalan, which has been observed and reported by other investigators (13). The potentiation of bone marrow toxicity was considered to be relatively slight, since the incidence was low (30%), the bone marrow lesions were not severe and were readily reversible, and there was no drug-induced mortality caused by this combination.

Surprisingly, the combination of melphalan with multiple 1600 mg/kg doses of BSO was nephrotoxic, as evidenced by increases in blood urea nitrogen and histopathological lesions. Evidence of renal toxicity did not occur with either agent alone. The incidence of the nephrotoxicity was very high in male mice (80%) and it was readily reversible. The only suggestion in the clinical literature that melphalan administration is linked to
renal toxicity appeared in a study in which high doses of melphalan were administered with other cytotoxic agents (cyclophosphamide, cisplatin, and bis-chloroethylnitrosourea) with bone marrow transplantation support (45). From this clinical study, it is impossible to determine whether melphalan is responsible for the observed renal toxicity: (a) melphalan administered as a single agent at similar doses has not been reported to be nephrotoxic (44); and (b) cisplatin, one of the other agents administered, is known nephrotoxic agent. The authors suggest that the addition of melphalan to the combination produced the nephrotoxicity but melphalan may be potentiating the nephrotoxicity produced by any or all of the agent(s) in this combination or, conversely, one or all of the other agent(s) may be inducing the nephrotoxicity by melphalan.

The renal toxicity produced by BSO pretreatment and melphalan administration did not appear to be related to the amount of glutathione depletion, since multiple doses of 400 to 1600 mg/kg/dose of BSO produced comparable reductions of tissue GSH concentrations. Renal toxicity was noted only after a combination of melphalan and 1600 mg/kg/dose of BSO; therefore, GSH depletion cannot explain the effect of this combination on the kidneys. This information suggests that, clinically, dose escalation of BSO above levels needed for maximal GSH depletion should be approached cautiously.

In summary, BSO did not alter the dose-limiting toxicity of melphalan in preclinical studies in CD2F1 mice except at extremely high (1600 mg/kg/dose) multiple i.v. doses of BSO. This combination of high multiple doses of BSO prior to a single dose of melphalan produced nephrotoxicity and increased the myelotoxicity of melphalan.

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