

Busulfan-Glutathione Conjugation Catalyzed by Human Liver Cytosolic Glutathione *S*-Transferases¹

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ABSTRACT

We have examined the catalytic activity of glutathione *S*-transferases (GST) in the conjugation of busulfan with glutathione (GSH) in human liver cytosol, purified human liver GST, and cDNA-expressed GST- α 1-1. Human liver microsomes and cytosol were incubated with 40 μ M busulfan and 1 mM GSH. Cytosol catalyzed the formation of the GSH-busulfan tetrahydrothiophenium ion (THT⁺) in a concentration-dependent manner, whereas microsomes lacked activity. The total and spontaneous rates of THT⁺ formation increased with pH (pH range, 6.50–7.75), with the maximum difference at pH 7.4. Due to the limited aqueous solubility of busulfan, a K_m for busulfan was not determined. The intrinsic clearance (V_{max}/K_m) of busulfan conjugation was 0.167 μ l/min/mg with 50–1200 μ M busulfan and 1 mM GSH. GSH V_{max} and K_m for busulfan conjugation were 30.6 pmol/min/mg and 312 μ M, respectively. Ethacrynic acid (0.03–15 μ M) inhibited cytosolic busulfan-conjugating activity with 40 μ M busulfan and 1 mM GSH. Enzyme-mediated THT⁺ formation was decreased 97% by 15 μ M ethacrynic acid with no effect on the spontaneous reaction. In incubations with affinity-purified liver GST and GST- α 1-1, the intrinsic clearance for busulfan conjugation was 0.87 and 2.92 μ l/min/mg, respectively. Busulfan is a GST substrate with a high K_m relative to concentrations achieved clinically (1–8 μ M).

INTRODUCTION

Busulfan is an alkylating agent widely used in preparative chemotherapy regimens for peripheral blood stem cell and bone marrow transplantation. Systemic exposure to busulfan is correlated with regimen-related toxicity and graft rejection. At the standard 16-mg/kg total dose of busulfan, average steady-state plasma busulfan levels range 10-fold in children and adults, indicating that busulfan clearance and/or bioavailability after oral drug administration is highly variable (1).

Conjugation with GSH³ is the primary route of busulfan elimination. Initial work done by Roberts and Warwick (2) identified sulfur-containing busulfan metabolites in rat urine and demonstrated that busulfan reacted with GSH spontaneously at pH 8.0. Following administration of [¹⁴C]busulfan to the isolated perfused rat liver, γ -glutamyl- β -(*S*-tetrahydrothiophenium)-alanyl-glycine, a sulfonium ion of GSH, was found in bile (3). Thirty-eight percent of the [¹⁴C]busulfan dose was recovered in bile during the 4-h perfusion as the THT⁺ conjugate. No other metabolites were identified. THT⁺ was also produced following i.v. administration of busulfan to rats (4). Urinary sulfur-containing metabolites of busulfan after intrapleural administration to rats and oral administration to humans have been identified,

and they include the *N*-acetyl-cysteinyl-sulfonium ion, tetrahydrothiophene 1-oxide, sulfolane, and 3-hydroxysulfolane (5, 6). On treatment of urine with base, THT was produced. Each of these results is consistent with the initial formation of a GSH conjugate.

GST is a supergene family of homodimers and heterodimers that catalyze the conjugation of GSH with many reactive electrophiles. GST nomenclature has been described by Mannervik *et al.* (7). The human cytosolic GST expressed in liver include classes α , π , and μ . A membrane-bound form, microsomal GST, has been characterized (8). In human liver, 3–4% of cytosolic proteins are GST, and 80–90% of this is GST- α (9). Approximately 45% of the Caucasian population does not express GST- μ (8).

Marchand and Abdel-Monem (10) investigated the metabolism of busulfan in rat liver cytosol incubations. Busulfan conjugation was not demonstrated. However, a structural analogue, diiodobutane, was shown to be a substrate for GST. We sought to reexamine the role of GST catalysis, because 200 μ M ethacrynic acid, an inhibitor of GST and a depletor of GSH, inhibits busulfan conjugation with GSH in the isolated perfused rat liver (3).

The purpose of this investigation was to determine whether the formation of the busulfan conjugate is catalyzed by human liver cytosolic GST. We report that in incubations with human liver cytosol, affinity-purified human liver GST, and cDNA-expressed human GSTA1-1, human liver GST catalyzes busulfan conjugation with a K_m at least 120 times concentrations achieved with the use of busulfan in the bone marrow and peripheral blood stem cell transplantation setting.

MATERIALS AND METHODS

Reagents and Chemicals. GSH, THT, busulfan, CDNB, 1-bromopentane, and ethacrynic acid were purchased from Sigma Chemical Co. (St. Louis, MO). GC-MS grade hexane was purchased from Burdick and Jackson (Muskegon, MI). All other solvents were analytical grade or higher.

Preparation of Cytosol and Microsomes. Human liver was obtained from the human liver bank in the Departments of Pharmaceutics and Medicinal Chemistry at the University of Washington. Cytosol and microsomes were prepared from human liver by differential centrifugation. All steps were performed at 4°C. Briefly, frozen liver was weighed and allowed to thaw in a 5-fold dilution of 100 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4). This mixture was homogenized over ice with six 3-s strokes of a VirTishear (Virtis, Gardiner, NY) homogenizer. Following centrifugation at 10,000 \times *g* for 25 min, the supernatant was filtered through gauze and centrifuged at 100,000 \times *g* for 70 min, and cytosol was decanted. The microsomal pellet was resuspended by hand in 100 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4) in a 10-ml Thompson tube and repelleted by centrifugation at 100,000 \times *g* for 70 min. Liver cytosol and microsomes were stored at –80°C until incubations were performed. Protein concentrations were determined with the Bio-Rad (Oakland, CA) protein assay with BSA as the standard (11). CDNB was used to characterize GST activity of all preparations as described by Habig *et al.* (12).

Affinity-purified GSTA1-1 and Human Liver GST Enzymes. *GTH1* cDNA cloned into expression vector pKK332-2 was the kind gift of Dr. C-P. David Tu (Pennsylvania State University; Ref. 13). The cDNA was expressed in *Escherichia coli*, essentially as described by Van Ness *et al.* (14). The overnight bacterial culture was sonicated and centrifuged at 9000 \times *g* for 25 min. *GTH1* was purified on a GSH-Sepharose affinity column (Pharmacia

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³ The abbreviations used are: GSH, glutathione; THT⁺, tetrahydrothiophenium ion; THT, tetrahydrothiophene; GST, glutathione *S*-transferase; GSTA, GSTM, and GSTP, GST- α , GST- μ , and GST- π , respectively, numerals refer to specific monomers in the dimeric enzyme; CDNB, 1-chloro-2,4-dinitrobenzene; GC-MS, gas chromatography-mass spectroscopy.

Biotech, Piscataway, NJ), washed with PBS, and eluted in 10 mM GSH and 50 mM Tris (pH 8.0). *GTH1* has been identified as *GSTA1-1* by Hayes *et al.* (15) and confirmed in our own laboratory based on isoelectric point, immunoreactivity, and CDNB K_m (16). Human liver GST was purified from cytosol as described (17). Fractions containing CDNB activity were pooled and stored at -80°C .

Incubations. All incubations were performed in 100 mM potassium phosphate buffer and 1 mM EDTA (incubation buffer) in 13×100 -mm glass test tubes at 37°C in a shaking water bath. In cytosolic incubations, 1–2 mg cytosolic protein were added to each test tube and kept on ice. Appropriate control incubations (no protein or boiled protein) were performed to determine the rate of spontaneous busulfan-GSH conjugate formation. A GSH stock solution was prepared fresh daily in incubation buffer and added to incubations to yield a final concentration of 1 mM. Additional incubation buffer was added to make the final volume 1 ml. The incubation mixture was preincubated for 4 min at 37°C before starting the reaction with the addition of busulfan in 10 μl acetonitrile [final acetonitrile concentration, 1% (v/v)]. In all incubations, conditions were optimized to maintain linear product formation with respect to time and protein concentration.

The effect of protein concentration (1–4 mg/ml) was determined in respective incubations with cytosolic and microsomal protein. The substrate concentrations in these incubations were 1 mM and 40 μM for GSH and busulfan, respectively, and the incubation time was 15 min. The CDNB specific activities for cytosol and microsomal protein were 540 and 230 nmol/min/mg protein, respectively. These values include the spontaneous reaction rate of 89.6 nmol/min.

The influence of pH on the THT⁺ formation rate was determined in incubations with and without cytosol by adjusting the incubation buffer pH with NaOH to make solutions with pH values ranging from 6.5 to 8.0. The concentrations of busulfan and GSH were 200 μM and 1 mM, respectively. The pH of each reaction mixture was determined during a 10-min preincubation period at 37°C . Incubations were carried out for an additional 5 min to determine the THT⁺ formation rate.

The K_m for busulfan was sought by varying busulfan concentrations between 50 and 1200 μM in the presence of 1 mM GSH. The maximum busulfan concentration was limited by solubility. The THT⁺ formation rate was determined in cytosol and boiled cytosol. The spontaneous formation rate for THT⁺ was subtracted from the total formation rate to yield the enzyme-mediated rate. Similar experiments were performed to determine the K_m for GSH at 600 μM busulfan. GSH concentrations were 100–5000 μM . Incubation times varied from 2.5 to 30 min to maintain linear product formation rates. PCNONLIN (SCI Software, Lexington, KY) was used to estimate V_{max} and K_m for the GSH concentration-dependent THT⁺ formation rate from nontransformed data.

Ethacrynic acid was included in selected incubations at final concentrations of 0.30–15 μM with cytosol or boiled cytosol. Substrate concentrations were 1 mM GSH and 40 μM busulfan. The enzymatic contribution to THT⁺ formation was calculated as the difference between the cytosol and boiled cytosol THT⁺ formation rates. The reaction was carried out for 15 min, and the percentage of inhibition of busulfan conjugation was determined by comparison to incubations without ethacrynic acid.

Incubations to confirm GST-catalyzed conjugation of busulfan were conducted with Sepharose affinity-purified human liver GST and *GSTA1-1* expressed in *E. coli*. Substrate concentrations were 5–80 μM busulfan and 7.5 mM GSH. The incubation time was 44 min, and 310 μg *GSTA1-1* and 440 μg liver GST were used in each 1.0-ml incubation. The CDNB specific activities of *GSTA1-1* and liver GST were 44.9 and 5.90 $\mu\text{mol}/\text{min}/\text{mg}$ purified enzyme, respectively. The human liver from which GST was purified did not contain GSTM1.⁴ Linear regression was used to determine the slope of the velocity *versus* substrate concentration plot, which at low busulfan concentrations relative to the K_m is defined as the intrinsic clearance (V_{max}/K_m).

Analysis of THT⁺. Following incubation, samples were placed in an ice water bath, and 5.5 ml ice-cold methylene chloride were added to stop the reaction and to extract unreacted busulfan. This mixture was sealed, shaken for 5 min, and centrifuged at $1000 \times g$ for 5 min. An aliquot of the aqueous layer (0.7 ml) was transferred to a clean test tube, and 0.22 ml hexane, 10 ng internal standard 1-bromopentane, and 0.10 ml 1 N NaOH were added. Base converts

THT⁺ to THT. After 5 min at room temperature while sealed, the mixture was shaken and centrifuged as before. Tubes were placed on dry ice, and the hexane layer was removed from the frozen aqueous phase for analysis of THT.

THT was analyzed by a Hewlett-Packard 5890 gas chromatograph with a 5970 mass selective detector with electron impact ionization (70 electron volts) operated in selective ion monitoring mode for ions at m/z 60 and 71 (fragment ions of THT and 1-bromopentane, respectively) and m/z 88 for the THT parent ion. The system was equipped with a split-splitless pressure programmable injector, 7673 autosampler, and Pascal workstation. The column was a DB5, 20 m \times 0.18 mm with a 0.4- μm film thickness (Supelco, Bellefonte, PA), to which helium was supplied as carrier gas using the constant flow mode with a column head pressure of 26 p.s.i. The heated zones were: oven, 34°C for the first 2 min, then ramped at $5^{\circ}\text{C}/\text{min}$ for 8 min; injector, 150°C ; and mass spectrometer source, quadrupole, and transfer line temperatures, 250, 100, and 200°C , respectively. An aliquot (5 μl) of hexane was injected in the splitless mode. THT and 1-bromopentane eluted at 7.96 and 8.34 min, respectively. Standard curves were prepared by spiking the incubation buffer with THT (30–1300 pmol/ml added) and extracting with hexane as described above. The linear relationship between the peak height ratio and THT concentration was used to calculate the concentration of THT in samples.

RESULTS

The subcellular fraction containing busulfan-conjugating activity in human liver preparations was determined by incubating 1–4-mg/ml concentrations of either cytosolic or microsomal protein with 1 mM GSH and 40 μM busulfan. THT⁺ was not formed in incubations without GSH (data not shown). Fig. 1 shows that THT⁺ formation was proportional to cytosolic protein concentration, whereas microsomal protein did not catalyze THT⁺ formation. Cytosolic protein enhanced the amount of product formed by 3-fold at 4 mg/ml protein. The amount of product formed in microsomal incubations was not different from control incubations without protein (42.5 ± 1.3 pmol without protein *versus* 47.0 ± 0.5 pmol with 4 mg microsomal protein).

Fig. 2 shows the effect of pH on the spontaneous and total (enzymatic plus nonenzymatic) rates of THT⁺ formation. The total THT⁺ formation rate increased linearly with pH over the range of 6.50–7.75 in cytosolic incubations. The spontaneous rate increased slightly from pH 6.50 to 7.40 and then increased sharply from pH 7.50 to 7.75. Subsequently, all incubations were performed at pH 7.40 because of its physiological relevance and because it is the pH at which the maximum difference between spontaneous and total product formation velocities was observed.

In experiments to determine the K_m for busulfan, there was a linear increase in the velocity of THT⁺ formation with busulfan concentration up to the limit of busulfan solubility (1200 μM), which is approximately 120 times the observed plasma busulfan concentrations

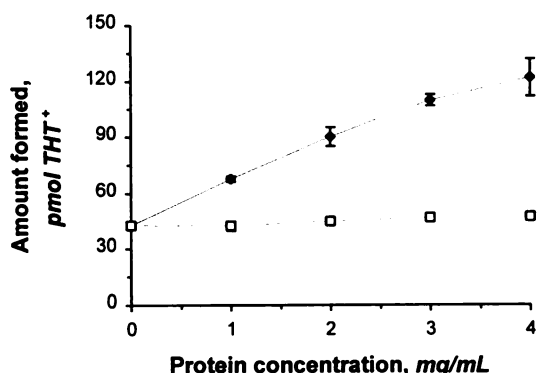


Fig. 1. Effect of cytosolic (◆) and microsomal (□) protein on the amount of THT⁺ formed in a 15-min incubation with 40 μM busulfan and 1 mM GSH. The data are the means of triplicate determinations. Bars, SD.

⁴ F. Farin, unpublished data.

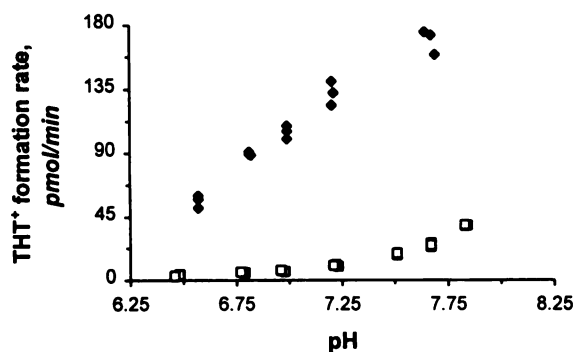


Fig. 2. Influence of pH on the THT⁺ formation rate was determined in 100 mM phosphate buffer and 1 mM EDTA. pH was adjusted prior to addition of protein; shown is the pH at the beginning of the 5-min incubation after the addition of protein. Substrate concentrations were 200 μ M busulfan and 1 mM GSH. The spontaneous reaction rate (\square) was determined in incubations without cytosol, and the total reaction rate (\blacklozenge) was determined in incubations with 1.4 mg/ml cytosolic protein.

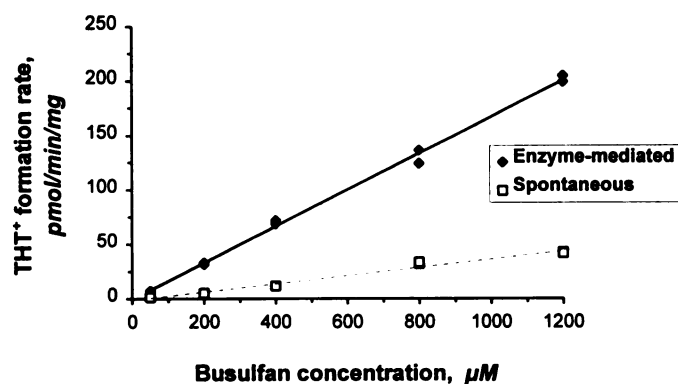


Fig. 3. THT⁺ formation rate as a function of busulfan concentration. Incubations contained 1.4 mg/ml cytosol or boiled cytosol and 1 mM GSH. Duplicate rate determinations at each busulfan concentration are shown. The slopes for cytosol and boiled cytosol regression lines are 0.167 and 0.038 μ L/min/mg, respectively.

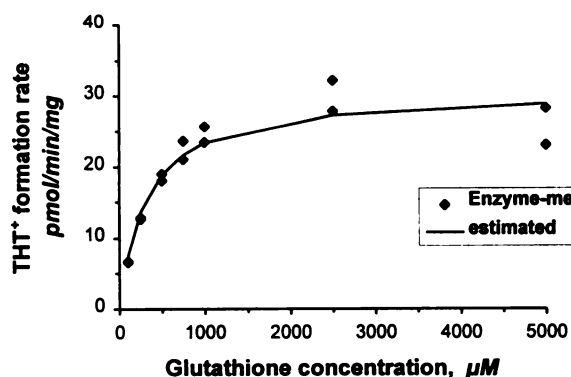


Fig. 4. THT⁺ formation rate as a function of GSH concentration. The incubation contained 1.1 mg/ml cytosol or boiled cytosol and 600 μ M busulfan. Individual values for duplicate determinations are shown. Solid line, difference between total and spontaneous THT⁺ formation rate fitted to the Michaelis-Menten equation. V_{max} and K_m were 30.6 pmol/min/mg and 311 μ M, respectively.

following doses of 1 mg/kg (Fig. 3; Ref. 1). The intrinsic clearance rates for busulfan (V_{max}/K_m) were determined by linear regression to be 0.167 and 0.038 μ L/min/mg protein for cytosol and boiled cytosol, respectively. Thus, the addition of 1.4 mg/ml cytosol increased the spontaneous reaction rate by 4.4-fold. Fig. 4 shows the relationship between GSH concentration and busulfan conjugation velocity from which the apparent K_m for GSH was obtained. The spontaneous rate of THT⁺ formation was linear with GSH concentration, and the slope

was 0.00832 μ L/min/mg protein. GSH had an apparent K_m (mean \pm SE) of 311 \pm 60 μ M and a V_{max} of 30.6 \pm 1.6 pmol/min/mg protein at 600 μ M busulfan.

The nonspecific GST inhibitor ethacrynic acid inhibited the enzymatic formation of THT⁺ in a concentration-dependent manner (Fig. 5). Almost complete inhibition (97%) was observed at 15 μ M ethacrynic acid, without changing the spontaneous reaction rate. In control incubations without ethacrynic acid, the mean (\pm SD) enzymatic and nonenzymatic rates of THT⁺ formation (pmol/min/mg protein) were 10.3 \pm 0.3 and 1.74 \pm 0.02, respectively. The IC_{50} for ethacrynic acid in human liver cytosol was 1.5 μ M.

GST purified from human liver cytosol or from the *E. coli* expression system also catalyzed busulfan conjugation (Fig. 6). Saturation of product formation was not observed across the busulfan concentration range, 5–80 μ M, which encompassed concentrations observed in patients. The intrinsic clearances of GSTA1-1 and liver GST determined as the slopes of the velocity *versus* busulfan concentration regression lines were 2.92 and 0.87 μ L/min/mg protein, respectively.

DISCUSSION

We have developed a GC-MS assay to measure THT⁺ formation in metabolic studies performed *in vitro* following conversion of THT⁺ to THT by treatment with base. With GC-MS, the sensitivity limit for THT was lowered to 0.03 nmol/ml, representing a 1000-fold improvement over the previous detection limit (4). This method allowed us to demonstrate that busulfan conjugation is catalyzed by human liver

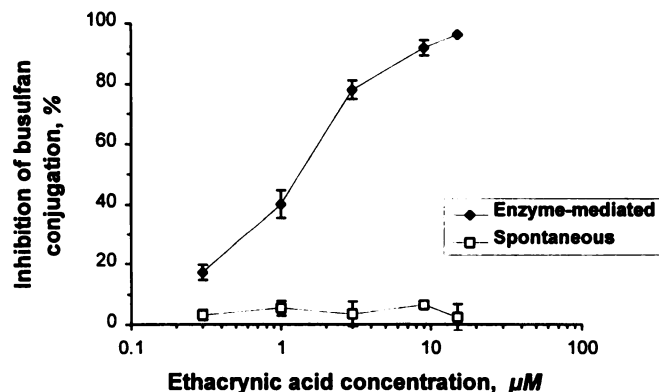


Fig. 5. Ethacrynic acid inhibition of THT⁺ formation in incubations with 1.8 mg/ml cytosol or boiled cytosol. Incubations were performed with 40 μ M busulfan and 1 mM GSH. The data are the means of triplicate determinations. Bars, SD.

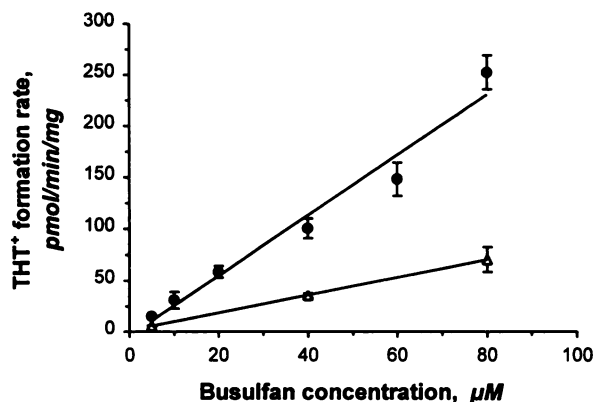


Fig. 6. Busulfan-conjugating activity of purified human liver GST (Δ) and *E. coli*-expressed human GSTA1-1 (\bullet) cDNA. Incubations contained 440 and 340 μ g/ml liver GST and GSTA1-1, respectively, and 7.5 mM GSH. Data are the means of quadruplicate determinations. Bars, SD.

GST in incubations with cytosol and affinity-purified GST. The K_m for busulfan conjugation was greater than 1200 μM , which is consistent with linear pharmacokinetics reported *in vivo* (18).

At physiological pH (7.4) and GSH concentration (1 mM) and 1.4 mg/ml human liver cytosolic protein, we have found a 4.4-fold enhancement of the formation of THT⁺ over that observed in the absence of active enzyme (Fig. 3). One might question whether this modest enhancement over the spontaneous rate is toxicologically or therapeutically significant. In our studies, liver was homogenized in phosphate buffer, resulting in a 5-fold dilution of cytosolic protein. Incubations were performed at an additional 4-fold dilution of cytosolic protein. Because a 4.4-fold rate enhancement was observed at a 20-fold dilution of cytosolic protein, the cellular cytosolic GST-mediated product formation is estimated to be approximately 90 times that of the spontaneous THT⁺ formation in a hepatocyte if velocity increases in proportion to GST concentration. In the hepatocyte, spontaneous conjugation of busulfan with GSH is apparently insignificant in comparison to the GST-catalyzed rate.

Even more modest enhancement of the rate of the spontaneous (noncatalyzed) GSH conjugation rate has been observed for other GST substrates. Dirven *et al.* (19) have commented on the catalytic activity afforded by GST on rates of nitrogen mustard-GSH conjugate formation. The formation rates of monochloro, monogluthionyl conjugates of chlorambucil, melphalan, and phosphoramidate mustard were enhanced only 2-fold by GST- α enzymes, and a 3–5 fold enhancement in the formation rate of monochloro, monogluthionyl ifosphamide mustard was seen with GST- π (19). In those studies, the GST concentrations were 4 (chlorambucil), 11 (phosphoramidate mustard), and 40 (melphalan and ifosfamide mustard) times the estimated total GST concentration at which we found a 4.4-fold enhancement of busulfan conjugation.⁵ Despite the low activity observed with the mustards, resistance to each of these compounds has been linked with enhanced GST (20).

Busulfan is perhaps more comparable to thiotepa, for which GSH conjugation is accelerated 30–35-fold by 40 μM GST, about 20% of the apparent activity we observed with busulfan. Interestingly, neither busulfan nor thiotepa undergoes chemical transformation prior to GSH conjugation. The nitrogen mustards mentioned first rearrange to aziridinium ion intermediates. The rearrangement has been shown to be slow relative to the step of GSH conjugation of the aziridinium ion (19, 21–24).

Because busulfan is a relatively good substrate for GST in comparison to the nitrogen mustards, the potential for GST-mediated cellular resistance exists. This issue has been examined with the busulfan congener hepsulfam. Resistance to hepsulfam has been correlated with total cytoplasmic GST activity in six breast cancer cell lines (25). Hepsulfam also forms a GSH conjugate, but GST catalysis apparently has not been assessed. Our findings suggest that the GST-related cellular resistance to hepsulfam is due to GST-catalyzed conjugation of hepsulfam with GSH.

REFERENCES

- Slattery, J. T., Sanders, J. E., Buckner, C. D., Schaffer, R. L., Lambert, K. W., Langer, F. P., Anasetti, C., Bensinger, W. I., Fisher, L. D., Appelbaum, F. R., and Hansen, J. A. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant.*, 16: 31–42, 1995.
- Roberts, J. J., and Warwick, G. P. The mode of action of alkylating agents—III. The formation of 3-hydroxytetrahydrothiophene-1:1-dioxide from 1:4-dimethanesulphonyloxybutane (myleran), *S*- β -L-alanyl-tetrahydrothiophenium mesylate, tetrahydrothiophene and tetrahydrothiophene-1:1-dioxide in the rat, rabbit, and mouse. *Biochem. Pharmacol.*, 6: 217–227, 1961.
- Hassan, M., and Ehrsson, H. Metabolism of ¹⁴C-busulfan in isolated perfused rat liver. *Eur. J. Drug Metab. Pharmacokinet.*, 12: 71–76, 1987.
- Marchand, D. H., Rimmel, R. P., and Abdel-Monem, M. M. Biliary excretion of a glutathione conjugate of busulfan and 1,4-dibromobutane in the rat. *Drug Metab. Dispos.*, 16: 85–92, 1988.
- Hassan, M., and Ehrsson, H. Urinary metabolites of busulfan in the rat. *Drug Metab. Dispos.*, 15: 399–402, 1987.
- Hassan, M., Oberg, G., Ehrsson, H., Ehrnebo, M., Wallin, I., Smedmyr, B., Totterman, T., Eksborg, S., and Simonsson, B. Pharmacokinetic and metabolic studies of high-dose busulfan in adults. *Eur. J. Clin. Pharmacol.*, 36: 525–530, 1989.
- Mannervik, B., Awasthi, Y. C., Board, P. B., Hayes, J. D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W. R., Pickett, C. B., Sato, K., Widersten, M., and Wolf, C. R. Nomenclature for human glutathione transferases. *Biochem. J.*, 282: 305–308, 1992.
- Hayes, J. D., and Pulford, D. J. The glutathione *S*-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, 30: 445–600, 1995.
- Beckett, G. J., and Hayes, J. D. Glutathione *S*-transferases: biomedical applications. *Adv. Clin. Chem.*, 30: 281–380, 1993.
- Marchand, D. H., and Abdel-Monem, M. M. Glutathione *S*-transferases catalyzed conjugation of 1,4-disubstituted butanes with glutathione *in vitro*. *Biochem. Biophys. Res. Commun.*, 128: 360–367, 1985.
- Bradford, M. A. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248–254, 1976.
- Habig, W. H., Pabst, M. J., and Jakoby, W. B. Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130–7139, 1974.
- Tu, C.-P. D., and Qian, B. Human liver glutathione *S*-transferase: complete primary sequence of an Ha subunit cDNA. *Biochem. Biophys. Res. Commun.*, 141: 229–237, 1986.
- Van Ness, K. P., Buetler, T. B., and Eaton, D. L. Enzymatic characteristics of chimeric mYc/rYc1 glutathione *S*-transferases. *Cancer Res.*, 54: 4573–4575, 1994.
- Hayes, J. D., Kerr, L. A., and Cronshaw, A. D. Evidence that glutathione *S*-transferases B1B1 and B2B2 are the products of separate genes and that their expression in human liver is subject to inter-individual variation. *Biochem. J.*, 264: 437–445, 1989.
- Czerwinski, M., Gibbs, J. P., and Slattery, J. T. Busulfan conjugation by glutathione *S*-transferases α , μ , and π . *Drug Metab. Dispos.*, 24: in press, 1996.
- Soma, Y., Satoh, K., and Sato, K. Purification and subunit-structural and immunological characterization of five glutathione *S*-transferases in human liver, and the acidic form as a hepatic tumor marker. *Biochim. Biophys. Acta*, 869: 247–258, 1986.
- Hassan, M., Ljungman, P., Bolme, P., Ringden, O., Syruckova, Z., Bekassy, A., Stary, A., Wallin, I., and Kallberg, N. Busulfan bioavailability. *Blood*, 84: 2144–2150, 1994.
- Dirven, H. A. A. M., Dictus, E. L. J. T., Broeders, N. L. H. L., van Ommen, B., and van Bladeren, P. J. The role of human glutathione *S*-transferase isoenzymes in the formation of glutathione conjugates of the alkylating cytostatic drug thiotepa. *Cancer Res.*, 55: 1701–1706, 1995.
- Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res.*, 54: 4313–4320, 1994.
- Bolton, M. G., Hilton, J., Robertson, K. D., Streeper, R. T., Colvin, O. M., and Noe, D. A. Kinetic analysis of the reaction of melphalan with water, phosphate, and glutathione. *Drug Metab. Dispos.*, 21: 986–996, 1993.
- Ciaccio, P. J., Tew, K. D., and LaCreta, F. P. Enzymatic conjugation of chlorambucil with glutathione by human glutathione *S*-transferases and inhibition by ethacrynic acid. *Biochem. Pharmacol.*, 42: 1504–1507, 1991.
- Dirven, H. A. A. M., van Ommen, B., and van Bladeren, P. J. Involvement of human glutathione *S*-transferase isoenzymes in the conjugation of cyclophosphamide metabolites with glutathione. *Cancer Res.*, 54: 6215–6220, 1994.
- Dirven, H. A. A. M., Megens, L., Oudshoorn, M. J., Dingemans, M. A., van Ommen, B., and van Bladeren, P. J. Glutathione conjugation of the cytostatic drug ifosfamide and the role of human glutathione *S*-transferases. *Chem. Res. Toxicol.*, 8: 979–986, 1995.
- Armstrong, D. K., Gordon, G. B., Hilton, J., Streeper, R. T., Colvin, O. M., and Davidson, N. E. Hepsulfam sensitivity in human breast cancer cell lines: the role of glutathione and glutathione *S*-transferase in resistance. *Cancer Res.*, 52: 1416–1421, 1992.

⁵ Beckett and Hayes (9) found that the total hepatocyte concentration was 17 mg/g cytosolic protein. Based on this concentration, we estimate that the total GST concentration in our incubations was approximately 0.9 μM .

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