Glutathione and related enzyme activity in human lung cancer cell lines

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Summary  Glutathione levels were measured in 30 human lung cancer lines. Lower levels were detected in cell lines derived from small cell lung cancer specimens compared to non-small cell lines (mean 42 vs. 130 mmol mg⁻¹ protein, P = 0.005). However, no differences were detected between cell lines derived from previously untreated patients, compared to those derived from patients who had received chemotherapy. Non-small cell lines were found to have increased activity of 4 detoxification enzymes compared to small cell lines, although these differences did not reach statistical significance: glutathione transferase activity (69 vs. 36 units, P = 0.137), glutathione reductase (139 vs. 82 units, P = 0.05), γ-glutamyl transpeptidase (9.39 vs. 3.03 units, P = 0.072) and superoxide dismutase (20 vs. 13.6 units, P = 0.137). As the cell lines exhibit a similar chemosensitivity pattern to that observed in clinical practice, these differences in glutathione and detoxification enzyme levels may prove to be important indicators of intrinsic drug resistance often seen in patients with non-small cell lung cancer.

The tripeptide glutathione (GSH) has been implicated in the detoxification of a wide range of xenobiotics including many of the currently used cytotoxic drugs (Arias & Jakob, 1976; Meister, 1981). Detoxification is generally achieved through a substitution reaction with electrophilic compounds, often catalysed by the glutathione-S-transferases [GST] (Jakob & Habig, 1980; Wolf et al., 1987). GSH detoxifies oxygen-induced free radicals, a reaction catalysed by glutathione peroxidase (GPX), and in addition, it is important in transferring reducing equivalents in the cell. Modulation of GSH levels by either lowering levels with buthionine sulfoximine (Dethmers & Meister, 1981) or raising levels with oxothiazolidine-4-carboxylate (Williamson et al., 1982) or glutathione esters (Anderson et al., 1985) changes the response of cells to a number of cytotoxic drugs (Russo et al., 1984; Russo & Mitchell, 1985) and ionising radiation (Mitchell et al., 1983; Biaglow et al., 1983; Jensen & Meister, 1983; Russo & Mitchell, 1984).

Lung cancer exhibits an interesting spectrum of drug and radiation resistance, from small cell lung cancer (SCLC), which is relatively sensitive on presentation, but which exhibits an acquired resistance pattern on relapse, to the intrinsically resistant non-small cell lung cancer (NSCLC) (Bergsagel & Feld, 1986; Ruckdeschel et al., 1986). In view of the importance of GSH and related enzymes in the response of cells to both chemotherapy and radiation therapy, measurements of these levels were performed on a panel of human cell lines, including all of the major histological sub-types of lung cancer.

Materials and methods

Cell lines

A panel of 30 human lung cancer cell lines was used. These cell lines were derived from patients exhibiting a variety of histological sub-types of lung cancer. Some of these patients had received prior chemotherapy or radiotherapy. The majority of SCLC lines grew as floating aggregates with the exception of NCI-H841 which grew as a loosely adherent monolayer. In contrast, all NSCLC lines grew as monolayers. Cell lines were grown in RPMI 1640 medium containing 10% (v/v) foetal calf serum with penicillin and streptomycin. For experimental procedures cell lines were maintained in exponential growth and had been refed 48 h prior to harvest. They were grown at 37°C in humidified conditions in 7% CO₂/93% air.

Glutathione assay

Cells were seeded in five 100 mm Petri dishes at a cell density to ensure that cells were in exponential growth phase at the time of assay, with all cell lines refed with fresh medium 48 h prior to harvest. Cells were washed twice in ice-cold PBS, with the cells from 3 plates lysed using 0.6% sulfosalicylic acid at 4°C. The supernatant was then aspirated from each plate and assayed individually for total GSH content as previously described (Tietze, 1969). The remaining 2 dishes were assayed for protein content using the method of Bradford (1970).

Enzyme assays

Cells were washed twice in PBS, resuspended in 2 ml PBS with 0.005 M EDTA, lysed by sonication and the supernatant stored at -80°C prior to use, with all experiments performed in triplicate.

Glutathione transferases

GST activity was measured using 1-chloro-2,4 dinitrobenzene (CDNB) as substrate, as previously described by Habig et al. (1974), monitoring spectrophotometric absorbance at 340 nm.

Glutathione reductase

Glutathione reductase (GR) activity was measured as previously described (Massey & Williams, 1965), following coupling of the substrate to GSH, with the reaction monitored at a wavelength of 412 nm.

γ-glutamyl-transpeptidase

γ-glutamyl transpeptidase activity was measured using L-γ-glutamyl-p-nitroanilide as substrate as described by Sasz (1969), monitoring the reaction at an absorbance of 412 nm.

Superoxide dismutases

Superoxide dismutase activity was estimated using pyrogallol, as previously described (Marklund & Marklund, 1974), with inhibition of the autooxidation of pyrogallol monitored at a wavelength of 420 nm.
Table I Characteristics of lung cancer cell lines: C-SCLC = classic small cell lung cancer, V-SCLC = variant small cell lung cancer, LCC = large cell anaplastic lung cancer. T = previous chemotherapy, U/T = untreated patient, XRT = previous radiotherapy treatment status unknown. Chemosensitivity expressed as the IC\textsubscript{50} (± 1 s.d.), however where no bracket is shown this represents the results of one experiment.

| Lung cancer cell line | Histologic type | Patient treatment status | Adriamycin (nM) | Melphalan (μM) |
|-----------------------|-----------------|--------------------------|----------------|---------------|
| (a) Small cell lung cancer lines | | | | |
| NCI-H60 | C-SCLC | T | 171.0 (134) | 2.8 (2.9) |
| NCI-H69 | C-SCLC | T | 127.0 (46) | 10.1 (4) |
| NCI-H82 | V-SCLC | T | 94.0 (71) | 7.9 (6.2) |
| NCI-H128 | C-SCLC | T | 110.0 (126) | 20.8 (8) |
| NCI-H1146 | C-SCLC | T | 144.0 (86) | 13.8 (8) |
| NCI-H1187 | C-SCLC | U/T | 25.5 (22) | 1.8 (2) |
| NCI-H209 | C-SCLC | U/T | 24.8 (7.2) | 0.2 (0.1) |
| NCI-H249 | C-SCLC | T | 127.0 (123) | 10.2 (11) |
| NCI-H417 | V-SCLC | U/T | 12.9 (3) | 0.8 |
| NCI-H524 | V-SCLC | T | 15.8 (3.9) | 2.8 (2.4) |
| NCI-H526 | V-SCLC | U/T | 37.0 (17) | 0.1 (0) |
| NCI-H678 | C-SCLC | U/T | 61.0 | 6.3 (8) |
| NCI-H1719 | V-SCLC | U/T | 10.1 (7.7) | 0.1 |
| NCI-H841 | V-SCLC | T | 231.0 (162) | 84.0 (23) |
| NCI-H889 | C-SCLC | U/T | 13.4 (6.5) | 3.6 (2) |
| (b) Non-small cell lung cancer lines | | | | |
| NCI-H23 | Adenocarcinoma | U/T | 38.7 (26) | 8.7 (7) |
| NCI-H125 | Adenocarcinoma | U/T | 216.0 (58) | 8.2 (3) |
| NCI-H157 | LCC | U/T | 238.0 (167) | 102.0 (60) |
| NCI-H226 | Squamous | U/T | 221.0 (90) | 56.3 (17) |
| NCI-H290 | Mesothelioma | U/T | 26.8 (14) | 3.7 (6.6) |
| NCI-H322 | Adenosquamous | U/T | 173.0 (165) | 75.5 (51) |
| NCI-H338 | Adenocarcinoma | U/T | 85.0 (56) | 31.0 (9) |
| NCI-H460 | LCC | U/T | 16.5 (8) | 1.6 (0.4) |
| NCI-H520 | Squamous | U/T | 411.0 (132) | 23.3 (9) |
| NCI-H522 | Adenocarcinoma | U/T | 197.0 (72) | 30.3 (18) |
| NCI-H596 | Adenosquamous | XRT | 813.0 (207) | 60.7 (30) |
| NCI-H647 | Adenocarcinoma | XRT | 115.0 (10) | 55.3 (32) |
| NCI-H661 | LCC | U/K | 130.0 (57) | 20.4 (19) |
| A549 | Adenocarcinoma | U/K | 57.7 (32) | 11.4 (7) |
| JMN | Mesothelioma | U/K | 40.2 (13) | 3.3 (1) |

Results

Details of the cell lines used are shown in Table I (Carney et al., 1985, Gazdar et al., 1985). For the purpose of these studies, the in vitro histology was used, with the in vivo histology from the original biopsy different in only 3 cell lines: NCI-H322, NCI-H338 and NCI-H522 (Carmichael et al., 1988). In addition, this table shows the sensitivity of these cell lines to 2 commonly used cytotoxic drugs, adriamycin and melphalan, whose effects are known to be modulated by GSH (Green et al., 1984; Russo et al., 1984; Russo & Mitchell, 1985). Chemosensitivity assays were performed using the MTT assay (Carmichael et al., 1987), and form part of a more detailed study of the chemosensitivity profile of these cell lines (Carmichael et al., 1988). SCLC lines derived from untreated patients were more sensitive to adriamycin than cell lines established from previously treated SCLC or NSCLC patients respectively: (mean IC\textsubscript{50} 26 vs. 127 vs. 185 nM). Similar differences were observed for melphalan (mean IC\textsubscript{50} 1.8 vs. 19.0 vs. 33.0 μM).

GSH levels and activities of the various enzymes are listed in Tables II and III for SCLC and NSCLC lines respectively. Groups were compared and analysis performed using the Kolmogorov-Smirnov test. In general, levels of all of these parameters were lower for SCLC lines than for NSCLC lines. The mean GSH level for the SCLC lines was 42 nmol mg\textsuperscript{-1} protein compared to 130 nmol mg\textsuperscript{-1} cytosolic protein for NSCLC lines (P = 0.005). Similarly, in NSCLC lines enzyme activity was increased for all 4 enzymes compared to SCLC lines although these changes did not achieve statistical significance: mean GSH 68 ± 37 units (P = 0.137), GR 139 vs. 82 units (P = 0.05), γ-GT 9.39 vs. 3.03 units (P = 0.072) and SOD levels 20 vs. 15.6 units mg\textsuperscript{-1} protein (P = 0.137). Of interest, there were no differences in GSH or enzyme activities comparing SCLC lines derived from untreated patients with lines established from patients who had previously received chemotherapy. No significant difference in any parameter was observed between histological sub-types of NSCLC.

Discussion

Thirty human lung cancer cell lines were analysed for GSH content, and for levels of a number of related detoxification enzymes. These cell lines exhibited a similar histological profile to that observed clinically, and their chemosensitivity profile has been shown to closely resemble that seen in clinical practice (Carmichael et al., 1988).

GSH levels were found to be significantly lower in SCLC lines with no detectable difference between variant small cell lines and cell lines exhibiting the classical phenotype (Carney et al., 1985). Surprisingly, no differences in GSH levels were detected comparing SCLC lines established from treated and previously untreated patients despite the fact that differences in chemosensitivity were maintained between the groups in vitro. The lack of correlation between GSH levels and treatment status is particularly surprising, in that many of these patients had been heavily pretreated with classes of cytotoxic agents which have been shown to interact with GSH (Doroshow et al., 1980; Berrigan et al., 1982; Dulik et al., 1986). However, it is possible that GSH levels decreased on serial passage in tissue culture. Certainly, in some malignant ascites samples obtained in this laboratory (JBM), GSH levels had fallen to less than 30% of primary tumour levels within 5 passages (unpublished data).
The difference in GSH levels between SCLC and NSCLC cell lines is extremely interesting in view of the importance of this tripeptide in the cellular response to cytotoxic drugs, although probably of more importance is whether there are similar differences between normal and neoplastic tissues. Of interest, buthionine sulfoximine (Dethmers & Meister, 1981) is shortly to enter clinical trial in the USA, making modulation of GSH levels a realistic possibility clinically. Marked differences in enzyme activity were noted between SCLC and NSCLC lines. Some cell lines exhibited very high levels of one or more of these enzymes, but there was no apparent correlation with histological type, although it should be emphasised that numbers in these groups were small.

Of particular interest were the 2 mesothelioma cell lines which have previously been shown to be relatively sensitive in vitro to a number of cytotoxic drugs (Carmichael et al., 1988) and to radiation (Carmichael, unpublished), in contrast to clinical experience with this tumour type (Chahinian, 1982). No obvious difference in GSH levels was observed in these cell lines compared to other NSCLC lines, although GST and SOD activity were reduced in both lines.

GST activity was measured in this study, using CDNB as substrate. However, it is known that these proteins exhibit variable substrate specificity (Mannervik, 1985) and are representative of a multigene family. An acidic transferase has been implicated in the resistance associated with pre-neoplasia (Kitahara & Satoh, 1983; Faber, 1984) and in MCF 7 cells in vitro, that express the multidrug resistance phenotype (Batist et al., 1986). In an attempt to identify a resistance ‘marker’ for lung cancer, it is intended to extend these studies to include an analysis of the GST isoenzyme pattern of these cell lines. In addition, it is intended to extend these studies to include an assessment of other detoxification enzymes. In view of the multi-drug resistance pattern exhibited by some of these lines (Bech-Hansen et al., 1976). It is possible that differences in expression of multidrug resistance genes (Fojo et al., 1987) account for some of the observed differences in chemosensitivity and this is currently being assessed.

In a panel of 30 cell lines covering the major histological sub-groups of lung cancer, significant differences were observed in GSH levels and in the activity of a number of detoxification enzymes. These differences were apparent in cell types where drug resistance is considered intrinsic. In contrast, in acquired drug resistance, such as in cell lines derived from previously treated patients, these differences were no longer apparent. Whether these differences are an in

### Table II: Glutathione levels and related enzyme activity of human small cell lung cancer cell lines

| Cell line | GSH (nmol mg⁻¹ protein) | GST (nmol CDNB conjugated mm⁻¹ mg protein⁻¹) | GR (nmol NADPH utilised mm⁻¹ mg protein⁻¹) | y-GT (nmol L-glutamyl-p-nitroanilide metabolised mm⁻¹ mg protein⁻¹) | SOD (unit/mg protein) |
|-----------|------------------------|---------------------------------------------|---------------------------------------------|-------------------------------------------------|-----------------------|
| NCI-H117  | 41±9                   | 44±30                                       | 51±30                                       | 3.9±2.3                                         | 15±11                 |
| NCI-H209  | 56±7                   | 48±41                                       | 67±20                                       | 1.9±1.6                                         | 10±7                  |
| NCI-N417  | 24±0                   | 4±2                                         | 102±17                                      | 0.5±0.3                                         | 8±3                   |
| NCI-H526  | 54±15                  | 107±85                                      | 106±43                                      | 1.8±1.3                                         | 21±11                 |
| NCI-H678  | 32±10                  | 41±22                                       | 115±38                                      | 1.9±1.5                                         | 17±10                 |
| NCI-H719  | 54±20                  | 67±14                                       | 72±69                                       | 6.2±1.5                                         | 16±2                  |
| NCI-H889  | 66±20                  | 15±3                                        | 92±17                                       | 2.6±1.6                                         | 13±15                 |
| NCI-H60   | 43±28                  | 15±6                                        | 57±26                                       | 2.2±1.4                                         | 15±17                 |
| NCI-H69   | 48±14                  | 32±37                                       | 108±43                                      | 1.7±0.8                                         | 10±3                  |
| NCI-H82   | 31±5                   | 25±12                                       | 83±59                                       | 2.1±1.9                                         | 10±8                  |
| NCI-H116  | 26±28                  | 45±35                                       | 50±35                                       | 6.0±6.6                                         | 32±28                 |
| NCI-H146  | 50±14                  | 34±29                                       | 83±29                                       | 4.6±5.0                                         | 10±7                  |
| NCI-H249  | 24±15                  | 28±26                                       | 87±41                                       | 1.9±1.6                                         | 8±5                   |
| NCI-H524  | 25±19                  | 6±3                                         | 48±36                                       | 4.5±4.0                                         | 9±2                   |
| NCI-H841  | 58±1                   | 37±9                                        | 114±46                                      | 3.1±2.7                                         | 8±1                   |

GSH = Glutathione content – nmol mg⁻¹ protein; GST = Glutathione transferase activity – nmol CDNB conjugated min⁻¹ mg prot⁻¹; GR = Glutathione reductase activity – nmol NADPH utilised min⁻¹ mg prot⁻¹; y-GT = y-glutamyl transpeptidase activity – nmol L-glutamyl-p-nitroanilide metabolised min⁻¹ mg prot⁻¹; SOD = Superoxide dismutase activity – 1 unit=Concentration of SOD causing 50% reduction in the autoxidation of pyrrogallol. Levels represent the mean of 3 determinations ±1 s.d.

### Table III: Glutathione levels and related enzyme activity of human non-small cancer cell lines

| Cell line | GSH (nmol mg⁻¹ protein) | GST (nmol CDNB conjugated mm⁻¹ mg protein⁻¹) | GR (nmol NADPH utilised mm⁻¹ mg protein⁻¹) | y-GT (nmol L-glutamyl p-nitroanilide metabolised min⁻¹ mg protein⁻¹) | SOD (unit/mg protein) |
|-----------|------------------------|---------------------------------------------|---------------------------------------------|-------------------------------------------------|-----------------------|
| NCI-H223  | 103±26                 | 117±0                                       | 54±24                                       | 4.0±4.0                                         | 17±9                  |
| NCI-H125  | 100±33                 | 43±22                                       | 129±52                                      | 5.5±2.9                                         | 14±9                  |
| NCI-H157  | 65±22                  | 46±17                                       | 75±16                                       | 3.6±1.2                                         | 16±12                 |
| NCI-H226  | 210±51                 | 39±37                                       | 70±8                                        | 2.7±2.1                                         | 27±15                 |
| NCI-H290  | 140±10                 | 38±22                                       | 74±30                                       | 5.8±2.7                                         | 11±2                  |
| NCI-H322  | 150±27                 | 38±18                                       | 126±74                                      | 6.0±8.0                                         | 25±26                 |
| NCI-H358  | 100±51                 | 67±39                                       | 152±63                                      | 7.0±4.1                                         | 13±9                  |
| NCI-H460  | 220±90                 | 43±19                                       | 251±85                                      | 7.2±4.7                                         | 11±3                  |
| NCI-H520  | 160±61                 | 232±177                                     | 139±53                                      | 2.5±1.0                                         | 23±11                 |
| NCI-H522  | 80±42                  | 109±89                                      | 146±53                                      | 3.5±2.9                                         | 18±8                  |
| NCI-H596  | 140±70                 | 16±14                                       | 267±200                                     | 6.7±4.3                                         | 49±36                 |
| NCI-H647  | 160±43                 | 117±97                                      | 251±53                                      | 56.5±35                                        | 46±39                 |
| NCI-H661  | 100±16                 | 79±79                                       | 74±22                                       | 5.4±3.8                                         | 15±7                  |
| JMN       | 80±11                  | 24±24                                       | 44±9                                        | 3.3±2.6                                         | 8±1                   |
| A549      | 140±37                 | 24±16                                       | 235±144                                     | 12.1±4.3                                        | 10±8                  |

GSH = Glutathione content – nmol mg⁻¹ protein; GST = Glutathione transferase activity – nmol CDNB conjugated min⁻¹ mg prot⁻¹; GR = Glutathione reductase activity – nmol NADPH utilised min⁻¹ mg prot⁻¹; y-GT = y-glutamyl transpeptidase activity – nmol L-glutamyl-p-nitroanilide metabolised min⁻¹ mg prot⁻¹; SOD = Superoxide dismutase activity – 1 unit=Concentration of SOD causing 50% reduction in the autoxidation of pyrrogallol. Levels represent the mean of 3 determinations ±1 s.d.
vitro artefact remains unanswered, although it should be stressed that in the majority of in vitro derived resistance models changes in these enzymes are frequently observed. This cell line panel may prove to be a valuable tool for the study of clinical drug resistance in lung cancer.

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