Chemosensitization of L-Phenylalanine Mustard by the Thiol-modulating Agent Buthionine Sulfoximine

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ABSTRACT

Glutathione (GSH) plays a crucial role in the protection of normal and normal tissue against the toxic effects of numerous chemotherapeutic drugs. Therefore, the possible therapeutic benefit of thiol depletion in cancer treatment is dependent upon the relative degree to which tumor or normal tissue is sensitized to the toxic effects of subsequent chemotherapy. To address this issue, the following studies on the chemosensitization of melphalan (L-PAM) by the thiol-depleting agent buthionine sulfoximine (BSO) were conducted in vivo in BDF mice inoculated with L-PAM-resistant murine L1210 leukemia. Different dosing regimens of BSO were found to potentiate L-PAM toxicity in a manner that depended upon the degree of GSH depletion. Multiple i.p. injections of BSO (450 mg/kg every 6 h × 5) were found to reduce GSH concentrations in most tissues by 70-80%, and to decrease the LD50 for L-PAM from 22 to 14 mg/kg. No two organs were found to behave entirely the same with respect to the rate of depletion or recovery of GSH, or to the maximum depletion that could be attained by BSO. In this regard, the bone marrow was found to be the most resistant tissue to thiol depletion by BSO and was found to tolerate the combination of BSO and therapeutic doses of L-PAM. However, BSO pretreatment markedly inhibited the recovery of the peripheral WBC population at the LD50 dose of L-PAM. Differences also were found in the in vivo metabolism of GSH by L-PAM-sensitive and -resistant murine L1210 leukemia cells. The intracellular concentration of GSH in the resistant cell line was 1.6-fold higher than in the sensitive tumor. Moreover, GSH levels were depleted more rapidly in the resistant tumor relative to the sensitive cell line. A single injection of BSO decreased GSH concentrations in both tumors to equivalent levels (20 nmol/10^6 cells) within 24 h. However, multiple i.p. injections of BSO failed to produce a significant increase in the life-span of L-PAM-treated animals despite a 90% reduction in tumor GSH concentrations (5.5 nmol/10^6 cells). In contrast to the medians day survival data, BSO was found to enhance the antitumor activity of L-PAM as determined by an in vivo/in vitro clonogenic assay or by in vivo thymidine incorporation. Using decreased thymidine incorporation as an index of antitumor activity, BSO was found to increase the therapeutic index (1.1 LD50/KI)w) of L-PAM from 3.6 to 6.5. The present findings provide the first indication that tumor tissue may be more sensitive than normal tissue to L-PAM toxicity following GSH depletion and suggests that thiol modulation by BSO may be useful as an adjuvant in cancer chemotherapy.

INTRODUCTION

GSH,1 has been shown to play a crucial protective role against cellular injury produced by a number of toxic insults including ionizing radiation (1), reactive electrophiles (2, 3), and reactive oxygen intermediates generated by the respiratory burst of phagocytes (4) or the metabolism of quinone-containing drugs (5). The importance of GSH in mediating the therapeutic efficacy of cancer treatment strategies involving radiation ther-

MATERIALS AND METHODS

Male BDF mice (22-25 g) obtained from the Frederick Cancer Research Facility (Frederick, MD) were maintained in a temperature, humidity, and light controlled environment throughout the study. BSO (Chemical Dynamics Corp., South Plainfield, NJ) was administered either as a single (2 mm; 450 mg/kg) or by multiple i.p. injections (450 mg/kg every 6 h × 4). In some experiments, BSO was administered in the animals' drinking water (20 mm). Animals that had received BSO in their drinking water consumed approximately 2 ml/day/mouse (i.e., 40 μmol/mouse). L-PAM was provided by the Pharmaceutical Resources Branch, National Cancer Institute. A 30-mm stock solution...
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RESULTS

GSH Depletion. A single dose of BSO depleted GSH concentrations in all tissues examined (Fig. 1). However, no two organs behaved entirely the same with respect to the rate of depletion, or recovery of GSH, or the maximal depletion that was obtained. For example, kidney and liver GSH levels were maximally depleted within 2 h; however, renal GSH remained fully depressed at 24 h whereas liver GSH had returned to control values. The concentration of GSH in the heart also was markedly depleted at 24 h but required a much longer time to achieve maximum depletion (8 h, versus 2 h for kidney). These differences in the initial rate of GSH depletion reflect the rate of GSH utilization, or turnover of GSH, in a given tissue. In this regard, the lung appeared to turnover GSH only very slowly as reflected by the estimated biological half-life for GSH in the lung of 7.5 h. This compares to the estimated half-life for GSH in the liver and the kidney of 1.5 h. Bone marrow was found to have extremely low cellular concentrations of GSH (7 nmol/10^6 cells) and to be the most refractory to GSH depletion by BSO (Fig. 2). The maximal depletion that was obtained in bone marrow was only 20%. This compares to a 55% decrease in the lung or to the 70–80% decrease that was observed in the liver, kidney, heart, or stomach.

Differences also were found in the metabolism of GSH by L-PAM-resistant (L1210R) and -sensitive murine L1210 leukemia cells (Fig. 2). The intracellular concentration of GSH in L1210R cells was 1.6-fold higher than in the sensitive cell line. Moreover, GSH levels were depleted more rapidly in the resistant murine leukemia relative to the sensitive tumor. Such differences in the rate of GSH depletion may be due to differences in the rate of utilization or synthesis of GSH, or to differences in the uptake or retention of BSO by the resistant and sensitive tumor cell lines, respectively. However, GSH concentrations in both tumors were maximally depleted 24 h after BSO administration. At this time, GSH concentrations were equal in both cell lines (i.e., 20 nmol/10^6 cells).

Several different chronic dosing models for thiol depletion by BSO also were investigated (Fig. 3). MIs of BSO (450 mg/kg every 6 h × 5) resulted in a greater decrease in tissue GSH concentrations compared to an oral dosing regimen (p.o.) which consisted of a single i.p. dose of BSO (450 mg/kg) followed by 3 days of feeding BSO in the animals’ drinking water (approx-

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Fig. 1. Time-course for depletion and recovery of tissue GSH concentrations after a single i.p. injection of BSO (450 mg/kg). Values are expressed as a percentage of time-matched controls. Each value is the mean of five animals; standard errors were less than 10% of the respective means.

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Fig. 2. Time-course for depletion of GSH concentrations in bone marrow and in L-PAM-sensitive and -resistant murine L1210 leukemia cells after a single i.p. injection of BSO (450 mg/kg). L1210 cells were harvested from animals as described in "Materials and Methods" and GSH levels are expressed as the mean ± SE of five animals. A total of six nontumored animals per time point were used for determining bone marrow GSH levels. Each value is the mean of three determinations ± SE from bone marrow cells pooled from the femurs of two mice.

Fig. 3. The effect of multiple i.p. injections or chronic oral dosing of BSO on mouse tissue GSH levels. Injections of BSO (MIP) were given every 6 h x 5 (450 mg/kg). BSO was given orally (p.o.) in the animals' drinking water (20 HIM) for 3 days following a single i.p. injection of a loading dose of BSO (450 mg/kg). GSH concentrations were determined either 6 h after the last injection of BSO, or 6 h after withdrawal of BSO from the animal's drinking water. Each value is the mean ± SE of determinations on five animals.

Fig. 4. The effect of multiple injections of BSO on bone marrow and L-PAM-resistant murine L1210 leukemia GSH concentrations. BSO was administered every 6 h x 5 (450 mg/kg; i.p.) and cells were harvested and GSH levels determined as described in the legend to Fig. 2. Values are the mean ± SE of determinations on five tumored animals, and as three determinations on bone marrow samples pooled from the femurs of six nontumor bearing animals. * significantly different from control values (P < 0.01).

Fig. 5. The effect of chronic oral (p.o.) or multiple injection (MIP) of BSO on the toxicity of L-PAM in BDF mice. BSO was administered every 6 h × 5 (450 mg/kg; i.p.) and L-PAM was administered either 6 h after the withdrawal of BSO from the animals' drinking water or 6 h after the last injection of BSO. Lethality at 7 days was determined on groups of six animals.

The murine L1210a leukemia was the most sensitive tissue to GSH depletion by MIP injection of BSO. GSH levels were decreased by more than 90% to levels that were comparable to those found in marrow cells (Fig. 4). In contrast, the concentration of GSH in bone marrow was decreased only by 40%, making this the most resistant tissue to thiol depletion by BSO.

Toxicity. The degree of thiol depletion produced by these two dosing regimens translated into different susceptibilities to the toxicity resulting from the administration of L-PAM (Fig. 5). The dosing regimen which maximally depleted GSH (MIP) produced a marked shift of the lethality curve, resulting in a 1.6-fold reduction in the LD₅₀ for L-PAM. Oral dosing of BSO increased lethality only at high doses, resulting in a decrease in the LD₅₀ for L-PAM. The actual cause of death in these animals is not known. However, L-PAM was myelosuppressive as shown by the dose-dependent decrease in peripheral WBC counts (Fig. 6). BSO was found to have little effect on the myelosuppressive activity of L-PAM at the time of the WBC nadir, on day 4. However, BSO markedly inhibited the recovery of the WBC population that was observed on day 8 in animals that received only L-PAM. BSO alone was not found to be toxic by the
indices of toxicity reported in this study (i.e., lethality or WBC counts). Chronic BSO administration did result in a slight loss of weight that recovered rapidly after cessation of treatment (not shown). As in the previous experiments, L-PAM was administered 6 h after the last dose of BSO to minimize possible drug-drug interactions.

In Vivo Sensitization of L1210R to L-PAM by BSO. Pretreatment of animals with MIP injections of BSO prior to L-PAM administration was not found to have an appreciable effect on the antitumor activity of L-PAM as judged by median survival times (Table 1). The slight increase in life-span produced by BSO pretreatment (i.e., 10% relative to treatment with 10 mg/kg L-PAM alone) was equivalent to a log-fold increase in tumor kill as determined by the survival curve generated by plotting median survival versus the number of inoculated L1210R cells (not shown). However, BSO was found to increase toxicity at the highest tested dose of L-PAM (i.e., 13 mg/kg), shown by the reduction in mean survival (Table 1). The relative lack of effect that BSO pretreatment had on the antitumor activity of L-PAM occurred in spite of the fact that tumor GSH levels were decreased by more than 90% at the time of the L-PAM injection (Fig. 4). This observation prompted additional studies on the possible sensitization of L-PAM by BSO.

In this regard, MIP injections of BSO increased the cytotoxicity of L-PAM as shown by the decreased cloning efficiency of L1210R cells harvested from animals treated in vivo with BSO and/or L-PAM (Table 2). BSO treatment alone produced a slight reduction (i.e., 10%) in cloning efficiency. BSO in combination with L-PAM (2.5 mg/kg) decreased the clonogenicity of L1210R cells by 92% compared to the 78% decrease produced by treatment with L-PAM alone. Moreover, the recovery of tumor cells from the peritoneum of L-PAM-treated animals was far less than in control animals, and BSO pretreatment resulted in an even further decrease in tumor cell recovery (Table 2). This observation suggested that a considerable degree of toxicity, and cell death, may have occurred in the 18 h that had elapsed from the time of the L-PAM injection to the harvesting of the tumor cells for the clonogenic assay. In order to investigate the early effect of BSO/L-PAM treatment and to minimize the procedural artifacts that occur in the multiple manipulations required to perform such in vivo/in vitro experiments, an in vivo labeling assay was adopted.

[3H]dThd incorporation into L1210R cells was increased 3 h after administering a low dose of L-PAM (i.e., 0.5 mg/kg; Fig. 7). At higher doses, L-PAM produced a dose-dependent decrease in DNA synthesis as shown by the decrease in [3H]dThd incorporation. BSO pretreatment inhibited both the increase in [3H]dThd incorporation that occurred at low dose L-PAM, and at higher doses of L-PAM, caused a further reduction in DNA synthesis compared to treatment with L-PAM alone. The ED50 of L-PAM for inhibition of [3H]dThd incorporation was estimated.
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estimated to be 3.9 mg/kg in control animals and 1.8 mg/kg in BSO pretreated animals, a dose reduction factor of 2.2.

DISCUSSION

The results of the present study indicate that BSO effectively reduced GSH concentrations in normal and in tumor tissue without producing appreciable toxicity. BSO was, however, found to potentiate both the toxicity and antitumor activity of L-PAM. The increase in L-PAM toxicity depended upon the degree of thiol depletion (Fig. 5) and was observed only at much higher doses of L-PAM (Figs. 5 and 6) than required to produce an increase in antitumor activity (Table 2 and Fig. 7). Using decreased thymidine incorporation to estimate the antitumor activity of L-PAM, BSO was found to increase the therapeutic index (LD10/ED50) of L-PAM from 3.6 to 6.5. These results in vivo support in vitro studies which have shown that GSH depletion increased tumor sensitivity to a number of antineoplastic agents (6-8, 15-17). However, the present findings provide the first indication that tumor tissue may be more sensitive than normal tissue to L-PAM toxicity following GSH depletion by BSO, and suggests that thiol modulation by BSO may be useful as an adjuvant in cancer chemotherapy.

The observation that some tissues (e.g., bone marrow) were comparatively resistant to GSH depletion by BSO, whereas other tissues (e.g., kidney) were particularly sensitive to BSO, may provide a basis for the rational selection of drugs which can be used in conjunction with BSO. For example, recent studies have shown that BSO pretreatment markedly increased the renal toxicity of MeCCNU (18) and cis-platinum (25), two agents with a predisposition towards nephrotoxicity. In contrast, the bone marrow was found to tolerate, reasonably well, the combination of BSO and therapeutic doses of L-PAM (Fig. 6). It is possible that combination therapies involving anticancer agents that are predominantly myelosuppressive may be better tolerated than agents that are reported to cause renal, hepatic, or cardiac toxicities. This observation does not preclude the need to monitor the degree of myelosuppression, or other toxicities, that may result from combination therapies involving thiol depletion.

In the present study, BSO was found to be somewhat effective at sensitizing the L1210R tumor cells found within the peritoneal cavity to L-PAM (Table 2; Fig. 7). However, BSO failed to cause a significant increase in the life-span of tumor bearing animals (Table 1) despite a 90% reduction in tumor GSH concentrations (Fig. 5). This observation is not surprising in light of the well-known metastatic potential of the murine L1210 leukemia. The failure to observe a greater increase in the life-span of these animals probably was due to metastatic infiltration into specific host organs, notably the liver. Recent studies have shown that hepatic metastasis arising from i.p. inoculation of L1210 cells had elevated GSH levels and were more resistant to L-PAM compared to their counterparts found in the peritoneal cavity (26). Pharmacokinetin differences between the metastatic and primary tumor not withstanding, such differences in GSH metabolism may provide an explanation for the ineffectiveness of BSO at prolonging life-span, and suggests that thiol modulation by BSO may be useful as an adjuvant in cancer chemotherapy.

It is clear that GSH plays an important role in the cellular protection against a variety of antineoplastic agents. However, the potential usefulness of BSO or other thiol-modulating drugs is limited not only by a thorough understanding of possible drug interactions and/or host toxicities that may arise from combination therapies with various antineoplastic drugs, but also by an understanding of GSH metabolism by the tumor systems in which these therapies may be used.

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