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AN EVALUATION OF THE ROLE OF GLUTATHIONE AND ITS ASSOCIATED ENZYMES IN THE EXPRESSION OF DIFFERENTIAL SENSITIVITIES TO ANTITUMOUR AGENTS SHOWN BY A RANGE OF HUMAN TUMOUR CELL LINES

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Abstract—Glutathione and its associated enzyme activities have been quantitated in a series of human tumour continuous cell lines expressing a range of *in vitro* sensitivities to certain antitumour agents. Fourteen different parental lines and 15 various drug- and X-ray-selected resistant sublines have been studied. Quantitative relationships between total glutathione levels and related enzyme activities and sensitivities to six clinically-useful antitumour drugs or X-rays, as judged by colony forming assays, have been determined by linear regression analysis. A positive correlation has been identified between glutathione levels and sensitivities to cisplatin, Adriamycin®, or to X-rays. In addition, positive correlations were noted between cisplatin sensitivities and glutathione peroxidase and reductase activities and for Adriamycin® responses with respect to glutathione peroxidase activity, using cumene hydroperoxide as substrate. However, no positive correlations were noted for glutathione levels or these enzyme activities with differential methotrexate, etoposide, vincristine or 5-fluorouracil cytotoxicities. Furthermore, no direct relationship was apparent between total glutathione *S*-transferase activities and any of these drug or X-ray sensitivities in this series of cell lines. These data appear to provide further evidence linking altered glutathione metabolism with differential cytotoxicities of certain clinically-useful antitumour agents.

Over a decade ago Meister suggested that perturbation of glutathione (GSH) metabolism had the potential to modulate the effectiveness of certain therapeutic agents, including X-rays and various drugs, used in the treatment of cancer [1]. Since that time a number of laboratories have set out to examine the role of intracellular GSH and related enzymes as determinants of the therapeutic efficacy of a wide range of anti-neoplastic drugs. The GSH redox cycle can be altered at a number of points, including depletion of GSH pools, inactivation of glutathione reductase (GR; EC 1.6.4.2) or inhibition of the activities of glutathione peroxidase (GP; EC 1.11.1.9) or the glutathione *S*-transferases (GST; EC 2.5.1.18) (cf. reviews Refs 2–5). Data derived from a wide range of experimental tumour model systems soon became available. Initial results, summarized in Table 1, tended to support the contention that elevated GSH levels and, in particular, increased GST activities were associated with the expression of resistance to a wide range of chemotherapeutic drugs. In the light of certain of these data it was suggested [6, 7] that over-expression of GSH-based detoxification mechanisms may be characteristic of cells expressing the multidrug resistance (MDR) phenotype. However, more recent investigations [8–12] have led to the conclusion that whilst the GSH system is implicated in maintaining the resistance observed in certain MDR and non-MDR tumour cells, it is *not* a universal feature of the classic MDR phenomenon. Furthermore, others have suggested [13] that the quantitative relationship between GSH levels and cytotoxicity is different for primary selected resistance compared to cross resistance. In an attempt to clarify any possible relationship between GSH and its associated enzyme activities with antitumour agent cytotoxicities we have quantitated these parameters in a series of human tumour continuous cell lines. We have studied 14 different human tumour parental cell lines and 15 variously-selected drug resistant sublines. Correlations have been examined between their *in vitro* sensitivities, as judged by colony forming assays (CFA), to a range of clinically-useful antitumour drugs and to X-rays, and total GSH levels and activities of GR, GP or GST.

MATERIALS AND METHODS

Antitumour drugs. The following drugs were kindly donated for these studies: etoposide (VP-16) by Bristol-Myers (Slough, Bucks, U.K. and Evansville, IN, U.S.A.); vincristine (VCR) and methotrexate (MTX) by Lederle Laboratories (Gosport, Hants, U.K.); Adriamycin® (ADR) by Farmitalia Carlo Erba (St Albans, Herts, U.K.); 5-fluorouracil (5-FU) by Roche Chemicals (Welwyn Garden City, Herts, U.K.). Cisplatin (CDDP) was purchased from Sigma Chemicals (Poole, Dorset, U.K.). Immediately prior to use VCR and 5-FU were solubilized in phosphate

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Table 1. Summary of published data reporting perturbation of glutathione metabolism associated with the expression of antitumour drug resistance

Model system studied	Selecting drug(s)	Ref.
Elevated GSH levels		
Human ovarian cancer cells	ADR, L-PAM or CDDP	25
Human ovarian cancer cells	CDDP,* CHL* and 5-FU*	28
Murine L1210 leukemia cells	L-PAM	54
	CDDP	29
Chinese hamster ovary cells	L-PAM	49
Walker 256 rat carcinoma cells	HN-2	55
Elevated GST activity		
Human melanoma cell lines	Alkylating agents	46
Human breast carcinoma cells	ADR	6
	Colchicine	8
	VCR or VP16	9
P388 murine leukemia cell lines	ADR	42, 47
SEWA mouse cells	ACT-D	48
Walker 256 cells	HN-2	55
Chinese hamster ovary cells	HN-2 and CHL	50
Human ovarian carcinoma cells	CDDP* and ADR*	28
Human squamous cell carcinoma cells	CDDP	30
Human small cell lung cancer cell lines	CDDP	56

Abbreviations: ACT-D, actinomycin D; ADR, Adriamycin®; CDDP, cisplatin; CHL, chlorambucil; 5-FU, 5-fluorouracil; HN-2, nitrogen mustard; L-PAM, melphalan; VCR, vincristine; VP-16, etoposide.

* Cell lines established from a patient after the onset of clinical resistance to these drugs.

buffered saline (PBS), whilst ADR and CDDP solutions were prepared using water and saline respectively. Stock solutions of VP-16 and MTX obtained from the suppliers were diluted with PBS.

Cell lines and culture techniques. The cell lines used, their origins and the orders of resistance expressed by the variously derived sublines are listed in Table 2. The different media and sera used were supplied by Gibco-Biocult (Renfrewshire, U.K.).

Cytotoxicity assays. All cytotoxicity assays were carried out using CFA methodologies involving 0.3% soft agar [14], or 0.17% agarose [15] or cloning directly onto plastic. Details of the method used for each cell line and the colony forming efficiency (CFE) obtained are listed in Table 2. Logarithmically-growing cells were exposed to a range of concentrations of each drug for 24 hr prior to cloning. For radiation sensitivity assays logarithmically-growing cells were treated with graded doses of radiation using a Pantak HF320 X-ray machine (Astrophysics, Slough, U.K.). The culture dishes were mounted 14 in. from the source and using settings of 210 kV and 8 mA, a dose rate of 2.61 Gy/min was delivered. Cells were then trypsinized and counted before performing the CFA. The CFE of treated cultures was calculated and expressed as a percentage of non-drug treated controls. The mean ± standard error (SE) of these values from replicate cultures, with each experiment repeated at least twice, was plotted against drug concentration from which the IC_{50} values (concentration required to reduce survival by 50%) were interpolated. The mean ± SE of IC_{50} values from separate experiments was calculated and subsequently used in linear regression analyses. Survival curves after X-irradiation were fitted using a linear least-squares computer programme and X-ray doses reducing the survival fraction of cells to 0.37 on the exponential region of the survival curve (Do) and the surviving fraction after 2 Gy (SF2) were derived.

Total GSH, GR, GP and GST assays. Total GSH content and related enzyme activities were measured in cell lysates prepared from mid-log phase cells. Total GSH content was measured using the GR recycling procedure of Griffith [16]. GR activity was measured by the method of Horn [17]. GP activity was determined by the method described by Paglia and Valentine [18], using cumene hydroperoxide as substrate. Total GST activity was determined using 1-chloro-2,4-dinitrobenzene as substrate according to the procedure of Habig and Jakoby [19]. Duplicate estimations of all the parameters were determined in at least two separate experiments. Values normalized for cellular protein content using the assay of Lowry *et al.* [20] were expressed as the mean ± SE.

Statistical analyses. Quantitative relationships in the panel of cell lines between total GSH levels (independent variable) or associated enzyme activities and drug or X-irradiation cytotoxicities (dependent variables) were determined using linear regression analysis. The resulting positive correlation coefficients ($r > 0.6$) were subjected to a t-test as a measure of statistical significance.

RESULTS

The total GSH levels and associated enzyme actities for each of the cell lines studied are detailed in Table 3. Amongst the parental cell lines tested GSH levels, GR and selenium-independent GP activities varied by factors of 6–9, whilst a 31-fold difference in total GST activities was recorded. In the series of drug- and X-ray-selected resistant sublines established in our laboratory, which express relatively

Table 2. Derivation and clonogenicity of parental and drug resistant human tumour cell lines

Cell line	Origin/derivation (Ref.)	Fold resistance to selecting agent	Colony forming assay	Colony forming efficiency (%)	Media used
MCF-7P	Pleural effusion from metastatic breast carcinoma [57]	—	Plastic	50–60	Eagle's (E4) + 10% foetal calf
VCR6E	Six 24 hr exposures to 10 ng/mL VCR [9]	13.7	Plastic	50–60	serum (FCS) +
ADR6E	Six 24 hr exposures to 50 ng/mL ADR [9]	3.0	Plastic	50–60	10 μg/mL insulin
VP6E	Six 24 hr exposures to 1 μg/mL VP-16 [9]	3.0	Plastic	50–60	"
DXR-10	Ten fractions of X-rays [58] (5–6 Gy per fraction)	5.0 to VCR	Plastic	50–60	"
SuSaP	Primary testicular teratoma [59]	—	Agar	5–15	RPM1 1640
VP10	Six 24 hr exposures to 10 ng/mL VP-16 [58]	5.0	Agar	5–15	+ 10% FCS
VPC2	Continuous exposure to 50–80 ng/mL VP-16 [9]	8.8	Agar	5–15	"
VPC3	Continuous exposure to 50–200 ng/mL VP-16 [9]	21.0	Agar	5–15	"
DXR-10	Ten fractions of X-rays (3.0 Gy per fraction) [58]	3.0 to VP-16	Agar	5–15	"
DXR-13	Thirteen fractions of X-rays (1.5 Gy per fraction)	3.3 to VP-16	Agar	5–15	"
RT112P	Transitional carcinoma of the bladder [60]	—	Plastic	10–20	RMP1 1640 + 10% FCS
DXR-8	Eight fractions of X-rays (6 Gy per fraction) [31]	2.0 to VCR 2.5 to VP-16	Plastic	10–20	"
CP	Continuous exposure to 0.08–2.5 μg/mL CDDP [31]	2.0	Plastic	10–20	"
T24	Metastasis from differentiated transitional cell bladder carcinoma [60]	—	Plastic	61–70	"
HN-1P	Squamous cell carcinoma of the tongue [61]	—	Agar	5–20	E4 + 10% FCS
VP2	Continuous exposure to 100–200 ng/mL VP-16 [62]	4.0	Agar	5–20	"
DXR-11	Eleven fractions of X-rays (4.5 Gy per fraction) [63]	4.0 to VP-16	Agar	5–20	"
SKOV-3	Ovarian cystadenocarcinoma [64]	—	Agar	5–10	"
TR175	Ascites from ovarian cystadenocarcinoma [64]	—	Agar	0.1–0.2	Hams F-12 + 10% FCS +10% FCS
TR170	Ascites from ovarian cystadenocarcinoma [64]	—	Agar	1–4	"
TR170AD	Six 24 hr exposures to 25 ng/mL ADR [64]	2.0	Agar	1–4	"
TR170CP	Six 24 hr exposures to 150 ng/mL CDDP [64]	2.0	Agar	1–4	"
CHP100	Neuroblastoma [65]	—	Agarose	25–40	RPM1 1640 + 10% FCS
CHP212	Neuroblastoma [65]	—	Agarose	6–25	"
LAN-1	Neuroblastoma [65]	—	Agarose	12–20	Hams F-12 + 10% FCS
COLO 205	Colon carcinoma [66]	—	Agar	30–40	RPM1 1640 + 10% FCS
LOVO	Colon carcinoma [67]	—	Agar	29–35	Hams F-12 + 10% FCS
KS-1	Kruckenburg ovarian tumour arising from 1° gastric adenocarcinoma [68]	—	Agar	15–20	"

modest levels of resistance, i.e. 2- to 14-fold, a 22-fold range of GST activities were noted, but again the variation in total GSH content and GR activity was narrower (i.e. 2- to 7-fold). For these sublines, however, the range of GP activities was higher, i.e. 22-fold, than for the parental lines.

Results of the antitumour agent cytotoxicity assays are summarized in Tables 4 and 5. The range of

Table 3. Total glutathione content and some related enzyme activities in a panel of parental and drug resistant human tumour cell lines

Cell line	GSH (nmol)*	GR (units)*	GST (units)*	GP (units)*
Breast†				
MCF-7P	41.8 ± 2	33.3 ± 2	15 ± 2	2.0 ± 0.9
VCR6E	54.4 ± 9	19.2 ± 2	100 ± 5	10.5 ± 3.0
ADR6E	46.3 ± 3	11.3 ± 1	40 ± 2	2.3 ± 0.1
VP6E	52.2 ± 2	ND	93 ± 5	2.9 ± 0.1
DXR-10	52.3 ± 4	31.3 ± 3	16 ± 1	0.7 ± 0.4
Testicular†				
SuSaP	18.8 ± 3.0	23.3 ± 0.7	160 ± 4	4.5 ± 0.7
VP10	16.8 ± 2.0	26.9 ± 0.6	159 ± 11	3.3 ± 1.3
VPC2	20.2 ± 3.7	21.4 ± 1.9	136 ± 35	4.2 ± 0.6
VPC3	15.8 ± 1.4	28.2 ± 2.2	131 ± 17	6.3 ± 0.4
DXR-10	16.1 ± 2.1	24.4 ± 1.7	149 ± 20	4.6 ± 0.7
DXR-13	9.6 ± 1.9	19.7 ± 1.2	190 ± 46	4.3 ± 0.4
Bladder‡				
RT112P	71.3 ± 8.6	20.1 ± 3.0	246 ± 60	11.6 ± 0.1
DXR-8	59.3 ± 9.7	16.1 ± 3.0	353 ± 62	10.8 ± 0.7
CP	112.4 ± 15	37.3 ± 14	328 ± 44	15.2 ± 0.6
T24	56.8 ± 6.0	18.0 ± 4.0	282 ± 31	ND
Head and neck§				
HN-1P	49.3 ± 5	61.5 ± 8	467 ± 16	1.8 ± 0.2
VP-2	46.9 ± 2	73.1 ± 12	441 ± 42	2.6 ± 0.3
DXR-11	48.8 ± 4	72.4 ± 5	544 ± 20	2.6 ± 0.2
Ovarian				
SKOV-3	80.7 ± 18	127.0 ± 27	98 ± 17	16.0 ± 3.0
TR175	28.0 ± 4.0	20.0 ± 4.0	86 ± 31	8.8 ± 1.7
TR170	37.0 ± 2.0	38.0 ± 1.0	181 ± 21	1.7 ± 0.5
TR170AD	32.0 ± 3.0	31.1 ± 3.0	215 ± 6.0	0.8 ± 0.1
TR170CP	26.7 ± 5.0	41.7 ± 1.0	170 ± 12	1.3 ± 0.6
Neuroblastoma				
CHP100	27.9 ± 1.7	37.3 ± 0.8	ND	ND
CHP212	34.4 ± 0.7	32.5 ± 0.7	ND	ND
LAN-1	42.9 ± 1.6	28.3 ± 3.9	ND	ND
Colon				
COLO 205	32.5 ± 3.0	55.2 ± 3.0	ND	ND
LOVO	8.8 ± 3.0	45.5 ± 3.3	ND	ND
Stomach				
KS-1	32.0 ± 0.5	80.1 ± 1.0	ND	ND

Total GSH content, and related enzyme activities were determined spectrophotometrically in lysates prepared from logarithmically growing cell lines.

* All values represent the mean ± SE of at least two separate experiments involving duplicate estimations and have been expressed as per mg protein; GST: 1 unit conjugates 1 nmole CDNB per min at 25°; GR and GP: 1 unit oxidizes 1 nmole NADPH per min at 25°.

ND, not determined.

† Reproduced in part from Ref. 9.

‡ Reproduced in part from Ref. 31.

§ Reproduced in part from Ref. 62.

responses of the parental cell lines, as judged by IC_{50} concentrations, varied depending on the drug tested: 60-fold for ADR, 45-fold for CDDP, 15-fold for 5-FU, 20,000-fold for MTX, 100-fold for VP-16 and 45-fold for VCR. The IC_{50} values for the series of resistant sublines generally fell within those of the overall parental series. For radiation sensitivities Do values ranged from 0.41 to 1.53 and the SF2 values varied from as low as 2% to as high as 50%.

Correlation coefficients determined from linear regression analyses are listed in Table 6. These data show that significant positive correlations (i.e. $r > 0.6$ and $P < 0.05$) were noted with three of the parameters tested for CDDP (GSH, GR and GP), and for two of the parameters (GSH and GR) irrespective of whether the data from the parental lines only or from all the lines combined were analysed.

Significant positive correlations were also determined for ADR cytotoxicities and total GSH levels, whilst a positive correlation between ADR lethality

Table 4. Response of human tumour cell lines to ADR, CDDP and X-irradiation*

Cell line	ADR†	CDDP†	Do‡	SF2§
Breast				
MCF-7P	19.0	180	1.47	47
VCR6E	50.0	180	—	—
ADR6E	50.5	180	—	—
VP6E	51.0	180	—	—
DXR-10	20.0	190	1.38	37
Testicular				
SuSaP	1.9	40	0.55	4.0
VP10	1.5	—	0.52	3.5
VPC2	2.9	—	0.82	8.4
VPC3	4.4	—	1.03	6.1
DXR-10	1.1	—	0.41	2.0
DXR-13	2.7	—	0.41	2.0
Bladder				
RT112P	50	200	1.31	50
DXR-8	53	—	1.53	50
CP	—	—	—	—
T24	10	—	—	—
Head and neck				
HN-1P	8.8	230	1.39	43
VP2	23.4	300	—	—
DXR-11	10.0	173	0.97	34
Ovarian				
SKOV-3	30	450	—	—
TR175	7	—	—	—
TR170	9	50	—	—
TR170AD	21	50	—	—
TR170CP	8	100	—	—
Neuroblastoma				
CHP100	7.2	88	0.79	16.5
CHP212	5.8	31	—	42
LAN-1	8.2	25	—	8.8
Colon				
COLO 205	10	10	—	—
LOVO	6	40	1.05	28
Stomach				
KS-1	40	250	—	—

Survival of parental and resistant lines following exposure to antitumour agents was determined by CFA either in soft agar or on plastic.

* All values are the mean of at least two experiments involving full dose response curves assessing four doses in quadruplet. The SE of the means never exceeded 11%.

† IC_{50} values as judged by CFA following 24 hr drug exposures (ng/mL).

‡ Dose of X-irradiation reducing survival fraction (as judged by CFA) to 0.37 as defined using a multitarget equation and a linear least squares computer program.

§ Survival following 2 Gy X-irradiation as judged by CFA.

Table 5. Drug responses of human tumour cell lines*

Cell line	VP-16	VCR	MTX†	5-FU
Breast				
MCF-7P	100	0.8	50,000	1000
VCR6E	200	11.0	—	—
ADR6E	—	5.0	—	—
VP6E	507	2.0	—	—
DXR-10	543	3.0	—	—
Testicular				
SuSaP	7.5	0.22	—	—
VP10	35.0	0.40	—	—
VPC2	66.0	0.30	—	—
VPC3	157.5	0.90	—	—
DXR-10	21.2	0.45	—	—
DXR-13	23.3	0.50	—	—
Bladder				
RT112P	72	1.0	18	780
DXR-8	165	2.0	—	—
CP	335	—	—	—
T24	—	—	7.3	—
Head and neck				
HN-1P	97	1.7	500	2000
VP2	390	4.6	—	—
DXR-11	220	3.1	400	1000
Ovarian				
SKOV-3	78	7.8	—	—
TR175	—	—	—	—
TR170	145	1.1	—	—
TR170AD	115	1.0	—	—
TR170CP	—	—	—	—
Neuroblastoma				
CHP100	5.5	0.5	2.5	2200
CHP212	7.3	9.3	92	1900
LAN-1	6.5	6.9	60	1300
Colon				
COLO 205	350	7	25	180
LOVO	30	10	53	250
Stomach				
KS-1	—	—	—	2800

Survival of parental and resistant lines following exposure to antitumour agents was determined by CFA either in soft agar or on plastic.

* IC_{50} values as judged by CFA following 24 hr drug exposures (ng/mL). All values are the mean of at least two experiments involving full dose–response curves assessing four drug concentrations in quadruplet. The SE of the means never exceeded 11%.

† MTX, methotrexate.

and GP activity was shown. Figures 1 and 2 illustrate the various correlations for ADR and for CDDP with respect to each parameter measured. Positive correlations were also obtained for the relationship between total GSH content and cellular sensitivity to X-irradiation as measured either by Do or SF2 values, and these are illustrated in Fig. 3. The significance of these linear relationships, derived from analyses of all the cell lines tested, have been described as F values as judged by linear regression analysis. These values with respect to GSH levels are listed in Table 7 and all appear highly significant. For CDDP sensitivities the F values in relation to GR and GP activities are also significant being $F(1,17) = 9,9$, $P = 0.006$ and $F(1,12) = 6.95$, $P = 0.021$, respectively.

Table 6. Correlation coefficients determined from linear regression analyses

Antitumour agent	Parameter measured			
	GSH	GR	GST	GP
ADR	*0.679 (28) ***0.616 (14)†**	0.022 (27) **0.343 (14)†**	0.132 (22) **0.067 (8)†**	*0.454 (21) **0.635 (7)†**
CDDP	*0.771 (20) ***0.789 (11)†**	*0.608 (19) ***0.768 (12)†**	0.199 (14) **0.042 (6)†**	*0.590 (14) **0.736 (6)†**
Do	*0.777 (14) **0.648 (6)†**	0.153 (14)	0.152 (12)	0.169 (12)
SF2	*0.849 (17) ***0.755 (9)†**	0.242 (16)	0.324 (12)	0.218 (12)
VP-16	0.431 (24)	0.175 (23)	0.070 (19)	0.111 (19)
VCR	0.272 (24)	0.227 (23)	0.144 (19)	0.502 (19)
MTX	0.018 (10)	0.166 (10)	ND	ND
5-FU	0.021 (10)	0.304 (10)	ND	ND

Figures in parentheses represent the number of cell lines analysed.
* Statistically significant as judged by *t*-test analysis (P values ranged between <0.05 and <0.001).
† Values represent analysis of parental cell lines only.
ND, not determined.

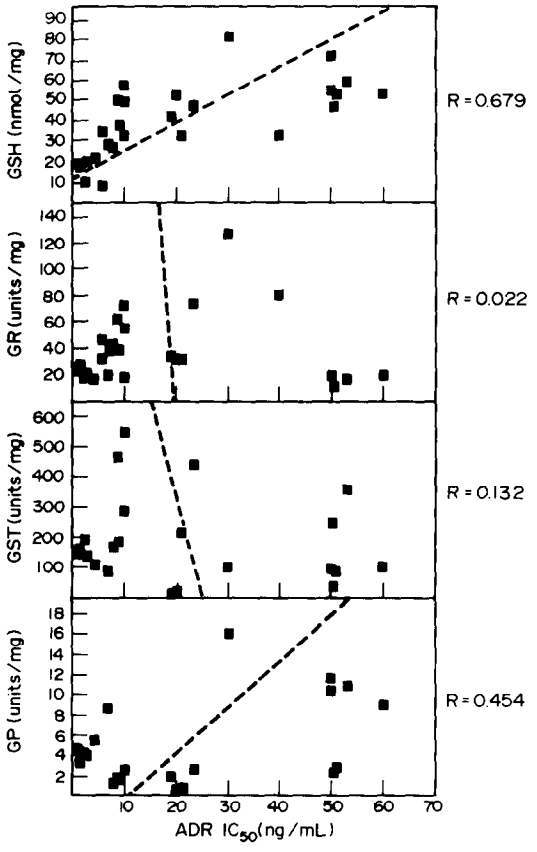

Fig. 1. Linear regression analysis data of possible relationships between GSH metabolism and Adriamycin® sensitivity in a panel of human tumour cell lines and various drug resistant sublines.

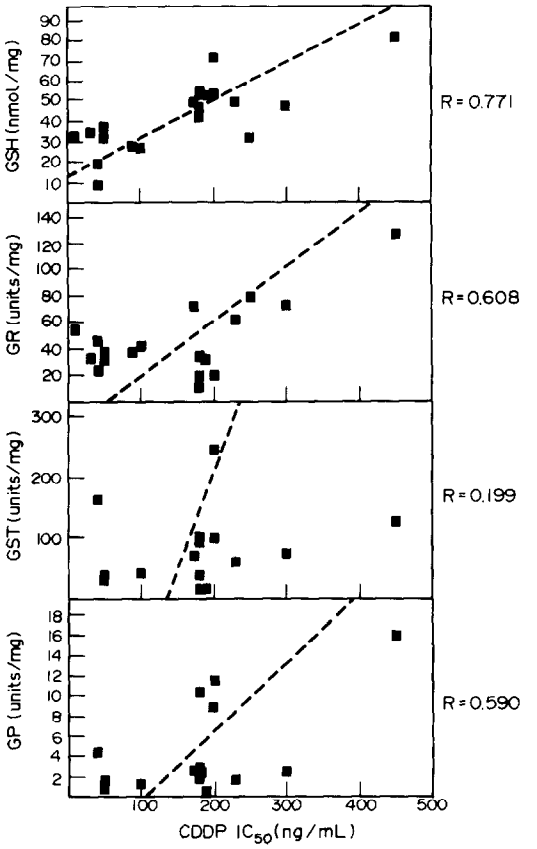

Fig. 2. Linear regression analysis data of possible relationships between GSH metabolism and cisplatin sensitivity in a panel of human tumour cell lines and various drug resistant sublines.

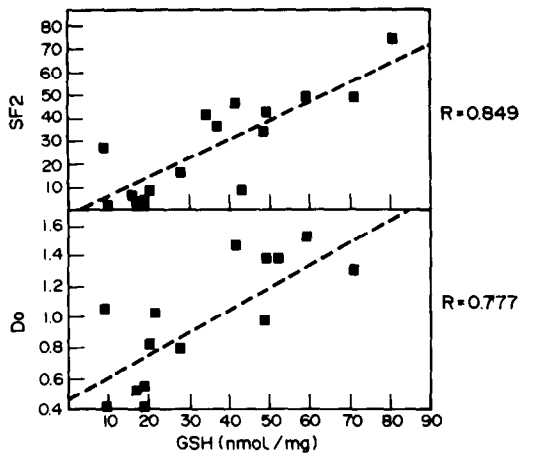

Fig. 3. Linear regression analysis data of relationships between X-ray response and total GSH content in a panel of human tumour cell lines and various drug resistant sublines.

Table 7. F values as judged by linear regression analysis

Antitumour agent	GSH level
ADR	$F(1,26) = 22.25$, $P < 0.0001$
CDDP	$F(1,18) = 26.4$, $P < 0.0001$
X-rays	
Do	$F(1,12) = 18.24$, $P = 0.001$
SF2	$F(1,15) = 38.84$, $P < 0.0001$

No positive correlations were noted for any of these parameters and differential VP-16, VCR, MTX or 5-FU cytotoxicities. It is also noteworthy that no positive correlations were noted in this overall series in terms of total GST activities.

DISCUSSION

We have investigated the relationship between total GSH levels and associated enzyme activities and sensitivities to various clinically-useful antitumour agents in human tumour cell lines. A series of parental cell lines established from a range of different tumour types and various drug- and X-ray-selected resistant sublines have been studied. The range of GSH levels and associated enzyme activities amongst these parental cell lines are similar to those reported by other groups using a series of lines from lung cancer [21], ovarian carcinoma [22, 23] and colorectal cancer [24], except that in this latter case a much wider range of GST activity, ranging from 1.63 to 218 units was documented. GSH levels of the variously-derived resistant sublines were not significantly different from those of their parental cell values, with the exception of the RT112-CP line. Although other groups have reported modified GSH levels in various cell lines resistant to ADR [7, 10, 13, 25–27] or to CDDP [25, 26, 28–32], certain workers in line with our observations have shown no such changes [10, 16, 33, 34]. However, it is generally accepted that since CDDP reacts with thiols such as GSH at physiologically relevant concentrations [35], GSH might play a role in determining cellular sensitivity to CDDP by preventing platination of critical loci [2, 3]. Consistent with this proposal, therefore, is our identification of a clear correlation between GSH levels and CDDP sensitivities in this series of 14 parental lines derived from a range of human tumour types. We have also shown a similar positive correlation between GSH levels and sensitivities to ADR. This appears to contrast with the data reported on seven ovarian lines expressing an inherent 3-fold range of ADR responses [23], where a poor correlation was noted. However, this group did report a good correlation between ADR sensitivity and the area under the GSH concentration–time curve, a parameter not quantitated in our analyses [22]. In addition, when we carried out a linear regression statistical analysis on the data published by Carmichael *et al.* [21] a poor correlation was noted between GSH levels and ADR responses in 15 small cell ($r = 0.046$) and 15 non-small cell ($r = 0.049$) lung cancer lines. Overall these results may add weight to the contention that in the light of the diverse spectrum of GSH levels identified in human tumour cell lines, xenografts and biopsies (cf. for example, Refs 21–24, 28 and 36) elevated GSH levels conferring drug resistance may be tumour specific. Therefore, the role of GSH in intracellular drug metabolism may vary between lines. Certainly our data, using a wide range of different tumour types, would indicate that GSH levels are not universally or automatically important determinants of the sensitivity of cells to all cytotoxic drugs since no clear correlations between GSH levels and sensitivities to VCR, VP-16, 5-FU or to MTX were noted.

Although in the past decade evidence has emerged concerning the role of endogenous thiols, mainly GSH, as modulators of response to irradiation [26, 37], the precise mechanism(s) involved have not been determined [2]. The lack of any distinct relationship between radiation sensitivity and related enzyme activities amongst the 17 lines we have studied, appears similar to that reported in human colorectal and lung cancer cell lines [24, 38, 39]. However the positive correlation between responses to X-irradiation, as judged in terms either of Do or SF2 values, and total GSH levels shown here clearly contrasts with data from these same studies [24, 38, 39] and a recent report on a series of murine tumour cell lines [40]. Further studies are needed to resolve this issue.

The other positive correlations noted in our study, in terms of CDDP and ADR sensitivities, were with selenium-independent GP activity measured using cumene hydroperoxide as substrate. GP is another of the GSH-dependent enzymes which are known to be involved in protection from cytotoxic chemicals [28, 41]. GP has a primary role in the reduction of organic and lipid hydroperoxides and such intermediates have been implicated in the mechanism of action of various anticancer drugs, particularly ADR [4, 28, 42, 43]. Kramer and his colleagues [7] demonstrated the importance of selenium-dependent GP

activity and of the associated redox capacity as biochemical mechanisms that contribute to ADR resistance in sublines of the MCF-7 breast carcinoma and P388 murine leukemia. However, Singh *et al.* [42] implicated an increase in selenium-independent activity in their ADR-resistant P388 subline, whilst Bellamy *et al.* [10] found no change in GP activity in a 40-fold ADR-resistant human myeloma subline, using either cumene hydroperoxide or hydrogen peroxide as substrates. In our series, increased selenium-independent GP activity was associated with CDDP resistance in the RT112-CP line [31], but was not characteristic of our ADR-resistant MCF-7 subline [9]. Unchanged GP activities were also reported in the ovarian 2780 sublines resistant to either CDDP or ADR [25]. Somewhat unexpectedly, an increase in this GP activity was noted in both our MCF-7 and SuSa sublines selected for VCR resistance [9] but the correlation coefficient for VCR sensitivities and GP activities in our main series of lines was <0.6. In view of the positive correlation noted here between responses to ADR or CDDP and selenium-independent GP activity, further quantitation of both selenium-dependent and -independent peroxidase activities appears indicated in the range of wild-type and drug-selected sublines already available.

Although GR has a key role in the regulation by GSH of the bioreductive activation of drugs [4], relatively few investigators have reported on GR activities in human tumours. No changes were recorded in ovarian carcinoma sublines resistant to CDDP and ADR [25, 28] and whilst increased activity was noted in non-small cell carcinoma versus small cell lung lines, these changes did not achieve statistical significance [21]. These data are therefore consistent with the apparent lack of correlation noted between GR activity and the various drug sensitivities studied here, except for CDDP where again a positive correlation was noted when either all the cell lines or parental lines only were tested.

In view of the vast literature describing the central role that the GSTs play in the detoxification of electrophilic xenobiotics (cf. for example Refs 5, 12, 44 and 45), our observation of a lack of any significant correlation between total GST activities and drug sensitivities was unexpected. Whilst we and others have identified modified GSTs in a range of multidrug-resistant human tumour sublines [6–9, 27, 28, 42, 46, 48], it should be stressed that this is not by any means a universal finding [7–11, 25]. However, in the light of the knowledge that these transferases are a multigene family of dimeric proteins (cf. Refs 5, and 44, 45), it remains a strong possibility that whilst changes in total GST activity, as measured here, may not reflect altered drug sensitivities, differences in GST isozyme composition may determine the development of a particular phenotype of resistance. For example, the elevated expression of the GST-α family of isozymes has been firmly correlated with increased resistance to alkylating drugs (cf. Refs 49–51). Whilst a more recent study evaluating GST-π as a determinant of drug resistance in transfectant cell lines has concluded that increased levels of GST-π are not *per se* sufficient to convey resistance to cisplatin, alkylating agents or radiation, although increased resistance to ADR was recorded [51]. In addition, differences in intracellular compartmentalization may have a role to play [52], in the light of the report that a specific nuclear GST is responsible for the "repair" of DNA peroxides resulting from exposure to ionizing radiation [53]. Studies aimed at providing evidence to support or refute this contention and to identify the role of each particular subunit in the metabolism of different classes of anticancer drugs are under way.

In summary, we have shown a positive correlation between GSH levels and sensitivities to CDDP, ADR, or X-irradiation in a series of human tumour cell lines, expressing a range of sensitivities to these agents. In addition, positive correlations were noted in terms of CDDP cytotoxicities for both GP and GR activities and for ADR lethality with respect to GP activity. These data add weight to the proposal that perturbation of GSH metabolism has the potential to modify the effectiveness of certain antitumour agents currently used in the clinical management of malignant disease.

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