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GLUTATHIONE AND GLUTATHIONE *S*-TRANSFERASES IN A HUMAN PLASMA CELL LINE RESISTANT TO MELPHALAN

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Abstract—We report the development of a melphalan-resistant HS-Sultan human plasma cell line. The melphalan-resistant [MEL(R)] cell line was 16.7-fold more resistant to melphalan *in vitro* than the parent cell line [MEL(S)]. The wild type and MEL(R) HS-Sultan cell lines formed localized plasmacytomas when injected into nude mice. A dose–response effect of melphalan against the drug-sensitive plasmacytomas was present *in vivo*. A dose of 10 mg/kg of melphalan, which caused a 90% regression of MEL(S) plasmacytomas, had no effect on the MEL(R) plasmacytomas *in vivo*. In contrast to previous reports, there was no increase in the levels of glutathione (GSH) in the MEL(S) and MEL(R) plasmacytomas, suggesting that the association of elevated glutathione levels and melphalan resistance may not be common to all drug-resistant lines. In the MEL(R) plasmacytomas, there was a 1.5-fold induction of a π type glutathione *S*-transferase (GST) as evidenced by isoelectric focusing (IEF) and Western blotting. This GST isoenzyme was purified and, although immunochemically similar to the π type isoenzymes induced in other drug-resistant cell lines, was noted to have different functional characteristics. These data suggest that, depending on cell type and the drug studied, functionally different GST isoenzymes may be induced and they could be of importance in the development of drug resistance.

Melphalan is an alkylating agent with good activity in a variety of malignancies such as multiple myeloma [1] and ovarian [2] and breast [3] carcinomas. More recently, it has been used at high doses to treat patients who have become refractory to standard chemotherapy regimens [4, 5]. Some success has been reported using this approach, suggesting a dose–response effect in potentially drug-resistant cell populations. Recent studies have suggested that glutathione (GSH‖) levels and enzymes related to GSH metabolism are important variables affecting melphalan cytotoxicity as well as the metabolism of melphalan [6–9]. Thus, GSH levels have been reported to be elevated in melphalan-resistant cell lines, and depletion of GSH levels by DL-buthionine-*S,R*-sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase, results in increased cell killing of drug-resistant cells by melphalan [10–12]. This strategy has been proposed as one potential method of clinically overcoming resistance to melphalan. Because of the well known activity of melphalan towards multiple myeloma, we decided to study whether some of these findings were applicable to multiple myeloma cells.

MATERIALS AND METHODS

Materials. Sources of the chemicals used in this study were similar to those described by us previously [13].

In vitro *cell lines*. A human myeloma cell line, HS-Sultan, was obtained from the American Tissue Culture Collection, Rockville, MD, and maintained in RPMI tissue culture medium containing 15% fetal serum (FCS) and 1% penicillin–streptomycin. These cells grow as colonies in suspension culture. A single cell suspension was obtained by gentle pipetting, and the cells were passaged three times a week. The original cell line was sensitive to melphalan and designated as MEL(S).

Development of drug resistance. A melphalan-resistant cell line, MEL(R), was obtained by treatment of the HS-Sultan cell line with incremental doses of melphalan. Preliminary experiments with melphalan (1.5 μg/ml) had indicated that cells in tissue culture did not recover from the lethal effects of melphalan. Therefore, initial melphalan treatment was started with 0.5 μg/ml × 3 and increased to 0.75 × 2, 1.0 × 2, 1.5 × 3 and 2.0 μg/ml after 12, 3, 7 and 8 weeks respectively. These drug concentrations would have resulted in 1–4 log cell kill based on the cell survival curve in Fig. 1. Cells surviving these drug concentrations grew rapidly and were presumed to be drug resistant. They were passaged three times per week as were the wild type cells. After 8 months,

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‖ Abbreviations: GSH, glutathione; GST, glutathione *S*-transferase; MEL(R), melphalan resistant; MEL(S), melphalan sensitive; IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis; CDNB, 1-chloro-2,4-dinitrobenzene; SDS, sodium dodecyl sulfate; BSO, DL-buthionine-*S,R*-sulfoximine; and FCS, fetal calf serum.

cell survival assays (Fig. 1) confirmed the presence of significant drug resistance *in vitro*. The MEL(R) cells were maintained melphalan resistant by treatment with melphalan (2 μg/ml) every 2 weeks. If the cells were left untreated with drug for an 8-week period, the MEL(R) cells would revert to a drug-sensitive state. The morphology of the MEL(R) and MEL(S) cells was that of plasmablasts. The cell sizes of the two cell lines appeared to be similar morphologically. After establishing that these MEL(R) cells were significantly resistant to melphalan *in vitro*, the MEL(R) cells were then injected into nude mice for demonstration of *in vivo* resistance to melphalan.

Cell survival assay. For each experiment, a fresh 100–1000 × concentration of melphalan was dissolved in 0.1 N HCl. This was then diluted with tissue culture medium. Addition of melphalan solution did not alter the pH of the final tissue culture medium used in the cytotoxicity experiments. Relative drug sensitivity was tested by incubating melphalan with the cells at a density of 5×10^5/ml at 37° for 1 hr in RPMI medium containing 15% FCS. After washing the drug out, the cells were plated in 0.3% agarose, RPMI medium containing 15% FCS in 35 mm Petri dishes for colony formation. The same protocol was used for induction of drug resistance as outlined above except that cells were incubated in tissue culture flasks. The Petri dishes containing cells for cell survival assays were incubated in a humidified atmosphere containing air and 5% CO_2 at 37° for 1 week. Aggregates of more than 50 cells were scored as colonies. Control plating efficiencies were 10% for both MEL(S) and MEL(R) cell lines. The ID_{50} was defined as the dose of the drug resulting in a 50% decrease in cell survivial.

Response in nude mice. Cells (1×10^7) were injected s.q. into male Balb/C nude mice (Life Sciences, St Petersburg, FL). The nude mice were housed in the Nude Mouse Facility, Cancer Center, The University of Texas Medical Branch, Galveston. The characteristics of the localized, subcutaneous plasmacytomas will be reported elsewhere (manuscript in preparation).

Tumor volume was calculated using the formula [14]:

$$V = \frac{\pi(\text{mean diameter})^3}{6}$$

where V is the volume. The data are plotted as the mean relative tumor volume from the start of drug treatment. MEL(S) tumors (fourth transplant generation) were treated with melphalan (5 and 10 mg/kg, i.p., × 1) when the mean tumor volumes were 1.23 ± 0.33 and 1.34 ± 0.44 cc^3, respectively. MEL(R) tumors (first transplant generation) were treated with melphalan (10 mg/kg, i.p. × 1) when the mean tumor volume was 0.32 ± 0.06 cc^3. Each experiment was repeated at least once to ensure reproducibility.

Preparation of tumor supernatant fractions. The tumor tissues were homogenized using a PT 10–35 Polytron (Kinematica GmbH, Litau), and a 10% (w/v) homogenate was prepared in10 mM potassium phosphate, pH 7.0, containing 1.4 mM 2-mercaptoethanol. For acid-soluble thiol determination, the tumor tissue was homogenized in 10 mM potassium phosphate, pH 7.0. The homogenate was centrifuged at 14,000 *g* for 40 min.

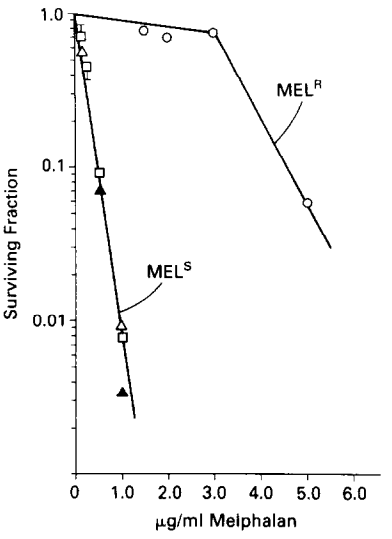

Fig. 1. Sensitivity profiles of MEL(S) and MEL(R) cell lines. Key: (□) MEL(S) cell line; (△) cells from the plasmacytomas in the sixth transplant generation; (▲) cells from the plasmacytomas in the twelfth transplant generation; and (○) MEL(R) cells. Points are means ± SE. Error bars are shown for three or more experiments and if larger than symbols.

Enzyme assays. Glutathione *S*-transferase (GST) activity towards different substrates was determined according to the method of Habig *et al.* [15]. GSH peroxidase and GSH reductase activities were determined according to the procedures described by Awasthi *et al.* [16] and Beutler [17] respectively. NADPH-cytochrome *c* reductase, ethoxycoumarin-O-deethylase, and benzo[*a*]pyrene hydroxylase activities were determined according to the methods of Hrycay *et al.* [18], Greenlee and Poland [19], and Nebert and Gelboin [20] respectively. The acid-soluble thiol content was determined according to the method of Beutler *et al.* [21]. Protein content was determined according to the method of Bradford [22].

Isoelectric focusing (IEF). The IEF of 14,000 *g* supernatant fractions of 10% (w/v) homogenate of tumors grown in nude mice was carried out in an LKB 8100-1 IEF column in a 0–50% sucrose density gradient with Ampholines in the pH range 3.5 to 10. After IEF at 1600 V for 18 hr, fractions of 0.8 ml were collected and monitored for pH and GST activity using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

Purification of GST from MEL(R) tumors. The GST from MEL(R) tumors was purified according to the protocol described previously [23]. The individual isoenzymes of GST were separated by IEF of the affinity purified GST [23].

Immunological studies. The antibodies against GST π of human placenta were the same as used by us previously [24]. The immunoblotting (Western

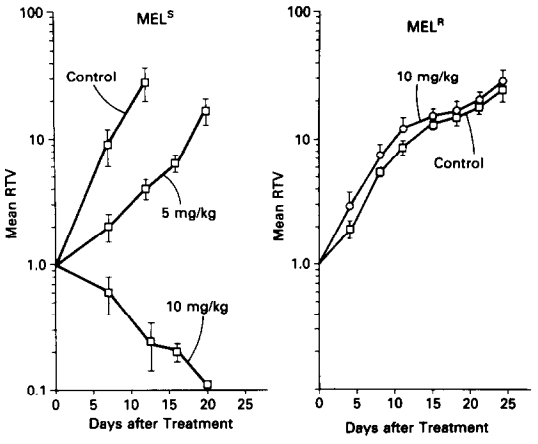

Fig. 2. *In vivo* response of tumor from MEL(S) and MEL(R) lines to melphalan during the third and first transplant generations respectively. Points are the means ± SE of three to ten tumors. RTV = relative tumor volume.

blotting) was performed according to the procedure of Towbin *et al.* [25] with slight modifications as described previously [13].

RESULTS

The data in Fig. 1 indicate that the wild type HS-Sultan cell line is extremely sensitive to melphalan *in vitro*. There was a 16.7-fold difference in the ID_{50} between the MEL(S) and MEL(R) cell lines. Both the MEL(S) amd MEL(R) cells when injected into nude mice formed localized subcutaneous plasmacytomas that were indistinguishable from each other in cell morphology or size. When cells from the MEL(S) plasmacytomas in the sixth (open triangles, Fig. 1) and twelfth (closed triangles, Fig. 1) generations were tested for *in vitro* sensitivity to melphalan, the data points indicate that the inherent drug sensitivity to melphalan had not changed on serial passage. These data argue against the selection of a more melphalan-sensitive cell population *in vivo*. The MEL(S) plasmacytomas were treated first with melphalan *in vivo* when the tumor volumes were 1.2–1.3 cc^3 in size (Fig. 2). The data indicate that (1) there was a dose response to melphalan, and (2) at a dose of 10 mg/kg of melphalan there was a 90% regression of *large* size tumors [14]. Because it is generally accepted that small size tumors respond better to chemotherapy drugs than larger size tumors, we reasoned that the data would be more significant if we could demonstrate *in vivo* resistance to melphalan of the MEL(R) plasmacytomas at a small tumor volume. The MEL(R) tumors were therefore treated with melphalan at 10 mg/kg when their tumor volume was 0.3 cc^3. Figure 2 shows that there was no regression of the *small* MEL(R) plasmacytomas *in vivo*, indicating a high degree of drug resistance *in vivo* to melphalan. These data clearly indicate that a 16.7-fold melphalan resistance observed *in vitro* with the MEL(R) cell line was sufficient to result in complete melphalan resistance *in vivo*.

The growth rate of serially transplantable tumors is highly passage dependent. In general, the growth rate increases with passage number [14]. The experiments shown with MEL(S) tumors were performed in the fourth transplant generation while the MEL(R) tumors were treated in the first transplant generation. These biological differences may account for the fact that the doubling time of the MEL(S) tumors (2.5 days) was shorter than that of the MEL(R) tumors (4.5 days). Since slowly growing tumors (i.e. longer doubling time) may respond to a lesser extent to chemotherapy, it is possible that this may have contributed to the unresponsiveness of the MEL(R) tumors to melphalan. However, in a subsequent study, when the same experiment was repeated with MEL(R) plasmacytomas in the second transplant generation, the tumor doubling time was 2 days; the MEL(R) tumors were still resistant to melphalan treatment *in vivo*. Therefore, biological factors such as doubling time cannot alone explain the observed resistance to melphalan *in vivo* with the MEL(R) plasmacytomas.

The resistance to melphalan *in vivo* of MEL(R) plasmacytomas was stable through the second transplant generation. Cell survival studies performed from cells of the MEL(R) plasmacytomas from the third transplant generation indicated that the MEL(R) cells had reverted to a melphalan-sensitive stage (data not shown). This is consistent with the stability of the *in vitro* MEL(R) cell line which is not periodically exposed to melphalan. These data as a whole suggest that melphalan treatment *in vitro* and *in vivo* is necessary for continued expression of melphalan resistance for the HS-Sultan plasma cell line. The instability of MEL(R) tumor and cell line in the absence of melphalan exposure is similar to that described in some other reports of clinically and experimentally induced drug resistance [26, 27].

We have not performed cross-resistance studies to other alkylating agents with MEL(R) plasmacytomas or cell lines. However, the MEL(S) plasmacytomas are resistant *in vivo* to maximally tolerated doses of *cis*-platinum and to the nitrosourea, 1,3-bis-(2-chloroethyl)-1-nitrourea (BCNU) (V Gupta, unpublished observations).

The acid-soluble thiol content was determined in 14,000 *g* supernatant fractions of MEL(S) tumors and MEL(R) tumors (Table 1). The acid-soluble thiol (GSH) content of MEL(R) tumors was found to be slightly lower than the GSH content of MEL(S) tumors.

The GSH-related enzymes were also determined in MEL(S) and MEL(R) tumors (Table 1). GSH reductase activity was approximately 1.3-fold higher in MEL(R) tumors when compared to that in MEL(S) tumors. The GST activity towards CDNB was also elevated about 1.5-fold in MEL(R) tumors. It is well documented that multiple GST isoenzymes arising from binary combinations of a number of distinct subunits belonging to three major classes, α, μ and π, are present in mammalian tissues [28]. The affinities of these isoenzymes towards different substrates depends on the type of subunits which form the dimeric holoenzyme [28, 29]. For example, the μ class subunits have comparatively higher activity towards *p*-nitrobenzyl chloride and subunits

Table 1. Thiol content and activities of GSH *S*-transferase and GSH reductase in tumors grown in nude mice from MEL(S) and MEL(R) plasmacytomas

Enzyme activity and thiol content	MEL(S)	MEL(R)
Acid-soluble thiol content (μmol/mg protein)	0.0329 ± 0.004	0.0287 ± 0.001
Glutathione reductase (units/mg protein)	0.0735 ± 0.010	0.0976 ± 0.016
Glutathione *S*-transferase (units/mg protein)		
CDNB	0.0770 ± 0.004	0.1184 ± 0.008
p-Nitrobenzyl chloride	0.0039 ± 0.001	0.0035 ± 0.001
Ethacrynic acid	0.0039 ± 0.0008	0.0073 ± 0.002

Values are means ± SD of three determinations. One unit of glutathione reductase is defined as the amount which reduces 1 μmol of oxidized glutathione utilizing 1 μmol of NADPH/min at 37°. One unit of GST is defined as the amount required to catalyze the formation of 1 μmol of GSH conjugate/min at 25°.

of the π class isoenzymes show maximum activity with ethacrynic acid [28, 30]. We, therefore, determined the GST activities towards these substrates in MEL(R) and MEL(S) tumors to investigate if any specific class subunits of GST were affected by melphalan. Whereas GST activities towards *p*-nitrobenzyl chloride were similar in both tumors, the activity towards ethacrynic acid in MEL(R) tumors was 1.9-fold higher compared to that in MEL(S) tumors. Since ethacrynic acid is a preferred substrate for π class isoenzymes, increased activity towards this substrate in MEL(R) tumors indicates elevation of π type GST isoenzyme in MEL(R) tumors. GST activity was also measured in the MEL(S) and MEL(R) cell lines in culture. Specific activities of GST using CDNB as substrate were 0.0575 ± 0.002 (N = 2) and 0.067 ± 0.001 (N = 2) for MEL(S) and MEL(R) cell lines, respectively, indicating a mild induction of GST in the resistant cells. This is consistent with the results obtained from tumor tissues.

To examine whether the treatment with melphalan *in vivo* had any effect on GSH content or the levels of related enzymes in MEL(R) and MEL(S) tumors in nude mice, we determined these variables in the tumors of mice treated with melphalan and in the tumors of mice not treated with melphalan *in vivo*. The GSH contents and the activities of GSH-related enzymes in MEL(R) tumors treated with melphalan (Table 1) and those not treated with melphalan but melphalan resistant in the first and second passages (data not presented) were found to be similar. These data indicate that *in vivo* treatment with melphalan had no effect on GSH and GSH-related enzyme activities of MEL(R) tumors in the first and second passages. However the MEL(R) plasmacytomas that were not drug treated in the third passage reverted to a melphalan-sensitive stage, and the GST activities in these tumors returned to wild type levels.

Preferential induction of GST π in MEL(R) tumors from the first and second passages *in vivo* was also indicated by the results of IEF studies with 14,000 *g* supernatant fraction of 10% (w/v) homogenates prepared from tumors. When the supernatant fractions containing equal amounts of protein were subjected to IEF, one major (pI 4.7) and two

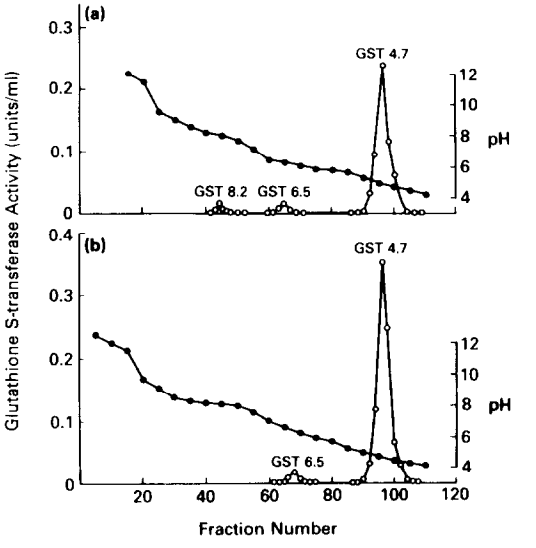

Fig. 3. IEF profile of GST in 14,000 *g* supernatant fractions of tumor grown from (a) MEL(S) and (b) MEL(R) plasmacytomas. Experimental details are given in the text. Key (●) pH; and (○) GST activity with CDNB as the substrate.

minor peaks (pI 8.2 and 6.5) of GST activity were observed in the IEF profiles of MEL(S) tumors (Fig. 3). On the other hand, in the IEF profiles of MEL(R) tumor supernatant fractions, the GST activity peak at pH 8.2 was absent, indicating possible suppression of a cationic GST isoenzyme in MEL(R) tumor. The enzyme activity peak corresponding to pH 4.7 was found to be elevated about 1.5-fold in the IEF profile of MEL(R) tumors, indicating the induction of this isoenzyme. The GST isoenzyme having pI 4.7 (GST 4.7) obtained from both MEL(R) and MEL(S) tumors cross-reacted with antibodies raised against GST π of human placenta, indicating it to be immunologically similar to GST π. To determine the functional relationship of GST 4.7 of MEL(R) tumor with GST π of human placenta, we purified GST 4.7 from MEL(R) tumor. The specific activities of GST

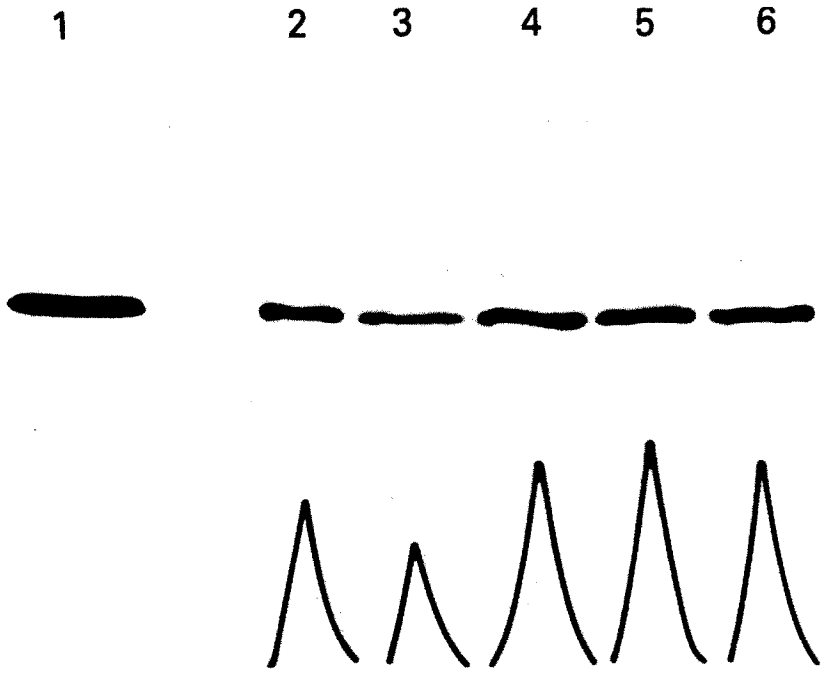

Fig. 4. Immunoblot analysis of GST in 14,000 *g* supernatant fractions of MEL(S) and MEL(R) tumors using antibodies raised against GST-π of human placenta. Lane 1 contained 5 μg of homogenous preparation of GST-π of placenta. Lanes 2 and 3 contained aliquots of supernatant fractions (10 μg protein) from two different MEL(S) tumors. Lanes 4 to 6 contained aliquots of supernatant fractions (10 μg protein) from three different MEL(R) tumors. The densitometer scanning of bands in Lanes 2–6 is shown below the bands.

Table 2. Activities of phase I drug-metabolizing enzymes in supernatant fractions of tumors grown in nude mice from MEL(S) and MEL(R) plasmacytomas

Enzyme	MEL(S)	MEL(R)
NADPH-cytochrome *c* reductase (μmol/mg protein)	0.0024 ± 0.0002	0.0033 ± 0.0005
Aryl hydrocarbon hydroxylase (pmol/mg protein)	0.087 ± 0.003	0.052 ± 0.011
Ethoxycoumarin-*O*-deethylase (pmol/mg protein)	0.1381 ± 0.016	0.1427 ± 0.015

Values are means ± SD of three determinations.

4.7 towards different electrophilic substrates were similar to those reported for GST π of human placenta. GST 4.7 did not express GSH peroxidase II activity which indicates functional differences between this π type enzyme and the π type GST reported to be induced in a multi-drug-resistant breast cancer cell line [31].

Western blotting experiments were performed (Fig. 4) to further examine whether or not the observed increase in GST activity of MEL(R) tumors (first and second passages) was due to the induction of a π type GST isoenzyme. Aliquots containing 10 μg protein from tumor supernatant fractions were subjected to urea/SDS/PAGE, and Western blots were developed with antibodies raised against GST π of human placenta. In Western blots (Fig. 4), the intensity of bands from MEL(S) tumors was less when compared to that of MEL(R) tumors as evidenced by densitometer scanning of bands, indicating the presence of an increased amount of GST π type antigen in the MEL(R) tumors. Western blot studies with the MEL(R) and MEL(S) cell lines in culture which were used to develop tumors in the nude mice also yielded similar results (data not presented). The results of Western blot analysis correspond to the enzyme activity and IEF data and indicate that the increased GST activity in MEL(R) tumor was due to an increase in a π type isoenzyme. This induction of GST π isoenzyme was further supported by the observation that MEL(R) plasmacytoma from the third transplant generation which had reverted to a melphalan-sensitive state in

the absence of continued exposure to melphalan *in vivo* had GST π type isoenzyme levels similar to those of the wild type MEL(S) plasmacytomas (data not shown).

We also determined the activities of some phase I drug-metabolizing enzymes in the MEL(S) and MEL(R) tumors (Table 2). The NADPH-cytochrome *c* reductase activity in MEL(R) tumors was slightly higher than that in MEL(S) tumors, whereas insignificant change in the ethoxycoumarin-*O*-deethylase activity was observed between the MEL(R) and MEL(S) tumors. However, the aryl hydrocarbon hydroxylase activity was found to be suppressed markedly in MEL(R) tumors.

DISCUSSION

Intracellular levels of thiols, which include both non-protein glutathione and protein bound thiols are known to influence the response of cells sensitive and resistant to melphalan or other nitrogen mustards and alkylating agents [6,8–12, 32, 33]. This effect has been demonstrated for a wide variety of murine and human carcinoma cell lines. Some investigators have reported that cellular thiols may also influence cell killing by other agents such as *cis*-platinum [33]. In the latter case it was reported that a *cis*-platinum-resistant squamous cell line was found to have a 2-fold increase in protein sulfhydryl content but no increase in the free sulfhydryl content when compared to the *cis*-platinum-sensitive cell line. In general, a 2- to 3-fold increase in GSH levels in melphalan-resistant cells has been reported. Conversely, depletion of GSH levels by nutritional deprivation or chemical means, as with BSO, has been shown to result in increased cytotoxicity to melphalan for both sensitive and resistant cells [9–11]. In addition, it is suggested that the elevated GSH levels in drug-resistant cells may convert melphalan to an inactive compound more efficiently [7, 10]. The formation of GSH-melphalan conjugate catalyzed by GST has been demonstrated recently [34]. Thus, both elevated GSH and GST levels may contribute to drug resistance to melphalan. Consistent with this finding is the reported decrease in DNA crosslinks formed in a Chinese hamster cell line resistant to nitrogen mustard with cross-resistance to melphalan and elevated GST levels [35, 36]. In most of the reports of elevated GSH levels in melphalan drug-resistant cells, there were no significant changes in the uptake or efflux of melphalan, suggesting that the elevated GSH levels may have been the major cause of drug resistance to melphalan. This was also true for human ovarian multi-drug-resistant cells but not Chinese hamster cells [37]. Our data suggest that the association of melphalan-induced drug resistance with elevated GSH levels may not be common to all cell lines and implies that the mechanism(s) of drug resistance to melphalan in the human plasma cells may be different from that of human carcinoma cells. These data do not negate the possibility that varying levels of GSH may influence cell resonse to melphalan. Whether or not the present findings are applicable to other human myeloma cells should be explored because of the potential therapeutic implications.

The role of GST, particularly GST π or GST-P, has received much attention recently because of several reports on the elevated levels of this type of GST in drug-resistant tumor cells as well as in chemically induced preneoplastic nodules [31, 38–40]. A 45-fold increase in GST π has been demonstrated in an MCF-7 breast cancer cell line selected for adriamycin resistance [31] with a strong correlation between the extent of resistance and the elevation of GST π. Transfection of this GST π gene into drug-sensitive cells resulting in a 10-fold increase in GST π activity produces a 1.5-fold increase in resistance to melphalan and BCNU [41]. Therefore, the small increase (1.5-fold) in GST π activity in the present study may not be quantitatively sufficient to explain the high degree (17-fold) of melphalan resistance. These GST π type isoenzymes although immunochemically similar (antibodies used by Batist *et al.* [31] were supplied by this laboratory) appear to have functional differences. For example, GST π isoenzyme isolated from the MEL(R) tumors did not express GSH peroxidase II activity, whereas the one from the MCF-7 Adriamycin®*-resistant cells has very high activity [31]. Whether or not these functional differences are sufficient to explain melphalan resistance in the Sultan plasma cells is unknown. The induction of this GST π isoenzyme and its potential role in melphalan resistance are consistent with our observation that, when the MEL(R) plasmacytomas revert to a drug-sensitive state, the levels and activity of this GST π isoenzyme return to the level observed with wild type, MEL(S) plasmacytomas. It is of some interest that Walker 256 rat mammary carcinoma cells with acquired resistance to chlorambucil [42], an alkylating agent similar to melphalan, are accompanied by the induction of another class (Yb type subunit of μ class) of GST isoenzyme rather than the GST π class or GST-P. However, upon purification of the GSTs from the same drug-resistant Walker 256 cells, overexpression of one GST having Yc type subunits was observed [43].

The decrease in aryl hydrocarbon hydroxylase activity indicates a possible suppression of this phase I enzyme. Cowan *et al.* [44] have studied a human breast cancer cell line, MCF-7, selected for Adriamycin® resistance and have reported a decrease in the phase I enzyme activities in resistant cells, when compared to the sensitive cells, with a simultaneous increase in GST activity.

The results of the present study in conjunction with other studies [42, 43] suggest that, depending upon the origin of the cell line and the nature of the chemotherapeutic agent, both the GSH levels and GST isoenzymes are differentially affected during the development of drug resistance. This may be one of the mechanisms by which cells acquire drug resistance. However, other factors including increased drug efflux or decreased nuclear binding may also be contributing towards the development of drug resistance. Extensive studies on the levels of GSH, the patterns of GST isoenzymes, and alternate mechanisms of melphalan resistance should, therefore, be conducted in a number of cell lines of

* Adriamycin is a registered trademark of Farmitalia Carlo Erba.

different origins with acquired drug resistance to various chemotherapeutic agents. Such studies will help immensely in understanding the role of glutathione-mediated detoxification mechanisms in the acquired resistance to chemotherapy.

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