The relationship between tumour glutathione concentration, glutathione S-transferase isoenzyme expression and response to single agent carboplatin in epithelial ovarian cancer patients

S Ghazal-Aswad1, L Hogarth2, AG Hall3, M George3, DP Sinha3, M Lind1, AH Calvert1, JP Sunter4 and DR Newell1

1Cancer Research Unit and 2Leukaemia Research Fund Unit, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE2 4HH; 3Regional Department of Gynaecological Oncology and 4Department of Pathology, Queen Elizabeth Hospital, Gateshead NE9 6SX UK.

Summary There is evidence to suggest that glutathione (GSH) and glutathione-S-transferases (GST) are important factors in determining sensitivity to cytotoxic drugs in vitro and in preclinical in vivo model systems. To define the relationship between tumour GSH concentration, GST isoenzyme expression and response to carboplatin in epithelial ovarian cancer (EOC), tumour samples from 39 patients with assessable disease after primary surgery were analysed for GSH content and GST expression. Response was assessed using six courses of single agent carboplatin therapy. GSH was measured by high performance liquid chromatography (HPLC) in fresh tumour samples taken at primary laparotomy. GST isoenzyme expression was assessed by immunohistochemistry of fixed tumour material using antibodies specific for ξ, η and μ classes. GST isoenzyme expression was defined as positive if the staining intensity was strong and more than 10% of tumour cells were involved. The mean GSH concentrations were: 831 ± 4496, 7211 ± 5026, 6559 ± 4573 and 3758 ± 1885 (nmol g⁻¹ tissue dry weight mean ± s.d.) for tumours from patients who subsequently achieved a complete response (CR, n = 18), partial response (PR, n = 10) or who had static disease (SD, n = 7) or progressive disease (PD, n = 4) respectively. There was no relationship between GSH concentration and response (ANOVA, P = 0.32). There were also no relationship between GST isoenzyme expression and response to carboplatin in both the concentration of GSH or expression of GST isoenzymes and response to single agent carboplatin in primary previously untreated EOC.

Keywords: glutathione; glutathione S-transferase; carboplatin; resistance; ovarian cancer

Single agent carboplatin therapy after primary surgery has been standard treatment in the Northern Region (UK) for patients with advanced epithelial ovarian cancer (EOC stages Ic–IV). A meta-analysis indicates that 56% of patients with advanced ovarian cancer will respond to single agent carboplatin as first-line therapy, suggesting that the remainder have tumours which are intrinsically resistant (Rozenwieg et al., 1990). Of the patients who do respond to first-line carboplatin, some 60% will subsequently relapse and hence acquired resistance is also a significant problem. Resistance to platinum complexes is multifactorial and includes: decreased accumulation of drug, increased intracellular detoxification, which can involve glutathione (GSH), glutathione S-transferases (GSTs) and metallothioneins, increased DNA repair and increased tolerance of unrepaired DNA damage. Notably, several mechanisms may operate concomitantly (De Graeff et al., 1988). Furthermore, essentially all of the data on resistance to platinum compounds are derived from cell lines or experimental animal systems, and it is uncertain whether the mechanisms defined in these model systems are relevant to the clinical setting.

Of all the mechanisms implicated in preclinical studies, GSH and its metabolism is the one most commonly identified in resistance to cisplatin and carboplatin. GSH is tripeptide, gamma-glutamylcysteinylglycine, and is the most abundant non-protein thiol found in the cell. GSH is present in millimolar concentrations and is found throughout the cell, with the bulk in the cytoplasm; subcellular particles, such as the nucleus and mitochondria, having smaller amounts (Biaglow and Tuttle, 1993). GSH plays a role in the detoxification and repair of cell injury induced by a wide range of toxic agents which include cytotoxic drugs, radiation and hyperthermia (Biaglow and Tuttle, 1993). GST functions primarily through nucleophilic thioether formation and peroxidation/reduction reactions. The exact mechanism by which GSH decreases platinum complex cytotoxicity is not well established; but the following possibilities have been proposed (reviewed by De Graeff et al., 1988) alteration of platinum complex transport, drug inactivation through the formation of an inactive GSH–Pt complex, decreased binding of platinum to DNA and increased DNA repair. More recently data have been published which indicate that cisplatin may be extruded from cells as a glutathione conjugate, and that increased expression of the GS-X pump may be associated with drug resistance (Ishikawa and Alim-Osman, 1993; Ishikawa et al., 1994).

The cytosolic GSTs are a multigene family of enzymes that exist as homo- and hetero-dimers each of which have a molecular weight of between 21 and 28 kDa (Mantle, 1990; Gullick and Fahl, 1995). Four different classes of enzyme have been described, ξ, μ, η and θ which differ in immunoreactivity. Monomers of one class will not dimerise with those of another. Within each class there may be several different isoforms which share amino acid homology but may differ significantly in substrate specificity. GSTs have been shown to detoxify a wide range of xenobiotics (reviewed by Ciaccio and Tew, 1993), including cytotoxic drugs, through both the catalysis of glutathione conjugate formation (Hall et al., 1994a; Bolton et al., 1991) and by sequestration (Meyer et al., 1992).

Although there is evidence from studies of both cell lines and clinical material that increased expression of GSTs and raised levels of GSH may be associated with resistance to cytotoxic drugs, the relationship between response and
expression in patients remains unclear. The aim of this study was to relate response to single-agent carboplatin to tumour levels of GSH and GST in EOC.

Materials and methods

Patients and tumour samples

EOC tumour samples were collected from 48 patients. Of these, only 39 patients were included in the current analysis. The remainder either had no residual disease after primary laparotomy and hence were non-assessable for response to chemotherapy (six patients) or GSH concentrations that varied by more than 2-fold between different tumour pieces (three patients). Samples (median = 1 and range 1–5 samples from each patient) were collected immediately after primary laparotomy, stored in liquid nitrogen within 10 min and analysed in batches. The histology of the 39 tumour samples analysed was reported as follows: adenocarcinoma in 12 cases (1 moderately and 11 poorly differentiated), serous cystadenocarcinoma in 12 (one well, seven moderately and four poorly differentiated), clear cell carcinoma in five (two well, two moderately and one poorly differentiated), endometroid carcinoma in seven (two well, three moderately and two poorly differentiated) and mucinous cystadenocarcinoma in three cases (one well, one moderately and one poorly differentiated). There was no significant trend for the relationship between tumour grade and response (chi-squared test P = 0.87) and too few samples to study the relationship between response and histological subtype.

Chemotherapy

All patients were treated with carboplatin as a single agent and the dose was calculated either according to the surface area (i.e. 400 mg m\(^{-2}\) in 13 patients) or according to renal function with a target area under the plasma concentration vs time curve of 7 mg ml\(^{-1}\) min\(^{-1}\) (Calvert et al., 1989; in 26 patients). Response was assessed after completing six courses of single-agent carboplatin and classified as a response (complete or partial) or non-response (static or progressive disease) according to standard criteria (UICC).

Chemicals

Chemicals were obtained from Sigma Chemical Co. Ltd. (Poole, Dorset, UK), Aldrich Chemical Co. (Gillingham, Dorset, UK) and BDH Chemical Co. (Poole, Dorset, UK).

Sample preparation for GSH measurements

Tumour tissue was removed from storage in liquid nitrogen and cut while frozen into two sections. The first section was weighed wet and then dried in a heating block at 50°C until the weight was stable. The dry weight was recorded and the wet weight to dry weight ratio was calculated. The second tissue section was ground in liquid nitrogen using a pestle and mortar. Once ground, the fragments were collected into a cooled Teflon-glass homogeniser and homogenised in 25 volumes of 6.5% (w/v) TCA. Three 450 \(\mu l\) samples of the homogenate were removed. An aliquot of 50 \(\mu l\) 0.5 mM GSH was added to the first of these aliquots and 50 \(\mu l\) 1.0 mM GSH to the second. An addition 50 \(\mu l\) of 6.5% (w/v) TCA was added to the third aliquot. After adding the GSH solutions, the samples were mixed and centrifuged (10 000 \(g\), 12 min, 4°C) using a Biofuge 15 (Heraeus Sepatech, Germany). An aliquot of 75 \(\mu l\) of 1 M potassium hydrogen phosphate was mixed with 75 \(\mu l\) of the supernatant, 150 \(\mu l\) of monobromobimane (mBrB) reagent was added (see below) and, after gentle mixing, the sample incubated at room temperature for 5 min in the dark. The reaction was stopped with 15 \(\mu l\) of 100% (w/v) TCA and the samples were kept at −80°C until analysed by HPLC within 2 weeks of preparation.

High performance liquid chromatography measurement of GSH in tumour samples

The method used was based upon the use of mBrB and is a modification of the one described by Cotgreave and Moldes (1986). This method results in the maximal recovery of both free and low molecular weight thiols from the tissue and, by virtue of the HPLC separation used, gives specific detection of GSH.

Monobromobimane reagent and standard curve preparation

The reagent used was 8 mM monobromobimane (Calbiochem, Nottingham, UK) in 50 mM N-ethylmorpholine (NEM). To prepare 25 \(\mu l\) of the mBrB reagent, 158 \(\mu l\) NEM was added to 23 ml deionised water in a 30 ml glass tube. MBrB (54.25 mg) was thoroughly dissolved in a minimum amount of acetonitrile, the solutions of NEM and mBrB were then mixed, and pH adjusted to 8.0, using 1 M hydrochloric acid, and then made up to 25 ml in a volumetric flask with deionised water.

For preparation of the GSH standard curve, eight different standard solutions were prepared (2.5, 5, 10, 20, 40, 60, 80 and 100 \(\mu M\) of GSH). GSH (64.53 mg) was dissolved in 50 ml of phosphate-buffered saline (PBS), giving a concentration of 4 mM GSH. A 0.25 ml aliquot of the solution was diluted up to 10 ml in a volumetric flask, giving a total concentration of 100 \(\mu M\) of GSH which was diluted serially to give the concentrations indicated above. Fifty \(\mu l\) of 30 mM dithiothreitol (DTT), made up fresh every day in PBS, was added to 1 ml of each of the above standard solutions and the standards were then left for 30 min at room temperature. MBrB reagent (130 \(\mu l\)) was mixed with 130 \(\mu l\) of each of the DTT-treated GSH standards and left in the dark for 5 min, after which 13 \(\mu l\) of 100% (w/v) TCA was added. These final solutions were analysed by HPLC without any further dilution.

To assess the reproducibility of the assay, two different solutions of GSH (5 and 80 \(\mu M\) in PBS) were prepared. These quality control standards were aliquoted and stored at −80°C. An aliquot of each concentration was analysed with each assay.

HPLC apparatus

Analysis of GSH was performed using a Waters model 625 low-pressure mixing gradient HPLC system fitted with a model 470 fluorescence detector (Waters, Watford, UK). Samples were injected using a Waters model 712 Wisp auto-injector and the results analysed using Millipore Maxima chromatography software.

HPLC conditions

The GSH mBrB adduct was analysed using a 5 \(\mu m\) octadecylsilica (ODS) reverse-phase column (4.6 mm inside diameter x 15 cm length; 4M15115, Jones Chromatography, Hengoed, UK). The solvent flow rate was 1 ml min\(^{-1}\), giving a back pressure of less than 2000 p.s.i. The GSH peak (retention time 11.1 min) was measured by a fluorescence detector (excitation 394 nm, emission 480 nm). Chromatography was performed using a gradient of 10% (v/v) acetonitrile, 0.25% (v/v) acetic acid in water against 75% (v/v) acetonitrile, 0.25% (v/v) acetic acid. Table I gives the gradient conditions used for the HPLC separation of the GSH mBrB adduct.

Calculation of final GSH concentration per gram of tissue dry weight

In this method, maximum efforts were made to minimise and correct for any loss of GSH during the process of the tissue collection or processing. The samples were collected in less than 10 min after removal of the tumour from the pelvis and selection of the tumour samples was performed by an experienced pathologist (JPS). The tumour tissue was handled and ground in liquid nitrogen before homogenisation with 6.5% TCA. A method of additions analysis was used, i.e. the addition of 0, 0.5 and 1.0 mM GSH to each tumour sample homogenate, so that recovery of GSH could be calculated for each individual sample and any loss
of GSH during processing compensated for. Figure 1 shows the result for the method of addition in one patient sample. The GSH concentration is given by the extrapolated x-axis intercept. The recovery is given by the slope of the line which was fitted by unweighed linear regression analysis \( (r^2 > 0.99 \text{ in all cases}) \). When possible (18 patients) more than one sample \((2–5)\) was collected from different locations within the tumour. After measurement of GSH concentration in each sample separately, the mean value for all of the samples was used as the GSH concentration value for the patient, unless there was a wide variation \((>2\text{-fold})\), in which case the patient was considered un evaluable for GSH determination. The original GSH content of the tumour tissue was expressed per gram of tissue dry weight.

**Immunohistochemical detection of glutathione-S-transferase expression**

The glutathione S-transferase (GST) isoenzyme distribution was determined using three rabbit polyclonal antibodies raised against the human \( \pi, \sigma \) and \( \mu \) forms of the enzyme \((\text{Hall et al., } 1990)\). The use of polyclonal antibodies to detect different GST isoenzymes has been described previously \((\text{Hayes et al., } 1989; \text{Harrison et al., } 1989; \text{Murphy et al., } 1992)\). The principles of the methods of immunohistochemical staining have been described by \( \text{Burn (1979)} \), and for GST assessment in particular, by \( \text{Randall et al. (1990)} \).

Paraffin wax sections \((4 \mu\text{m} \text{ thickness})\) were cut from the tissue blocks and mounted on slides. The sections were dried, dewaxed and rehydrated. After washing with water, the sections were washed in Tris-buffered saline \((\text{TBS})\) \((150 \text{ mM sodium chloride, } 5 \text{ mM Tris, pH } 7.9 \text{ with } 1 \text{ M hydrochloric acid})\). The excess TBS was wiped and the sections were incubated in normal swine serum \((\text{NSS})\) \((\text{Dako, High Wycombe, Bucks, UK})\) for 10 min. The excess NSS was removed and the primary anti-GST antibody was added at a dilution of \(1:400\) in NSS. After overnight incubation at \(4^\circ \text{C}\) the sections were rinsed with TBS. The excess TBS was wiped and the sections were incubated in biotinylated anti-rabbit secondary antibody \((\text{Dako})\) at a dilution of \(1:20\) in TBS. Following incubation for 30 min at room temperature, the sections were washed with TBS and then incubated in streptavidin–biotin horseradish peroxidase \((\text{Dako})\) \((1:200 \text{ in NSS})\) solution for 30 min. The sections were washed and immersed in TBS for 5 min. After wiping the slides, the peroxidase reaction was developed using \(3,3',\text{diaminobenzidine tetrahydrochloride solution (DAB) for 10 min (80 mg DAB in 100 ml TBS containing 0.086 mg imidazole)}\). The sections were washed with distilled water and treated with copper sulphate enhancer for 1 min, thoroughly washed in water and counter-stained with haematoxylin and mounted.

Tumour slides were stained in batches \((10–12 \text{ each})\). In each batch, and to control the quality of the staining, positive control slides were added to the batch (breast tumour for \( \pi \), kidney for \( \sigma \) and positive liver control for \( \mu \) GSTs; supplied by Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK).

Scoring was performed jointly \((\text{JPS and SGA})\) and the tissue sections selected for evaluation were those that only contained malignant changes as determined by an experi-

![Figure 1](image-url)  
**Figure 1** Calculation of GSH concentration in tumour samples by the method of standard additions \((r^2 = 0.999; \text{recovery } = 83\%\).

**Statistical analyses**

The relationship between GSH concentration and response was studied statistically by analysis of variance. The relationship between GST isoenzyme expression and response to carboplatin was analysed according to both Fisher’s exact and chi-squared tests.

**Ethical committee approval**

A copy of the study protocol was submitted to the regional ethics committee and approval was obtained before commencement of the study.

**Results**

**Tumour glutathione concentration in relationship to carboplatin**

GSH concentrations were measured and analysed in tumour samples from 39 patients \((78 \text{ different pieces})\). The recovery of GSH varied between 62 and 111\%, and the mean recovery rate was 93\%. Figure 2 represents the individual values for GSH concentrations in the tumours of 39 patients and their relationship to response to subsequent carboplatin therapy. Each point represents one sample or the mean of \(2–5\) samples from the same point. The GSH values in the tumour samples were: \(8351 \pm 4496, 7211 \pm 5026, 6559 \pm 4573\) and \(3758 \pm 1885 \text{ (nmol g}^{-1} \text{ tissue dry weight, mean } \pm \text{s.d.) for complete responders (CR, } n = 18\), partial responders \((PR, n = 18)\) and patients with static disease \(\text{(SD, } n = 7)\) or progressive disease \((PD, n = 4)\) respectively. These mean data are presented in Figure 3 and a one-way analysis of the variance did not show any significant relationship between GSH concentration and response \((\text{ANOVA, } P = 0.32)\).

**Tumour GST isoenzyme expression and its relationship to response to carboplatin**

GST isoenzyme expression was assessed by immunohistochemistry using polyclonal antibodies on fixed tumour samples from the same 39 patients whose tumours were analysed for GSH content. For each isoenzyme, the intensity of the staining and the percentage of tumour cells stained was
been used.

expression was recorded. For the purpose of this study, and in analysing the results in relation to response, patients were divided into two groups: responders (complete and partial) and non-responders (static or progressive disease); GST isoenzyme expression was defined as positive if the staining intensity was strong and more than 10% of the tumour cells stained positive. Most of the tumour samples (34/39) had positive expression of \( \pi \) GST, while only two different tumours expressed \( \alpha \) or \( \mu \) GST. Table II summarises the results of GST isoenzyme expression and its relation to response to carboplatin. Analysis of this result by Fisher’s exact and chi-squared tests did not show any significant relationship between response to carboplatin and GST isoenzyme expression (\( P = 0.51 - 0.553, 0.98 - 0.99 \) for Fisher’s exact and chi-squared tests respectively).

Discussion

From the results presented it is clear that, in this clinical setting, there was no marked relationship between GSH concentrations in primary EOC tumour samples and subsequent response to carboplatin chemotherapy. Similarly, there was no relationship between GST isoenzyme expression and response.

Platinum compounds are among the most effective agents for the treatment of patients with EOC and carboplatin has been used as a single agent in the Northern Region (UK) for the last 5 years. However, the clinical usefulness of carboplatin is limited by intrinsic resistance at initial treatment and subsequently by the development of acquired resistance at relapse (Rozenwieg et al., 1990).

In numerous preclinical studies (Godwin et al., 1992; Hamilton et al., 1985; Hill et al., 1990; Lai et al., 1989; Leyland-Jones et al., 1991; Mistry et al., 1991; Nakagawa et al., 1990; Saburi et al., 1989) GSH and GSTs have been implicated as important factors in intrinsic and acquired resistance to platinum compounds. Furthermore, a number of clinical studies have been reported which linked increased expression of GSTs with resistance to cytotoxic drugs in a variety of tumours including colorectal (Mulder et al., 1995) and lung cancer (Inoue et al., 1995), in which expression of the \( \pi \) isofrom was implicated, and acute lymphoblastic leukaemia (Hall et al., 1994b) in which expression of \( \mu \) class GST was associated with an increased probability of relapse.

However, it should be noted that several other mechanisms of resistance have also been suggested from preclinical work, including altered metallothionen content, increased DNA repair and reduced cellular accumulation, and the relative contribution of these various parameters to clinical resistance is currently unclear.

In the clinical setting, Green et al. (1993) studied GST expression in benign and malignant ovarian tumours and, in agreement with the present study, found that \( \pi \) GST was the most prevalent, being present in the majority of the malignant epithelia and 83% of the non-malignant tissue. There was no significant difference in the overall distribution of positive staining for \( \pi \), \( \alpha \) or \( \mu \) in the malignant and non-malignant biopsies, although the intensity of staining was greater in malignant epithelia. There was no significant association between survival and the presence or absence of \( \alpha \) or \( \mu \) GST subtypes, but the patients who showed resistance to chemotherapy (oral chlorambucil, single-agent cisplatin or a combination of cisplatin and cyclophosphamide), as opposed to responding patients, were found to have higher staining intensity for \( \pi \) GST (a Kruskal–Wallis test applied to the data for tumour response against staining intensity gave a \( P \)-value of 0.003).

Van der Zee (1992) studied GST activity and isoenzyme expression in benign ovarian tumours, untreated malignant ovarian tumours and malignant ovarian tumours after platinum–cyclophosphamide chemotherapy. In untreated malignant tumours GST activity and \( \pi \) GST expression were not related to histological type, differentiation grade or tumour volume index. GST isoenzyme patterns were identical in benign tumours and in malignant tumours, both before and after platinum–cyclophosphamide chemotherapy, with \( \pi \) GST again being the predominant isofrom. As in the study reported here, no role for GSTs as a predictor of response and hence a drug resistance mechanism was suggested. A similar finding was reported by Murphy et al. (1992), who studied GST activity and isoenzyme expression in human ovarian cancer tumours both before and after cytotoxic chemotherapy. Again, there was no statistically significant difference between GST activity or isoenzyme distribution in the two groups of patients. These authors observed that GST activities in both pre-chemotherapy and post-chemotherapy tumours were significantly higher than GST activity in normal ovaries. Also, \( \alpha \) isoenzyme expression was higher in the normal ovary than in tumour tissue. In the work of
Murphy et al. (1992), cytotoxic chemotherapy consisted of five different regimes of drugs with a small number of patients in each group, which prevented an analysis of the relationship between GST expression and response to therapy.

In conclusion, the results presented in this study show that there was no significant relationship between GST expression and chemotherapy or GST isoenzyme expression and response to single-agent carboptin chemotherapy in EOC. These data do not support the hypothesis that GST content or GST isoenzyme expression are major determinants of sensitivity to carboptin chemotherapy in primary ovarian cancer. It is important to stress the point that this study only looked at the role of GST/GSTs in intrinsic resistance. The possibility still exists that when resistance develops subsequent to chemotherapy (acquired resistance), GST/GSTs may play an important role. However, this issue is difficult to address clinically as the only way to do so is by sequential biopsy before and after chemotherapy, which is not always practical in a routine clinical setting.

Acknowledgements

The authors would like to thank and acknowledge the financial support of the North of England Cancer Research Campaign, Northern and Yorkshire Regional Health Authority Research Committee, the Leukaemia Research Fund and the North of England Children’s Cancer Research Fund. The help of research nurses and data managers at Newcastle General Hospital, and Mrs MR Walker in the pathology department at Queen Elizabeth Hospital in performing this research is also gratefully acknowledged.

References

BIAGLOW JE AND TUTTLE SW. (1993). The role of glutathione and associated enzymes in the cellular response to radiation, peroxide and hydroperoxides. In Drug Resistance in Oncology, Teicher BA (ed.) pp. 309 – 337. Marcel Dekker: New York.

BOLTON MG, COLVIN OM AND HILTON J. (1991). Specificity of isoenzymes of murine hepatic glutathione S-transferase for the conjugation of glutathione with L-phenylalanine mustard. Cancer Res., 51, 2410 – 2415.

BURN J. (1979). Immunohistochemical methods and their application in routine laboratory. In Recent Advances in Histopathology 10, Anthony PP and MacSween RNW. (eds) pp. 337 – 350. Churchill Livingstone: Edinburgh.

CALVERT AH, NEWELL DR, GUMBRELL LA, O’REILLY S, BURNELL N, BOXALL FE, SIDDICK ZH, JUDSON IR, GORE ME AND WILTSHE W. (1989). Carboptin dosing: prospective evaluation of a simple formula based on renal function. J. Clin. Oncol., 11, 1748 – 1756.

CIACCIO PJ AND TEW KD. (1993). Glutathione S-Transferases. In Drug Resistance in Oncology. Teicher BA. (ed.) pp. 351 – 374. Marcel Dekker: New York.

COTGREAVE IA AND MOLDEUS P. (1986). Methodologies for the application of monobromobimane to the simultaneous analysis of soluable and protein thiol components of biological systems. J. Biochem. Biophys. Methods, 13, 231 – 249.

DE GRAEF R, JUILLARD MG AND ROY LA. (1984). Resistance to cisplatin and analogues: mechanisms and potential clinical implications. Cancer Chemother. Pharmacol., 22, 325 – 332.

GODWIN AK, MEISTER A, O’DWYER PJ, HUANG CS, HAMILTON CT AND ANDERSON ME. (1992). High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc. Natl Acad. Sci. USA, 89, 3070 – 3074.

GREEN JA, ROBERTSON LJ AND CLARK AH. (1993). Glutathione S-transferase expression in benign and malignant ovarian tumours. Br. J. Cancer, 68, 235 – 239.

GULICK AM AND FAHL WE. (1995). Mammalian glutathione S-transferase: regulation of an enzyme system to achieve chemotherapeutic efficacy. Pharmacol. Ther., 66, 237 – 257.

HALL A, FOSTERS S, PROCTOR SJ AND CATTAN AR. (1990). Purification and characterization of π class glutathione S-transferase from human leukemia cells. Br. J. Haematol., 76, 494 – 500.

HALL AG, MATHESON E, HICKSON ID, FOSTER SA AND HOGARTH L. (1994a). Purification of an α class glutathione S-transferase from melphalan-resistant Chinese hamster ovary cells and demonstration of its ability to catalyze melphalan-glutathione adduct formation. Cancer Res., 54, 3369 – 3372.

HALL AG, AUTSEN P, CATTAN AR, MALCOLM AJ, COLE M, KERNANAH J AND REID MM. (1994b). Expression of μ class glutathione S-transferase correlates with event-free survival in childhood acute lymphoblastic leukemia. Cancer Res., 54, 5251 – 5254.

HAMILTON TC, WINKER MA, LOUIE KG, BATIST G, BEHRENS BC, TSURU T, GROTTINGER KR, MCKOY WM, YOUNG RC AND OZOLS RF. (1985). Augmentation of Adriamycin, melphalan and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoxime mediated glutathione depletion. Biochem. Pharmacol., 34, 2583 – 2586.

HARRISON DJ, KHABRAND A, CUNNINGHAM DS, MCLELLAN LI AND HAYS JD. (1989). Distribution of glutathione S-transferase isoenzymes in human kidney: basis for possible markers for renal injury. J. Clin. Pathol., 42, 624 – 628.

HAYES PC, HARRISON DJ, BOUCHER IAD, MCLELLAN LI AND HAYS JD. (1989). Cytosolic and microsomal glutathione S-transferase isoenzymes in normal human liver and intestinal epithelium. Gut, 30, 854 – 859.

HILL BT, SHELLARD SA, HOSKING KL, FICHITINGER-SCHEPAM AJ, AND BEDFORD P. (1990). Enhanced DNA repair and tolerance of DNA damage associated with resistance to cis-diaminedichloroplatinum(II) after in vitro exposure of a human teratoma cell line to fractionated X-irradiation. Int. J. Radiat. Oncol. Biol. Phys., 19, 75 – 83.

IOUE T, ISHIDA T, SUGIO K, MAEHARA Y AND SUGIMACHI K. (1985). Glutathione S-transferase P is a powerful indicator in chemotherapy of human lung squamous-cell carcinoma. Respiration, 62, 223 – 227.

ISHIKAWA T AND ALI-OSMAN F. (1993). Glutathione-associated cis-diaminedichloroplatinum(II) metabolism and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione – platinum complex and its biological significance. J. Biol. Chem., 268, 20116 – 20125.

ISHIKAWA T, WRIGHT CD AND ISHIHUKA H. (1994). GS-X pump is functionally over expressed in cis-diaminedichloroplatinum(II)-resistant human leukemia HL-60 cells and down-regulated by cell differentiation. J. Biol. Chem., 269, 29085 – 29093.

LAI GM, OZOLS RF, YOUNG RC AND HAMILTON TC. (1989). Effect of glutathione on DNA repair in cisplatin-resistant human ovarian cancer cell lines. J. Natl Cancer Inst., 81, 535 – 539.

LEYLAND-JONES BR, TOWNSEND AJ, TU CD, HOWAN KC AND GOLDSMITH ME. (1991). Antineoplastic drug sensitivity of human MCF-7 breast cancer cell stably transfected with a human α class glutathione S-transferases gene. Cancer Res., 51, 587 – 594.

MANTLE T, MCCUSKER FM, PHILLIPS M AND BOYCE S. (1990). Glutathione S-transferases. Isoenzymes, 18, 175 – 177.

MEYER DJ, GILMORE KS, HARRIS JM, HAYLTE JA AND KETERER B. (1992). Chlormebucil-monoglutathionyl conjugate is sequestered by human alpha class glutathione S-transferases. Br. J. Cancer, 66, 433 – 438.

MISTRY P, KELLAND LR, AVEL G, SIDHAR S AND HARRAP KR. (1991). The relationships between glutathione, glutathione S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines. Br. J. Cancer, 64, 215 – 220.

MULDER TP, VERSPAEGT HW, SIER CFM, ROELOPS HMJ, GANESH S, CRIFITIO E AND PETERS WHM. (1995). Glutathion S-transferase π in colorectal tumors is predictive for overall survival. Cancer Res., 55, 2696 – 2702.

MURPHY D, MCGOWN AT, HALL A, CATTAN A, CROWTER D AND FOX BW. (1992). Glutathione S-transferase activity and isoenzyme distribution in ovarian tumour biopsies taken before or after cytotoxic chemotherapy. Br. J. Cancer, 66, 937 – 942.

NAKAGAWA K, SAJOI NO, TSUCHIDA S, SAKAI M, TSUNOKAWA Y AND TEW KD. (1990). Glutathione-S-transferase π as a determinant of drug resistance in transfected cell lines. J. Biol. Chem., 265, 4296 – 4301.
GST isoenzyme expression and response to carboplatin
S Ghazal-Aswad et al

RANDALL BJ, ANGUS B, AKIBA R, HALL A, CATTAN AR, PROCTOR SJ, JONES RA AND HORNE CHW. (1990). Glutathione S-transferase (placental) as a marker of transformation in the human cervix uteri: an immunohistochemical study. *Br. J. Cancer*, **62**, 614–618.

ROZENCWEIG M, MARTIN A, BELTANGADY M, BRAGMAN K, GOODLOW J, WILTSHAW E, CALVERT H, MANGIONI C, PECORELLI S, BOLIS G, ROCKER I, ADAMS M AND CANETTA R. (1990). Randomised trials of carboplatin versus cisplatin in advanced ovarian cancer. In *Carboplatin (JM8) Current Perspectives and Future Directions*, Bunn PA, Canetta R, Ozols RF and Rozencweig M (eds). pp. 175–186. W.B. Saunders: Philadelphia.

SUBURI Y, NAKAGAWA M, ONO M, SAKAI M, MURAMATSU M, KOHNO K AND KUMANO M. (1989). Increased expression of glutathione S-transferase gene in cis-diamminedichloroplatinum (II)-resistant variants of a Chinese hamster ovary cell line. *Cancer Res.*, **49**, 7020–7025.

VAN DER ZEE AGJ, VAN OMMEN B, MEIJER C, HOLLEMA H, VAN BALDEREN PJ AND DE VRIES EGE. (1992). Glutathione S-transferase activity and isoenzyme composition in benign ovarian tumours, untreated malignant ovarian tumours and malignant ovarian tumours after platinum/cyclophosphamide chemotherapy. *Br. J. Cancer*, **66**, 930–936.