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THE RELATIVE ABILITY OF BSO AND OTHER γ-GLUTAMYLCYSTEINE SYNTHETASE INHIBITORS TO BOTH DEPLETE GLUTATHIONE AND ALTER DRUG METABOLIZING ENZYME ACTIVITIES

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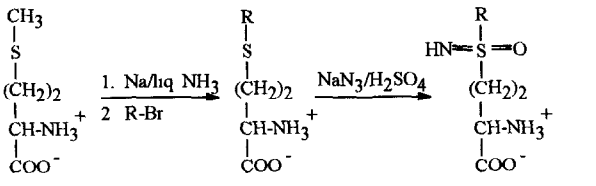
Abstract. Buthionine sulfoximine (BSO) is widely used for its ability to deplete glutathione (GSH). However, BSO also induces key drug metabolizing enzymes, potentially complicating the interpretation of experimental results. The prothionine and hexathionine analogs of BSO were synthesized and evaluated as alternative agents which might offer an advantage over BSO by depleting GSH without inducing the enzyme activities.

Introduction. L-Buthionine sulfoximine (BSO, **1**) is a potent, specific inhibitor of γ-glutamylcysteine synthetase, a key enzyme in glutathione (GSH) biosynthesis.[1,2] In addition, recent work has revealed that chronic treatment of animals with BSO also increases UDP-glucuronosyltransferase (UGT) and glutathione S-transferase (GST) activities in liver.[3-5] The induction of these enzymes occurs in the absence of any induction of cytochrome P450 (P450).[3] This unexpected induction of transferase activities necessitates careful interpretation of experimental results in studies where BSO is used to deplete GSH.

Analogs of BSO differ in the extent and duration for which they are able to deplete hepatic GSH. In general, they have been considered less suitable GSH depleting agents because of other biological effects, particularly CNS toxicity.[2] These studies were undertaken to determine whether two BSO analogs, L-prothionine sulfoximine (PSO, **2**) and L-hexathionine sulfoximine (HSO, **3**) might offer an advantage over BSO by depleting GSH without inducing drug metabolizing enzyme activities.



R = $CH_3(CH_2)_3$ BSO, **1**

$CH_3(CH_2)_2$ PSO, **2**

$CH_3(CH_2)_5$ HSO, **3**

Scheme 1

Materials and Methods TLC analyses utilized silica gel HL plates from Analtec, Inc (Newark, DE) with a solvent system consisting of 1-butanol·acetic acid·water (75 15:10); spots were visualized with ninhydrin. Melting points were determined on a MEL-TEMP II (Laboratory Devices, Holliston, MA) and are uncorrected. One- and two-dimensional NMR spectra were acquired on an IBM NR 200/AF spectrometer using either DCl (20% in D_2O) or *d*-trifluoroacetic acid (*d*-TFA) with 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external standard Optical activity was measured on a JASCO DIP-370 polarimeter (Tokyo, Japan) Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

BSO, L-methionine, NaN_3, NH_4Cl, sodium spheres, NMR solvents, and Dowex 50W were obtained from Sigma Chemical Co (St. Louis, MO) Anhydrous ammonia, 1-bromopropane, and 1-bromohexane were purchased from Aldrich Chemical Co. (Milwaukee, WI). All reagents were used without further purification

PSO and HSO were synthesized from L-methionine following the basic method of Griffith[2] (Scheme 1).

Prothionine: 14.5 g, 82% yield, mp=264°d. $[\alpha]_D^{25}$ +21.4° (c=0 2, 1 M HCl). TLC: R_f=0.39 NMR (DCl in D_2O) δ 0 94 (t, 3H, $C\underline{H}_3$-); 1.58 (m, 2H, $CH_3C\underline{H}_2$-), 2 31 (m, 2H, -$SCH_2C\underline{H}_2$-); 2.59 (t, 2H, -$C\underline{H}_2S$-); 2.77 (t, 2H, -$SC\underline{H}_2$-); 4 36 (t, 1H, -C<u>H</u>-) Anal Calculated for $C_7H_{15}NO_2S$· C, 47.43, H, 8.53, N, 7.90. Found: C, 47 35; H, 8.52; N, 7.92

PSO: 1 4 g, 23% yield; mp=209-210°d $[\alpha]_D^{25}$ +33 5 (c=0 2, 1 M HCl). TLC: R_f=0.09. NMR (DCl in D_2O) δ 1.16 (t, 3H, $C\underline{H}_3$-), 2.03 (m, 2H, $CH_3C\underline{H}_2$-), 2.74 (m, 2H, -S(O)(NH)$CH_2C\underline{H}_2$-); 4 00 (t, 2H, -$C\underline{H}_2$S(O)(NH)-), 4.31 (t, 2H, -S(O)(NH)$C\underline{H}_2$-), 4 51 (t, 1H, -C<u>H</u>-). Anal Calculated for $C_7H_{16}N_2O_3S$· C, 40 37; H, 7 74, N, 13.45. Found: C, 40.65, H, 7 85, N, 13.50

L-Hexathionine: 14 4 g, 66% yield; mp=251-252°d (Reported[6] 258-259°d). $[\alpha]_D^{25}$ +16.3° (c=0 2, 6 M HCl) (Reported[6] +18 3°). TLC. R_f=0.46 NMR (*d*-TFA) δ 0.84 (t, 3H, $C\underline{H}_3$-), 1 30 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2$-); 1.58 (m, 2H, -$C\underline{H}_2CH_2S$-); 2.44 (m, 2H, -$SCH_2C\underline{H}_2$-), 2 59 (t, 2H, -$C\underline{H}_2S$-), 2 88 (t, 2H, -$SC\underline{H}_2$-), 4 57 (t, 1H, -C<u>H</u>-). Anal Calculated for $C_{10}H_{21}NO_2S$· C, 54.76, H, 9 65; N, 6.39 Found· C, 53.42, H, 9.55, N, 6.33

HSO: 2.5 g, 33% yield, mp=225°d (Reported[6] 230-232°d). $[\alpha]_D^{24}$ +28.4° (c=0.2, 1 M HCl) (Reported[6] +28.2°). TLC: R_f=0.21. NMR (DCL in D_2O) δ 0 87 (t, 3H, $C\underline{H}_3$-), 1.38 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2$-), 1 54 (m, 2H, -$C\underline{H}_2CH_2S$(O)(NH)-); 2 70 (m, 2H, -S(O)(NH)$CH_2C\underline{H}_2$-); 4.00 (t, 2H, -$C\underline{H}_2$S(O)(NH)-), 4.25 (m, 2H, -S(O)(NH)$C\underline{H}_2$-), 4 50 (t, 1H, -C<u>H</u>-). Anal Calculated for $C_{10}H_{22}N_2O_3S$. C, 47.97; H, 8.86, N, 11.19 Found· C, 48 16, H, 9 03, N, 11 20.

Male Sprague-Dawley rats weighing 150-200 g were purchased from Simonsen Laboratories (Gilroy, CA) PSO, BSO, and HSO were given ig as suspensions in 1% methylcellulose of 125, 133, and 150 mg/mL, respectively, in volumes of 1 mL/100 g body weight, each animal thereby receiving a dose of 6 mmol/kg. For effects of chronic sulfoximine administration on hepatic drug metabolizing enzymes, drugs were administered daily for 3 d. Livers were removed 24 h after the last dose.

Hepatic microsomal and cytosolic fractions were prepared as described previously.[7] Protein concentrations were assayed according to the method of Lowry *et al*,[8] and P450 and GSH concentrations were assessed by the

methods of Omura and Sato[9] and Ackerboom and Sies,[10] respectively. 4-Nitroanisole demethylase activity was determined by the rate of p-nitrophenol generation,[11] and the mEH activity towards cis-stilbene oxide was determined by the radiometric partition method of Hammock *et al.*[12] Microsomal UGT and cytosolic sulfotransferase activities were determined from the disappearance of 4-nitrophenol.[13] Reverse-phase HPLC was employed for the direct quantitation of glucuronides from by UGT activities towards the aglycones, 1-naphthol, morphine, testosterone, and estrone.[14] Cytosolic GST activity towards 1-chloro-2,4-dinitrobenzene was analyzed spectrophotometrically according to Habig and Jakoby.[15] Statistical differences between treated and concomitantly assayed control groups were determined by one-way ANOVA and Fisher's PLSD multiple range test. Differences were considered significant at a p value of less than 0.05.

Results and Discussion. All three sulfoximines depleted hepatic GSH concentrations after a single dose (Figure 1). While statistical significance cannot be determined based on the small number of animals employed, some trends emerge from these data. BSO was the most effective agent, depleting GSH to below 50% of controls within 3 h and maintaining it below 50% for a least another 15 h. Maximal depletion of over 90% occurred at around 8 h. HSO exhibited similar properties, but was slower to deplete GSH, caused less maximal depletion, and allowed a quicker return of the GSH concentration toward normal. PSO was less effective than either BSO or HSO. It did not deplete hepatic GSH concentration below 50% at any time measured.

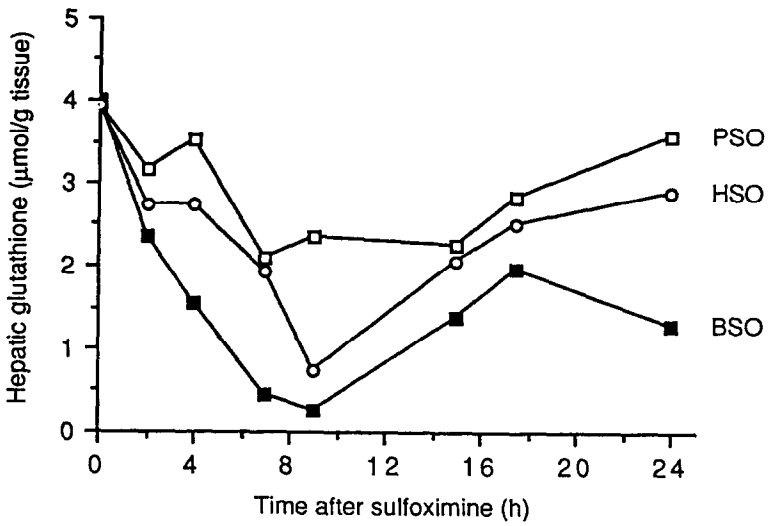


Figure 1. The effect of PSO, BSO, and HSO on hepatic GSH concentration. Livers were removed from rats at the times indicated following a single 6 mmol/kg dose of the sulfoximine, given ig in methylcellulose, and GSH concentrations determined. Each point is from a single animal except the control value (zero time), which is the mean of 7 rats.

When the sulfoximines were administered at the same dose for three consecutive days, and the drug metabolizing enzymes were examined 24 h after the last dose, PSO and HSO treatments did not significantly increase any activities (Table I). However, BSO treatment produced significant changes. a 42% increase in UGT (measured with 4-nitrophenol aglycone), a 30% increase in UGT (measured with 1-naphthol aglycone), and a 28% increase in GST activities UGT activities towards morphine, estrone, and testosterone were not significantly elevated by any treatment, nor were mEH activity, P450 concentration, P450-dependent 4-nitroanisol demethylation or cytoplasmic sulfotransferase activity. HSO treatment significantly decreased P450 (15%) and P450-dependent 4-nitroanisole demethylase activity (22%)

Table I

The Effect of PSO, BSO, AND HSO on Hepatic Drug Metabolizing Enzymes

Parameter	Treatment[a] None	PSO	BSO	HSO
P450 content[b]	0 72 ± 0 03	0 64 ± 0 03	0 63 ± 0.02	0.61 ± 0 02[c]
P450 activity[d,e]	0.49 ± 0 03	0 49 ± 0.03	0 46 ± 0 03	0 38 ± 0 02[c]
mEH	5 49 ± 0 93	6.67 ± 0.13	6 45 ± 0 94	6 75 ± 1 89
UGT towards.				
4-nitrophenol	7 00 ± 0 32	6 52 ± 0.50	9 90 ± 0 74[c]	7 51 ± 0 44
1-naphthol	37.1 ± 2 2	37.2 ± 1.9	48.4 ± 4.7[c]	35 0 ± 0 4
morphine	9 37 ± 0 66	7 91 ± 0.79	10.38 ± 0 76	8 04 ± 1.01
testosterone	3.37 ± 0 30	2 61 ± 0 43	3 03 ± 0.12	2.93 ± 0 36
estrone	0 097 ± 0 008	0.088 ± 0 003	0 100 ± 0.002	0 089 ± 0 007
Sulfotransferase	1 08 ± 0 07	0 74 ± 0 20	0 96 ± 0 05	0 95 ± 0 14
GST	1410 ± 86	1514 ± 120	1791 ± 140[c]	1295 ± 47
GSH[f]	4 37 ± 0.47	3 05 ± 0 63	2.97 ± 0 63	4 22 ± 0 62

[a] Animals received three daily doses of 6 mmol/kg, ig, of the sulfoximine in methylcellulose, and were euthanized 24 h after the last dose. Enzyme activities were determined in hepatic subcellular fractions All values represent the mean ± SE The number of animals used was 3 for PSO, 4 for BSO and HSO, and 5 for untreated controls

[b] P450 concentration is expressed in nmol/mg protein

[c] Significantly different from untreated controls, $p<0$ 05.

[d] All enzyme activities are expressed as nmol/mg protein/min

[e] Measured as 4-nitroanisole demethylase activity.

[f] GSH concentration is expressed in nmol/mg tissue

Since many xenobiotics can be bioactivated to electrophilic intermediates by P450, the nucleophile GSH is an important protective or sequestering agent. BSO has been used extensively in toxicological studies to determine whether the depletion of GSH is important in altering the toxicity of xenobiotics.

The ability of BSO to deplete GSH appears to be dependent on the route of administration and the nutritional status of the animal. At a dose of 4 mmol/kg, ip, BSO depleted hepatic GSH by 80% between 2 and 6 h in starved (12 h) mice,[1] but GSH was only depleted 60% at 4 h in fed mice.[16] The same 60% depletion, but over a slightly longer period (2 to 8 h), was seen when BSO was given by the po route to fed mice.[17] Previous reports show PSO to be less effective than BSO in depleting GSH In starved mice, when given at a dose of 4 mmol/kg, sc, PSO decreased GSH only 35% at 2 h,[18] whereas BSO at the same dose and by the same route caused 80% depletion in fed mice.[1] In rats, BSO at a dose of 4 mmol/kg, sc, depleted hepatic GSH 70% in 2 h and 73% in 4 h.[1] Previous studies in mice showed HSO to be more effective in depleting hepatic GSH than was BSO At a dose of 2 mmol/kg, ip, in mice, HSO maximally depleted GSH by 90% at 10 h, but allowed GSH to return to 75% of normal by 25 h. In contrast, BSO produced a maximal 55% depletion at 2 h, and allowed GSH to return to normal levels by 25 h.[2] From the above discussion, it is evident that variations in the treatment regimens and animal models used preclude determination of the relative effectiveness of route and dose on GSH depletion. The route, dose, and vehicle used in this study (6 mmol/kg, ig, in methylcellulose) were chosen as the most convenient manner of obtaining consistent hepatic GSH depletion by BSO in fed rats.[3] Using this regimen in rats, HSO was less effective than BSO in depleting hepatic GSH. This finding contrasts with a greater efficacy of HSO given by the ip route in the mouse. However, studies with PSO consistently point to its lack of effective GSH depleting activity.

The relationship, if any, between GSH depletion and enzyme induction by the sulfoximines remains unclear. All three agents depleted GSH in the following order of efficiency: BSO>HSO>PSO. However, only BSO caused increases in certain enzyme activities If enzyme induction is related to GSH depletion, then a threshold effect may be operating. The threshold could be related to the duration of GSH depletion or to the absolute maximum amount of GSH depletion; BSO exhibited the greatest effect on both these parameters. It is clear that chronic administration is required for induction; no effects on drug metabolizing enzymes were observed for up to 24 h following an acute dose of BSO in mice.[15,19]

No increase in P450 content or activity was observed in mice following chronic BSO treatment.[20] In fact, BSO and its analogs appear to slightly decrease P450, confirming previous information.[3] Thus, BSO is one of a few compounds reported to be selective inducers of Phase II, but not Phase I, drug metabolizing enzymes. Interestingly, another agent reported to have this property in both mice[21,22] and rats[23,24] is 2(3)-tert-butyl-4-hydroxyanisole (BHA). In contrast to BSO, however, BHA increases cellular GSH concentration.[25] This information tends to weaken the possibility of a general relationship between induction and GSH depletion.

These studies reproduce the previously demonstrated effect that several days of BSO administration induces microsomal UGT activity and cytosolic GST activity without inducing P450.[3] In the previous study, BSO was

given by the ıg route, but was dissolved in water rather than in methylcellulose. The choice of vehıcle affected a few of the parameters measured: In methylcellulose there was no significant decrease in 4-nitroanısole demethylatıon or sulfotransferase activity and no increase in the UGT actıvity towards morphine

The BSO effect is not a generalized response of all microsomal enzymes--P450, mEH, and some UGT activities are not ınduced. A similar situation exists for cytosolic enzymes, where GST activity is ınduced but sulfotransferase activity ıs not. The lack of co-induction of mEH and UGT ıs different from the effect elicited by numerous nitrogen-containing heterocycles.[26,27]

In summary, PSO and HSO are able to deplete hepatic GSH, but not to the same extent as BSO under the conditions employed. However, the propyl and hexyl analogs do not suffer from the liability of inducıng Phase II drug metabolizing enzymes and may make the interpretatıon of experimental results from complex pharmacological studies more straightforward.

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