[CANCER RESEARCH 47, 2525–2530, May 15, 1987]

Glutathione and Related Enzymes in Rat Brain Tumor Cell Resistance to 1,3-Bis(2-chloroethyl)-1-nitrosourea and Nitrogen Mustard[1]

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ABSTRACT

Reduced glutathione (GSH) and activities of several glutathione-related enzymes were measured in two 9L rat brain tumor cell lines with differing sensitivities to both 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and nitrogen mustard. GSH, measured by a specific high-performance liquid chromatographic method, was found to be approximately twice as high in 9L cells sensitive to BCNU but resistant to nitrogen mustard. The nitrogen mustard resistant cell line was also found to have 2.5-fold more bulk glutathione transferase activity and approximately 3-fold more γ-glutamyl transpeptidase activity. Glutathione reductase activity, protein thiol, and total protein content were similar in the two cell lines.

Pretreatment of 9L cells with 50 μM buthionine sulfoximine for 24 h to deplete GSH only slightly potentiated BCNU cytotoxicity in a clonogenic assay whereas that of nitrogen mustard was markedly potentiated in both cell lines. Similarly, buthionine sulfoximine pretreatment had little effect on the induction of sister chromatid exchanges by BCNU, but significantly increased the number of sister chromatid exchanges induced by nitrogen mustard in both cell lines. Depleting GSH also had no significant effect on the cytotoxicity of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea to 9L cells.

Pretreatment of 9L cells with 1 mM GSH significantly protected against nitrogen mustard cytotoxicity. Moreover, nitrogen mustard incubated with GSH and glutathione transferase was 4-fold less cytotoxic than nitrogen mustard incubated with GSH alone. Incubation of BCNU with GSH alone or with glutathione transferase had no effect on BCNU cytotoxicity. These results indicate that elevated GSH and glutathione transferase activity is one mechanism of cellular resistance to nitrogen mustard in the 9L cell line, but it does not correlate with resistance to BCNU or other clinically important nitrosoureas.

INTRODUCTION

BCNU[3] remains the chemotherapeutic agent of choice for human brain tumors. Nitrogen mustards could potentially also be used as there is evidence that they cross the blood-brain barrier (1). Tumor cell resistance is, however, a major problem in chemotherapy. Approximately 60% of human brain tumors are resistant to the cytotoxic effects of BCNU (2, 3). Due to their characteristic heterogeneity, tumors which initially demonstrate sensitivity to drug therapy often become repopulated by stem cells which were originally resistant or have acquired resistance to the drug(s) employed. An understanding of the biochemical mechanisms of cellular resistance to BCNU and other agents is therefore essential to the development of new and more effective therapies for human brain tumors.

GSH is a tripeptide thiol present at concentrations of 0.5 to 10 mM in most cells and serves many important functions in cellular metabolism and transport (4, 5). It also plays a key role in the protection against endogenous and exogenous free radicals and other electrophiles (6). The glutathione transferases catalyze the conjugation of electrophilic compounds, including many chemotherapeutic agents, with the sulfhydryl residue of GSH (6, 7). BCNU is inactivated by a glutathione transferase mediated denitrosation reaction (8, 9), and the nitrogen mustard L-PAM is detoxified through a GSH-dependent dechlorination reaction (10). There is growing evidence that GSH and GSH-related enzymes play an important role in cellular resistance to agents detoxified by reactions involving GSH. L1210 cells resistant to L-PAM contain 2–3 times more GSH than L1210 cells sensitive to L-PAM (11). Moreover, in a Walker 256 rat carcinoma cell line, resistance to bifunctional nitrogen mustards is accompanied by an approximate 2-fold increase in glutathione transferase activity (12). Selective modification of GSH metabolism is a useful approach to clarify the role of GSH in cellular resistance to drugs, and may also prove to be effective adjuvant therapy (13). Depletion of cellular GSH with BSO, an inhibitor of γ-glutamyl cysteine synthetase, has been shown to effectively sensitize L1210 cells resistant to L-PAM (14).

The 9L rat gliosarcoma cell line has been used extensively for both the *in vivo* and *in vitro* evaluation of chemotherapeutic agents (15). The 9L-2 subline which was developed from the 9L cell line is 3- to 4-fold more resistant to BCNU than the parent cell line (16). Comparison of 9L and 9L-2 cells treated with HN2 have shown that 9L cells are 3-fold more resistant to the cytotoxic effects and induction of SCEs than 9L-2 cells (17). The capacity to repair O^6-chloroethyl guanine adducts has been shown to be a major mechanism of resistance to BCNU (18). However, additional mechanisms of resistance to BCNU are likely. The experiments reported here were performed to determine the role of GSH and GSH-related enzymes in the response of 9L and 9L-2 rat brain tumor cells to BCNU and HN2.

MATERIALS AND METHODS

Cell Cultures

The 9L and 9L-2 rat gliosarcomas are well-established cell lines whose properties have been previously described (17, 18). Cell lines were maintained in 75- or 150-cm^2 tissue culture flasks in Dulbecco's modified Eagles' medium containing 1.0 g glucose per liter, 10% newborn calf serum, and 50 μg/ml gentamicin (Gibco, Grand Island, NY) under 5% CO_2 at 37°C.

Drugs

BCNU, CCNU, and MeCCNU were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) and D,L-buthionine-*S*, *R*-sulfoximine from Bachem Inc. (Torrance, CA). HN2, reduced glutathione, and glutathione transferase from equine liver were obtained from Sigma

Received 10/15/86; revised 1/27/87; accepted 2/16/87.

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[1] This research was supported by Grant CA 13525 from the National Cancer Institute.

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[3] The abbreviations used are: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; GSH, reduced glutathione; L-PAM, L-phenylalanine mustard; BSO, D, L-buthionine-*S*, *R*-sulfoximine; HN2, nitrogen mustard; SCE, sister chromatid exchange; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; MeCCNU, 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea; CFE, colony-forming efficiency; HPLC, high-performance liquid chromatography; CDNB, 1-chloro-2,4-dinitrobenzene.

Chemical Co. (St. Louis, MO). All drugs were dissolved in solvents immediately before use. BCNU, CCNU, and MeCCNU were dissolved in absolute ethanol. HN2, BSO, and GSH were dissolved in sterile Hanks' balanced salt solution.

CFE Assay

Single-cell suspensions of 9L and 9L-2 cells were obtained by incubation of confluent cultures with trypsin/EDTA; various numbers of cells (50 to 1 × 10^5) were plated into Flow 4-well plates in medium consisting of 60% fresh medium and 40% medium conditioned by log-phase cells for 2 days prior to filtration. Cells were subjected to treatment by one of the following protocols:

BSO Pretreatment. After 18 h of incubation, cells were treated with 50 μM BSO or solvent. After an additional 24 h of incubation, cells were treated with various concentrations of BCNU, CCNU, MeCCNU, or HN2 for 2 h followed by medium replacement.

GSH Pretreatment. 1 mM GSH or vehicle (control) was added to the medium immediately prior to treatment with HN2 (*i.e.*, at 42 h), followed by medium replacement after 2 h.

Preincubation of BCNU with GSH and Glutathione Transferase. The following solutions were prepared with Hanks' balanced salt solution with 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (necessary to maintain pH at 7.0–7.4). Upon addition of freshly dissolved BCNU, the solutions contained either: (*a*) 4.7 mM BCNU; (*b*) 4.7 mM BCNU + 20 mM GSH; (*c*) 4.7 mM BCNU + 20 mM GSH + 1 mg/ml glutathione transferase; or (*d*) 4.7 mM BCNU + 20 mM GSH + 1 mg/ml boiled glutathione transferase. Each solution was incubated for 15 min at 37°C prior to addition to the treatment plates. Cells were then treated for 2 h followed by medium replacement.

Preincubation of HN2 with GSH and Glutathione Transferase. Protocol was the same as for BCNU with the following changes: (*a*) compounds were dissolved in Hanks' balanced salt solution with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and (*b*) final concentrations of the incubation mixtures were 0.5 mM HN2, 2 mM GSH, and 1 mg/ml glutathione transferase.

Cultures were incubated for 10 to 14 days, then fixed with methanol and stained with crystal violet. Colonies containing 50 or more cells were scored. Each survival estimate was calculated from the mean of four determinations. Surviving fraction was calculated as the ratio of the CFEs of treated and untreated cells. The CFE for untreated cells ranged from 50 to 80%.

SCE Assay

9L and 9L-2 cells (7.5 × 10^5) were seeded into 75-cm^2 flasks. After 18 h incubation, BSO (50 μM) was added to some flasks for BSO pretreatment experiments. After an additional 24 h incubation, cells were treated with various concentrations of BCNU or HN2 for 2 h. After treatment, medium was removed and replaced with medium containing 10 μM bromodeoxyuridine, after which cells were incubated for two replication cycles (approximately 28 h). Mitotic cells were accumulated by treatment with colcemid (0.04 μg/ml) for 2 h. Flasks were shaken to dislodge the mitotic cells, medium was poured off, and mitotic cells were collected by centrifugation (1000 rpm for 5 min). The pellet was treated with 2.0 ml of 0.05 M KCl for 8 to 10 min, fixed twice with glacial acetic acid and methanol (1:3), and metaphase chromosomes were spread on glass microscope slides. The method of Perry and Wolff was used for differential staining of sister chromatids (19). For each experiment, the frequency of SCEs was determined in 25 metaphase cells.

Assay for GSH

Medium was aspirated from 75-cm^2 flasks containing log-phase cells, cells were rinsed with Dulbecco's phosphate buffered saline (PBS) containing 38 mg% EDTA, then scraped and suspended in PBS/EDTA at a concentration of 2–3 × 10^6 cells/ml. Intracellular GSH was measured by HPLC as described by Reed *et al.* (20).

To measure the depletion of intracellular GSH by BSO, log-phase cells were treated with 50 μM BSO in 75-cm^2 flasks. Cell suspensions were prepared and analyzed as above at 0, 2, 6, and 24 h after BSO treatment.

Assays for Glutathione-related Enzymes and Protein Thiols

Cell suspensions were prepared as above, then sonicated on ice with a Braun-sonic 1510 cell sonicator (2 min, 100 W). Glutathione transferase and glutathione reductase activities were measured according to Habig *et al.* (21) and Roos *et al.* (22), respectively. The method of Meister *et al.* (23) was used to measure γ-glutamyl transpeptidase activity. Protein thiols were determined using Ellman's reagent as described by Di Monte *et al.* (24). Protein values were determined by the method of Lowry *et al.* (25).

RESULTS

9L cells contain approximately twice as much GSH as do 9L-2 cells but the protein thiol and total protein contents are similar in the two cell lines (Table 1). Table 2 shows the activities of several GSH-related enzymes in 9L and 9L-2 cells. Bulk glutathione transferase activity was measured by reactivity with 1-chloro-2,4-dinitrobenzene (CDNB), a universal substrate for the many glutathione transferase isozymes. Activity was 2.5 times higher in 9L than in 9L-2 cells. 9L cells were also found to contain almost 3-fold more γ-glutamyl transpeptidase activity. Glutathione reductase activity was only slightly higher in 9L cells.

BSO was used to lower the reduced glutathione content of 9L cells in the following CFE and SCE assays. The time course of depletion of intracellular GSH with BSO (50 μM) was similar in 9L and 9L-2 cells. GSH levels in both cell lines dropped to approximately 50% of their initial values after 6 h and to below 10% after 24 h.

Survival curves for 9L and 9L-2 cells treated with BCNU for 2 h are shown in Fig. 1, *A* and *B*. Extrapolating from the curve, 90% of 9L cells would not be able to form colonies in the presence of 37 μM BCNU. In contrast, 70 μM BCNU produced less than 1-log cell kill in 9L-2 cells. The surviving fraction of 9L cells treated with BSO and BCNU was calculated as the ratio of the CFEs of cells treated with both drugs and cells treated with BSO only. BSO was found to be more cytotoxic to 9L than 9L-2 cells; the cell kill produced by treatment with 50 μM BSO for 26 h averaged 50 and 15%, respectively. In both 9L and 9L-2 cells, BSO pretreatment resulted in less than a 1/2-log potentiation of BCNU cytotoxicity.

Fig. 2 shows that BSO pretreatment also has essentially no effect on the cytotoxicity of two clinically important nitrosoureas, CCNU, MeCCNU, to 9L-2 cells. The resistance of 9L-2

Table 1 *Thiol and protein content of 9L and 9L-2 cells*

	9L	9L-2
GSH (nmol/mg protein)	53 ± 5[a]	25 ± 14
Protein-SH (nmol SH/mg protein)	90 ± 11	79 ± 7
Total protein (mg protein/10^6 cells)	0.38 ± 0.06	0.40 ± 0.06

[a] Mean ± SD of at least six determinations.

Table 2 *Activities of GSH-related enzymes in 9L and 9L-2 cell sonicates*

	9L	9L-2
Glutathione reductase (nmol NADPH oxidized/min/mg protein)	12 ± 3	9 ± 2
Glutathione transferase (nmol CDNB conjugated/min/mg protein)	75 ± 24	30 ± 12
γ-Glutamyl transpeptidase (nmol *p*-nitroaniline released/min/mg protein)	14 ± 4	5 ± 2

[a] Mean ± SD of at least 10 determinations.

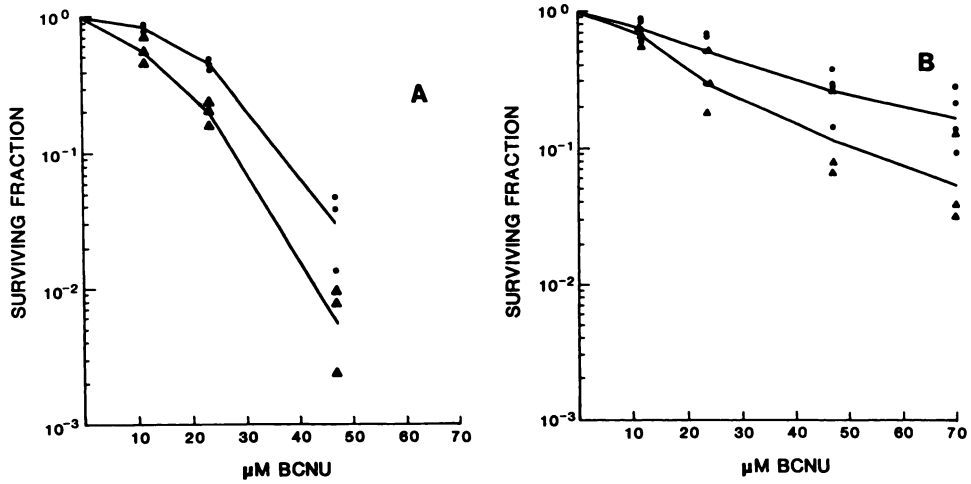

Fig. 1. Survival of 9L (*A*) and 9L-2 (*B*) cells treated for 2 h with 11.7, 23.4, 46.7, or 70.0 μM BCNU alone (●) or pretreated with 50 μM BSO for 24 h (▲). *Symbols*, mean values for single experiments.

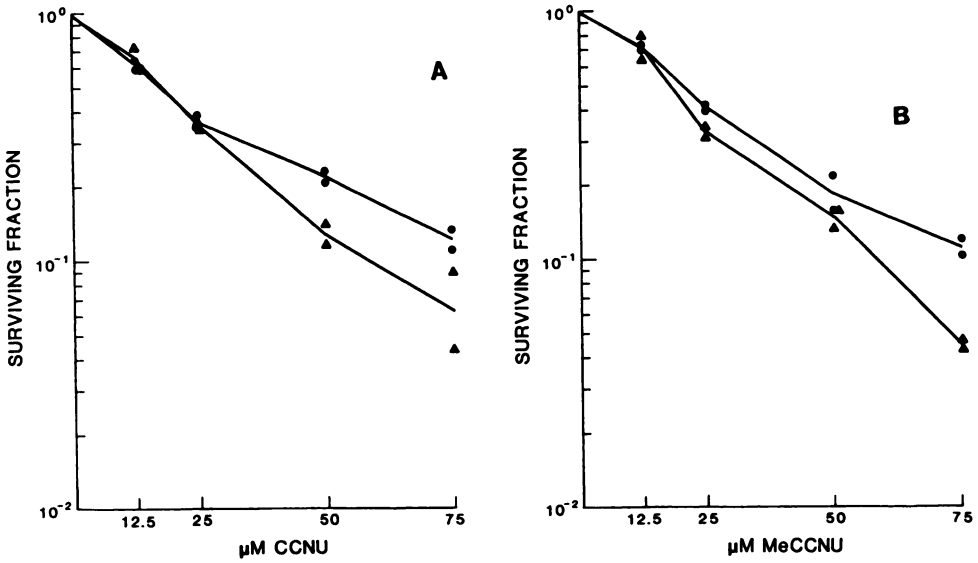

Fig. 2. Survival of 9L-2 cells treated for 2 h with 12.5 to 75 μM CCNU (*A*) or MeCCNU (*B*) alone (●) or pretreated with 50 μM BSO for 24 h (▲). *Symbols*, mean values for single experiments.

cells to nitrosoureas is therefore unlikely to be related to the concentration of GSH in these cells.

Survival curves for 9L and 9L-2 cells treated with HN2 are shown in Fig. 3, *A* and *B*. In contrast to results found for treatment with BCNU, 9L cells were approximately 2-fold more resistant to the cytotoxic effects of HN2 than were 9L-2 cells. Also, BSO pretreatment resulted in a significant potentiation of HN2 cytotoxicity. Across all doses of HN2 tested, BSO pretreatment of 9L cells produced a 2.5-log potentiation of HN2 cytotoxicity. BSO pretreatment of 9L-2 cells potentiated HN2 cytotoxicity by approximately 2 logs. In both cell lines, the combination of BSO and HN2 produced on average over 3-logs cell kill.

To contrast the effect of BSO pretreatment on HN2 toxicity, experiments were performed to determine the effect of enhanced GSH concentrations on HN2 cytotoxicity. In both 9L and 9L-2 cells, 1 mM GSH, added to the cultures just prior to HN2 treatment, provided significant protection from HN2 toxicity (Fig. 3, *A* and *B*). In order to clarify the role of the glutathione transferases in the resistance process, BCNU and HN2 were preincubated with GSH and equine liver glutathione transferase prior to their addition to the cultures (see "Materials and Methods" section). Table 3 shows that the presence of GSH and glutathione transferase had no effect on the cytotoxicity of 47 μM BCNU in 9L cells. However, 9L-2 cells sensitive to HN2 were significantly protected by all the preincubation combinations. Preincubation of 0.5 mM HN2 (4 μm final concentration) with GSH resulted in an 800-fold increase in cell survival compared with treatment with HN2 alone. The combination of GSH and glutathione transferase increased survival by 3450-fold over HN2 alone. The presence of glutathione transferases therefore decreased the cytotoxic effects of HN2 by a further 4-fold. Preincubation of HN2 with GSH and boiled glutathione transferase resulted in the cell survival being only slightly increased over survival with HN2 plus GSH (*i.e.*, 950-fold *versus* 800-fold, a 19% increase). Thus, the presence of active glutathione transferases provides additional protection against the cytotoxicity of HN2.

The SCE assay was performed in addition to the clonogenic assay as an indicator of cytotoxicity. A good correlation between SCE induction and cellular sensitivity to BCNU and HN2 has been shown previously (18, 26). Table 4 shows the number of SCEs induced by BCNU alone and with BSO pretreatment in 9L and 9L-2 cells. Four-fold higher doses of BCNU are required in 9L-2 cells in order to show SCE induction. Under these conditions, BSO pretreatment had no effect on the induction of SCEs by BCNU in either cell line. In contrast, BSO pretreatment resulted in a 27–66% increase in the number of SCEs induced by HN2 in 9L and 9L-2 cells. Furthermore, the increase in SCEs induced (ΔSCE) was dose related (Table 4). These results are in agreement with the findings in the clonogenic assay.

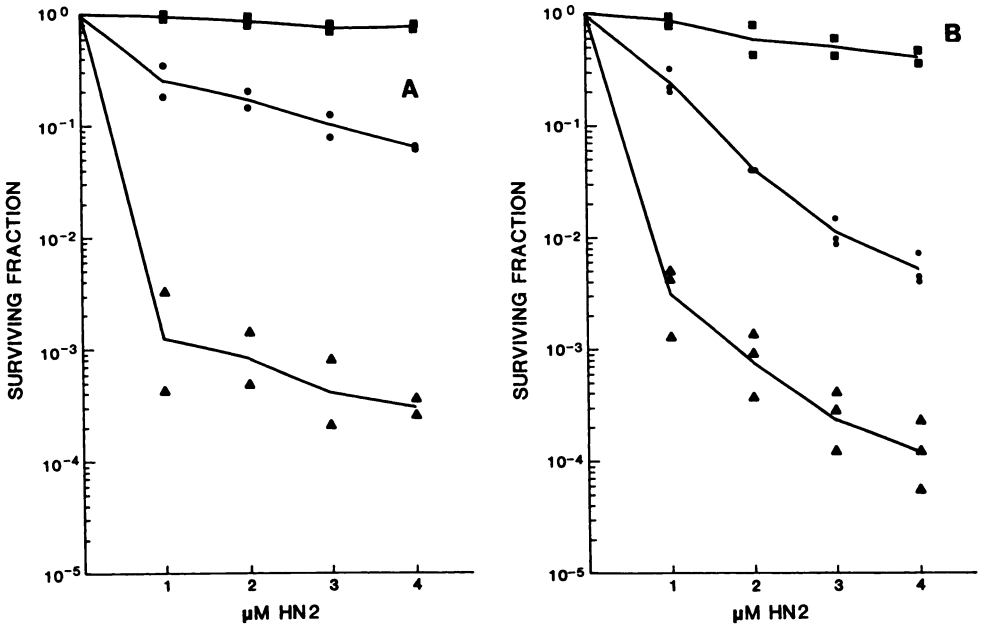

Fig. 3. Survival of 9L (*A*) and 9L-2 (*B*) cells treated for 2 h with 1–4 μM HN2 alone (●), pretreated with 50 μM BSO for 24 h (▲), or pretreated with 1 mM GSH (■) just prior to HN2 treatment. *Symbols*, mean values for single experiments.

Table 3 *Effect of preincubation with GSH and glutathione transferase on BCNU and HN2 cytotoxicity in 9L and 9L-2 cells*

BCNU and HN2 were preincubated at 37°C for 15 min with GSH (4× BCNU or HN2 concentration) and glutathione transferase (1 mg/ml) before addition to the test plates. (See "Materials and Methods" section.)

Drug	Cell line	Log cell kill: Drug alone (control)	Drug + GSH	Drug + GSH + GT[a]	Drug + GSH + boiled GT
47 μM BCNU	9L	1.41 (1)[b]	1.38 (1)	1.49 (0.8)	1.49 (0.8)
4 μM HN2	9L-2	3.69 (1)	0.79 (800)	0.16 (3450)	0.71 (950)

[a] GT, glutathione transferase (from equine liver).
[b] Numbers in parentheses, ratio of survival compared with control. The log cell kill was converted to surviving fraction ($1/10^x$) and divided by surviving fraction in the control.

Table 4 *Effect of BSO pretreatment on SCE induction by BCNU and HN2 in 9L and 9L-2 cells*

Cell line	Drug concentration (μM)	SCEs induced: −BSO	+BSO[a]	ΔSCE
BCNU				
9L	1.0	13.4 ± 7.1[b]	10.8 ± 4.5	−2.6
	2.0	21.4 ± 8.2	19.4 ± 4.1	−2.0
	4.0	38.2 ± 6.7	42.5 ± 13.2	4.3
9L-2	4.0	3.9 ± 3.1	3.5 ± 4.0	−0.4
	8.0	5.8 ± 3.0	6.5 ± 3.5	0.7
	16.0	17.9 ± 6.5	15.2 ± 5.0	−2.7
HN2				
9L	0.025	6.2 ± 5.0	8.5 ± 4.9	2.3
	0.050	10.9 ± 6.1	18.1 ± 8.4[c]	7.2
	0.100	21.6 ± 5.6	35.4 ± 16.1[c]	13.8
9L-2	0.025	15.0 ± 5.1	21.6 ± 5.7[c]	6.6
	0.050	34.5 ± 7.2	43.7 ± 9.5[c]	9.2
	0.075	47.6 ± 14.6	64.2 ± 14.8[c]	16.6

[a] SCEs induced by 24-h pretreatment with 50 μM BSO averaged 0.6–1.8. These values and background have been subtracted where appropriate.
[b] Mean ± SD of 25 determinations.
[c] Data for BSO-pretreated cells significantly different from cells not pretreated ($P < 0.05$).

DISCUSSION

9L rat brain tumor cells sensitive to BCNU but collaterally resistant to HN2 contain at least twice as much GSH, bulk glutathione transferase, and γ-glutamyl transpeptidase activities as do 9L-2 cells. Thus, an overall increase in GSH and related enzymes correlates with resistance to HN2 and not BCNU in these cells. Previous reports have shown that thiol compounds, including GSH, protect against HN2 cytotoxicity (27) and L-PAM-induced DNA alkylation (28), but there has been no direct evidence that glutathione transferases play a role in detoxifying mustard compounds. In this report we have shown that extracellular GSH protects against HN2 cytotoxicity in 9L and 9L-2 cells. In addition, we have demonstrated that HN2 incubated with GSH and glutathione transferase is significantly less cytotoxic to 9L and 9L-2 cells than HN2 incubated with GSH alone. This is the first direct evidence that the glutathione transferases contribute to the detoxification of HN2 and may explain their elevation in HN2-resistant cells. Our results suggest that in cells, HN2 can be inactivated by GSH directly or enzymatically via conjugation. Preliminary results indicate that only one arm of HN2 would have to be inactivated by GSH to dramatically reduce HN2 cytotoxicity. This conclusion is based on comparison of monofunctional and bifunctional nitrogen mustards, which shows that the monofunctional mustards are 900- to 2400-fold less cytotoxic than HN2.[4]

γ-Glutamyl transpeptidase, a membrane-bound enzyme involved in GSH metabolism, may also play a key role in cellular resistance to nitrogen mustard and other mustard compounds. This enzyme catalyzes the transfer of the γ-glutamyl moiety of GSH to amino acid acceptors which in turn are transported into the cell. As mentioned above, activity of this enzyme was almost three times higher in 9L than 9L-2 cells. This finding is in agreement with that of Ahmad *et al.* (29), who recently reported that γ-glutamyl transpeptidase was elevated 2- to 3-fold in L-PAM-resistant L1210 cells. They have postulated that this enzyme plays a key role in supplying cysteine to the glutathione precursor pool and maintaining elevated GSH levels (29), an idea supported by our data.

There is no apparent reason for the 9L-2 subline having developed lower levels of GSH, bulk glutathione transferase, and γ-glutamyl transpeptidase than the parent 9L cell line. One possibility is that 9L-2 cells may have developed a greater level

[4] K. Tokuda and W. J. Bodell, unpublished results.

of a glutathione transferase isozyme with a high specificity for BCNU. Recent results in our laboratory have shown that the inactivation of BCNU by glutathione transferase is highly isozyme dependent, being catalyzed predominantly by those isozymes containing the 4 subunit (Mu type) (30). These isozymes may be able to function very effectively at micromolar BCNU concentrations so long as GSH is not completely depleted. A Walker 256 carcinoma cell line resistant to nitrogen mustards has been found to have a different glutathione transferase isozyme profile from the parent line (12). Preliminary results in our laboratory also suggest that the glutathione transferase isozyme profiles of 9L and 9L-2 cells are different. The lack of protection against BCNU cytotoxicity by equine liver glutathione transferase in our study may therefore also be a function of isozyme profile. Final determination as to whether or not glutathione transferases are playing a role in BCNU resistance in 9L-2 cells is dependent upon quantitation of the isozymes present in 9L-2 cells, and the role of specific glutathione transferases in BCNU resistance in other tumor cell lines remains an open question. Studies are presently underway to quantitate the different glutathione transferase isozymes present in 9L and 9L-2 cells.

Decreased target availability is another possible mechanism of tumor cell resistance to chemotherapeutic agents. BCNU is an extremely potent inhibitor of glutathione reductase due to the significant carbamoylating activity of BCNU (31). The high affinity of BCNU for glutathione reductase could mean that in cells having high levels of cytoplasmic glutathione reductase, less isocyanate hydrolysis product from BCNU is available to bind to other critical cellular targets. A reduced level of glutathione reductase activity in the nitrogen mustard-resistant Walker 256 cell line is believed to be a determinate in the collateral sensitivity of this line to nitrosoureas (32). Our results show 9L and 9L-2 cells have similar levels of glutathione reductase, and thus this enzyme is not likely to be important in the resistance of 9L-2 cells to BCNU. Protein thiols, another possible target of BCNU, were also found to be at similar levels in the two cell lines. Levels of other potential targets of BCNU, including tubulin and the cation translocating ATPases, are currently under investigation.

BSO depletion of GSH in 9L and 9L-2 cells resulted in a small potentiation of BCNU cytotoxicity, but a very dramatic increase in HN2 toxicity. Several reports have previously described the potentiation of L-PAM cytotoxicity by BSO *in vitro* (14, 33, 34), and thus the result with HN2 is not surprising. Analysis of SCE induction showed that GSH depletion had very little effect on the induction of SCEs by BCNU but increased significantly the induction of SCEs by HN2. These results are in good agreement with our previous studies showing a correlation between susceptibility to the cytotoxic effects of BCNU and HN2 and the induction of SCEs by these agents. In addition, previous studies have shown that potentiation of BCNU cytotoxicity in 9L cells by pretreatment with either 6-thioguanine or difluoromethylornithine is associated with an increased level of SCE induction (35, 36).

The mechanism of increased HN2 cytotoxicity after GSH depletion is probably increased formation of DNA interstrand cross-links by HN2. This enhanced level of DNA cross-linking would also result in the observed increased level of SCE induction. This analysis is consistant with previous results obtained with 9L and 9L-2 cells (17), which demonstrated that the HN2-sensitive 9L-2 cells had a higher level of DNA cross-linking than the HN2-resistant 9L cells. Curent experiments aimed at determining whether GSH depletion results in a higher level of HN2-induced DNA cross-links in 9L and 9L-2 cells are underway.

Previous investigations of cellular resistance to BCNU in 9L-2 cells have shown that these cells have an increased repair capacity for O^6-methylguanine compared to 9L cells (18). However, the cellular repair capacity of 9L-2 cells for O^6-alkylguanine is low compared to BCNU-resistant human glioma cells (37). This originally led us to postulate that a combination of O^6-alkylguanine-DNA-alkyl-transferase and glutathione transferase may act to modify BCNU cytotoxicity in 9L-2 cells. The results of this investigation clearly show that GSH and glutathione transferase play an important role in modifying cellular cytotoxicity of HN2. Future investigations are needed to analyze the role of specific glutathione transferase isozymes in BCNU resistance. These studies will provide a better basis for understanding the biochemical mechanisms operating in cellular resistance of human tumors to treatment with chemotherapeutic agents, and may provide a biochemical rationale to modulate cellular resistance in clinical treatment programs.

ACKNOWLEDGMENTS

We thank Dr. Mark Rosenblum and Dolores Dougherty for providing the 9L cell lines and assistance with the clonogenic assay procedures. We also thank Mark Malamud for maintaining the cell lines.

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