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Evidence of differential cisplatin–DNA adduct formation, removal and tolerance of DNA damage in three human lung carcinoma cell lines

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The expression of intrinsic resistance to cisplatin in two lung cancer cell lines, one derived from a small cell carcinoma (SW1271) and the other from an adenocarcinoma (A549), relative to a drug-sensitive small cell line SW900, was characterized by: (i) expression of cross-resistance to mitomycin C and cadmium chloride, but increased sensitivity to adriamycin and etoposide; (ii) significantly decreased cisplatin uptake; (iii) elevated levels of glutathione which could be reduced by buthionine L-sulfoximine resulting in significant sensitization of the cells to cisplatin; (iv) a lack of consistent modification of metallothionein content and expression of levels of glutathione S-transferase, glutathione reductase and glutathione peroxidase or of activities of DT-diaphorase or catalase; (v) significantly reduced total DNA-platination levels immediately following a 1 h cisplatin treatment with 10 μg/ml (33.3 μM); (vi) increased removal of Pt–GG and Pt–AG adducts by the A549 cells, consistent with increased repair capacity, but a lack of removal of these major adducts by the SW1271 cells indicative of tolerance of this drug-induced DNA damage. These data therefore provide evidence of differential formation, repair and tolerance of DNA damage following exposure of three human lung carcinoma cell lines to cisplatin.

Key words: Cisplatin–DNA adducts, DNA repair, human lung carcinoma cell lines, metallothionein content.

Introduction

Approximately 25% of newly diagnosed lung cancers each year are small cell carcinoma, often characterized by rapid tumor progression and widespread metastatic disease.[1] Although cisplatin is an active drug for the treatment of small cell lung cancer (SCLC), its effectiveness is often limited by the development of drug resistance and non-small cell lung cancer (NSCLC) is considered intrinsically drug resistant.[2,3] Cisplatin can react with several cellular targets including membranes, proteins and RNA; however, its antineoplastic activity is generally associated with its bifunctional interactions with DNA.[4] Several mechanisms of cisplatin resistance have been proposed including alterations in drug accumulation, enhanced intracellular drug inactivation involving glutathione (GSH) and metallothionein (MT) metabolism, differential induction and/or repair of DNA damage, and tolerance of drug-induced DNA damage, as reviewed recently.[5,6] The development of polyclonal antibodies to digestion products of platinated-DNA (Pt–DNA)[7,8] has facilitated the study of the induction and removal of specific Pt–DNA adducts, so permitting an examination of the relationship between adduct formation and/or repair and cisplatin cytotoxicity. There are relatively few reports in which the mechanism(s) of 'inherent' drug resistance of SCLC and NSCLC have been analyzed.[9–11] In this present study our objective was to characterize two human lung carcinoma cell lines, one derived from a SCLC (SW900) and one from an adenocarcinoma of the lung (A549), with differential responses to cisplatin. In this study we have quantitated cisplatin uptake, the formation and removal of the four major Pt–DNA adducts, Pt–GMP, Pt–AG, Pt–GG and Pt–$(GMP)_2$, and examined GSH and MT contents and attempted to relate certain associated enzyme activities of the cells and their sensitivities to a range of other antitumor agents. In addition, we have assessed these same parameters in another SCLC cell line (SW1271) which expresses intrinsic cisplatin resistance relative to the most sensitive SW900 SCLC cell line.

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Materials and methods

Cell lines

The human lung adenocarcinoma cell line A549 was derived from a type II alveolar cell carcinoma.[12] The SCLC cell line SW900 was derived from a lymph node metastasis of a grade IV tumor whilst the SCLC SW1271 cell line was derived from a grade III tumor.[13] All three cell lines were derived from previously untreated patients. These lines, provided as a gift from Dr BH Long (Department of Experimental Therapeutics, Bristol-Myers Squibb Company, Wallingford, CN), were maintained as monolayer cultures in McCoys 5A medium supplemented with 10% fetal calf serum (Gibco, Paisley, UK), penicillin and streptomycin in a humidified atmosphere of 5% CO_2 in air. Characteristics of these three lines are listed in Table 1. Population doubling times were calculated from cell counts of duplicate 3 cm dishes (Nunc, Roskilde, Denmark) containing 10^4–10^5 cells, at daily intervals, using a Model ZBI Coulter Counter. Cell volumes were determined using the Coulter Counter, cellular protein content was estimated by the method of Lowry *et al.*[14] and DNA content was determined as described by Burton.[15]

Drug sensitivity assays

Drug sensitivities were evaluated by colony-forming assay following a 1 h [for cisplatin and mitomycin C (MMC)] or 24 h drug exposure [for adriamycin (ADR), etoposide (VP-16) and cadmium chloride] by plating control and drug-treated cells directly onto plastic. Cisplatin, buthionine L-sulfoximine (BSO) and cadmium chloride were purchased from Sigma (Poole, Dorset, UK), VP-16, ADR and MMC were kindly donated by Bristol-Myers (Evansville, IN), Farmitalia Carlo Erba (St Albans, Herts, UK) and Kyowa Hakko Kogyo (Tokyo, Japan), respectively. For cisplatin cytotoxicity potentiation experiments, cells were exposed to 20 μM BSO for 24 h with the addition of an IC_{50} concentration of cisplatin for the final hour of the BSO exposure. At the end of the incubation period cellular survival was assessed by colony-forming assay as detailed above and GSH levels were quantitated, as described below.

Total GSH content and glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GP) catalase and DT-diaphorase (DT-D) activities

Logarithmically growing cells were harvested 3 days after initial plating. These methodologies have been previously described: measuring GSH content by the GR method of Griffith,[16] GST according to the procedure of Habig and Jackoby,[17] using 1-chloro-2,4-dinitrobenzene as substrate, GR by the method of Horn[18] and GP using cumene hydroperoxide according to the modified method of Paglia and Valentine.[19] Cytosolic supernatants were prepared according to the procedure of Akman *et al.*[20] for DT-D estimations using the method of Ernster,[21] as modified by Benson *et al.*,[22] and for catalase activities using the method of Beutler.[23] Enzyme values were normalized for cellular protein content using methods described by Lowry *et al.*[14] or Bradford.[24] Data were compared statistically using Student's paired *t*-test.

MT content and gene expression

MT content was evaluated using a cadmium-binding assay procedure previously described by Eaton and Toal.[25] Briefly, samples were incubated at room temperature with ^{109}Cd solution for 10 min, followed by the addition of bovine hemoglobin solution to remove unbound labeled cadmium. Aliquots of the resultant supernatant were counted on a gamma-counter (LKB Climigamma model

Table 1. Cell lines characteristics

Parameter	SW900 cells	SW1271 cells	A549 cells
Population doubling time (h)	38 ± 2[a]	25 ± 3	17 ± 1
Colony-forming efficiency (%)	5–15	5–15	5–15
Cellular volume (μm^3)	2265 ± 72	3210 ± 80	3560 ± 59
DNA ($\mu g/10^6$ cells)	17.0 ± 0.8	16.7 ± 0.7	15.9 ± 1.2
Protein ($\mu g/10^6$ cells)	391 ± 4.7	350 ± 12.4	323 ± 19.6

[a] Mean values ± SE of at least two individual experiments.

1272). MT content was normalized for cellular protein by the method of Bradford[24] and total cell number per sample estimated by hemocytometer counting.

MT gene expression was examined by Northern blot analysis of total cellular RNA, isolated using guanidinium isothiocyanate[26] and separated by isopycnic centrifugation on a cesium trifluoroacetate gradient (Pharmacia, Milton Keynes, UK). RNA species were separated on a denaturing 1% agarose gel containing 6.7% formaldehyde and transferred to a nitrocellulose filter (Zetaprobe, Bio-Rad, Watford, Herts, UK) by capillary action in 10 × SSC. Hybridization of the filter was carried out at 42°C with 50% formamide using a 3.2 kb *Hin*dIII fragment of genomic human metallothionein II_A labeled with ^{32}P by random primer method of Feinberg and Vogelstein.[27] Following hybridization, filters were washed three times in 1 × SSC/1% SDS at room temperature for 30 min and twice in 0.1% SSC/1% SDS at 50°C for 30 min. To normalize for the amount of RNA loaded, the filter was stripped by incubation with 0.1 × SSC/0.5% SDS at 100°C for 30 min and then hybridized with a 40 nucleotide probe to 28S rRNA (Oncogene Sciences, Uniondale, NY) labeled on the 5′ OH with ^{32}P and T4 polynucleotide kinase (Gibco BRL, Gaitherburg, MD). Autoradiograms were scanned using an LKB Ultrascan XL laser densitometer and relative signal intensity was determined using the Gelscan HL software package (Pharmacia LKB Biotechnology, Piscataway, NJ).

Drug uptake studies

For analyses of cisplatin uptake, cells (2×10^6) were exposed to 0, 5, 10 or 20 μg/ml cisplatin for 1 h. Then, according to the procedure described earlier,[28] cells were harvested and washed twice in ice-cold PBS. Total cell number per sample was calculated by hemocytometer counting. Cell pellets were dried at 65°C for 2 h prior to solubilization in concentrated nitric acid. Drug uptake was quantitated by atomic absorption spectroscopy (Perkin Elmer model 4000) and results were expressed as pmol $Pt/10^6$ cells and then corrected for differences in cellular volume

Quantitation of Pt–DNA adducts

Cells were treated for 1 h with 10 μg ml (33.3 μM) cisplatin solubilized in 0.9% saline immediately prior to use, and then washed with PBS and harvested immediately or after an 18 h incubation in drug-free medium to permit 'repair' or adduct removal to occur.

Cells were lysed in the presence of 100 mM ammonium bicarbonate to inactivate monofunctionally bound drug and DNA was isolated using a phenol/chloroform procedure,[29] and enzymatically digested to nucleotides and platinated nucleotides. Separation of platinated products was carried out using an anion exchange chromatography column.[7] Details of the competitive enzyme-linked immunosorbent assay (ELISA) procedure using antiserum W101 to quantitate Pt–GG and $Pt–(GMP)_2$ have been described in an earlier publication.[30] Antisera 3/65 and 3/43 were used to detect Pt–AG and Pt–GMP adducts, respectively.[8] Since Pt–DNA adducts were quantitated per g DNA isolated, correction for DNA synthesis during the 18 h repair period was carried out using parallel cultures, as described earlier.[30] The dilution factor was calculated as the ratio of the specific activity of DNA (d.p.m. of [^{14}C]thymidine template-labeled DNA per μg DNA measured spectrophotometrically) at 18 h compared with that value at 0 h. The apparent number of lesions at 18 h divided by the dilution factor gave the 'true' level of Pt–DNA adducts.

Results

In vitro drug sensitivity evaluations

Figure 1 shows the dose–response curves following a 1 h exposure of logarithmically growing cultures of the three cell lines to cisplatin. From duplicate experiments the mean IC_{50} values were calculated. These are listed in Table 2 and indicate that line SW900 was most sensitive to cisplatin, whilst the adenocarcinoma line A549 was approximately 3-fold more resistant. The other SCLC cell line, SW1271, however, proved most resistant with an IC_{50} value differing by a factor of approximately 5 from that of the SW900 cells. These differences are statistically significant ($p < 0.002$) as judged by Student's *t*-test. A similar pattern of response was noted in terms of cadmium chloride sensitivities, with the resistant lines proving 2- and 2.7-fold less sensitive than the SW900 cells ($p < 0.02$ and $p < 0.002$, respectively). Line SW900 was also most sensitive to MMC, with lines A549 and SW1271 proving 2.2- and 8.7-fold more resistant, respectively. In contrast, SW900 cells were significantly

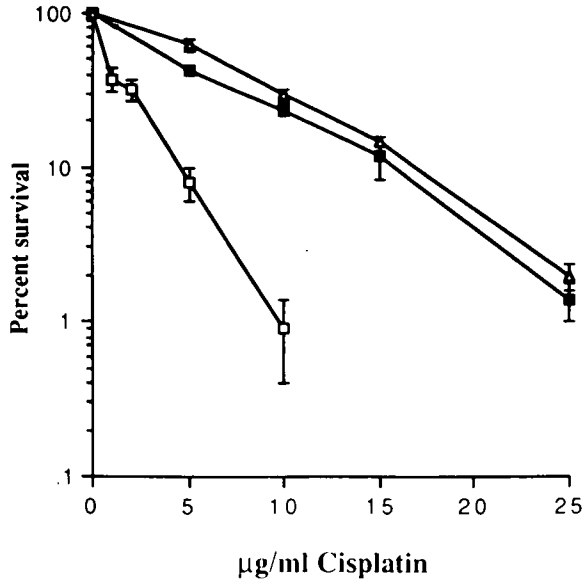

Figure 1. Survival curves for SW900 (□), SW1271 (△) and A549 (■) cells exposed to a range of concentrations of cisplatin for 1 h. Points represent the mean ± SE of two to three experiments in which replicate cultures were treated and duplicate samples from each were analyzed.

more resistant to ADR (~3-fold) and to VP-16 (~11-fold) than either of the other two lines tested. These variable responses to the range of cytotoxic agents tested clearly could not be correlated with the 2-fold difference in population doubling times noted amongst these three cell lines.

Cellular content of GSH, GR, GST, GP and MT, and activities of catalase and DT-D

Line SW900 had the lowest total cellular GSH content (see Table 3). In the more cisplatin-resistant lines A549 and SW1271, GSH content was significantly elevated by factors of 16 and 6, respectively ($p < 0.001$ and $p < 0.002$); however, it should be noted that this did not correlate with their relative cisplatin sensitivities. Depletion of total GSH content by 92–78% was achieved by incubation of the cells with 20 μM BSO for 24 h. When cisplatin was added during the last hour of the incubation period, IC_{50} values were reduced by

Table 2. *In vitro* drug sensitivity assay data

Agents tested	Clonogenic assay IC_{50} values (ng/ml)[a]		
	SW900 cells	SW1271 cells	A549 cells
Cisplatin (1 h)	1300 ± 200	6200 ± 100	4000 ± 100
Mitomycin C (1 h)	46 ± 5	400 ± 33	103 ± 10
Adriamycin (24 h)	29.2 ± 0.2	9.7 ± 1.0	12.4 ± 0.4
Etoposide (24 h)	1350 ± 10	107 ± 13	140 ± 7
Cadmium chloride (24 h)	700 ± 50	1900 ± 100	1400 ± 100

[a]Mean values ± SE from full dose–response curves obtained by colony-forming assays and generated from at least two repeat experiments. IC_{50} values represent the drug concentrations required to reduce cell survival by 50%.

Table 3. Glutathione levels and related enzyme activities, MT content and DT-D and catalase activities

Parameter measured[a]	SW900 cells	SW1271 cells	A549 cells
GSH (nmol)	16 ± 2	99 ± 14	262 ± 23
GR (nmol NADPH/min)	36 ± 2	11 ± 1	131 ± 14
GST (nmol CDNB/min)	218 ± 21	124 ± 17	166 ± 16
GP (nmol NADPH/min)	11.9 ± 1.4	9.1 ± 0.3	5.5 ± 1.0
MT (μg/10^7 cells)	10.7 ± 1.5	37.9 ± 2.0	0.35 ± 0.07
DT-D (μmol/min)	1130 ± 283	3.0 ± 2.0	4318 ± 676
Catalase (μmol H_2O_2/min)	2.1 ± 0.5	3.7 ± 0.5	9.5 ± 0.7

[a]All the values were normalized per mg of cellular protein content except for MT content and represent the mean of at least two experiments ± SE.

factors of 2.5 and 2.6 in the A549 and SW1271 cells, respectively, appearing to represent more efficient modulation of GSH levels in the latter cells, but no alteration was noted with the SW900 cells.

No consistent pattern of alterations in the GSH-associated enzymes was apparent in these three lines. The more resistant SCLC cell line SW1271 had significantly lower activities of GR ($p < 0.002$) and GST ($p < 0.01$) than the more sensitive SW900 line, while the adenocarcinoma line A549 expressed significantly increased GR activity ($p < 0.01$) and reduced GP levels ($p < 0.02$).

In an attempt to explain the differential responses of these lines to MMC, ADR and VP-16 (see Table 2), activities of DT-D and catalase were quantitated. The more resistant A549 cells had a 4-fold higher level of DT-D than the most sensitive SW900 cells ($p < 0.02$) (see Table 3). However, the most MMC-resistant line (SW1271) had minimal DT-D activity. Lines A549 and SW1271 had higher catalase activities than SW900 cells by factors of 4.5 and 1.8, respectively, suggestive of an increased capacity to detoxify free radicals, although this contrasted with their increased sensitivity to both ADR and to VP-16.

The differing sensitivities of these three lung cancer cell lines to cisplatin and to cadmium chloride were not reflected in their cellular MT contents, since the lowest level of MT was noted in the more resistant adenocarcinoma A549 line, although when comparing the two SCLC cell lines, the 5-fold more resistant SW1271 cells had a 3.6-fold higher MT content than the most sensitive SW900 cells. Data in Figure 2 illustrate that the varied MT content of the three lines was associated with differing levels of MT gene expression. By ascribing a value of 1.0 to the A549 cells (lane 1), the relative MT RNA levels of the SW900 and SW1271 cells were 9.6 and 22, respectively. Approximately equal loading was similarly demonstrated with 28S values of 0.422, 0.415 and 0.457 for lanes 1, 2 and 3, respectively (data not shown).

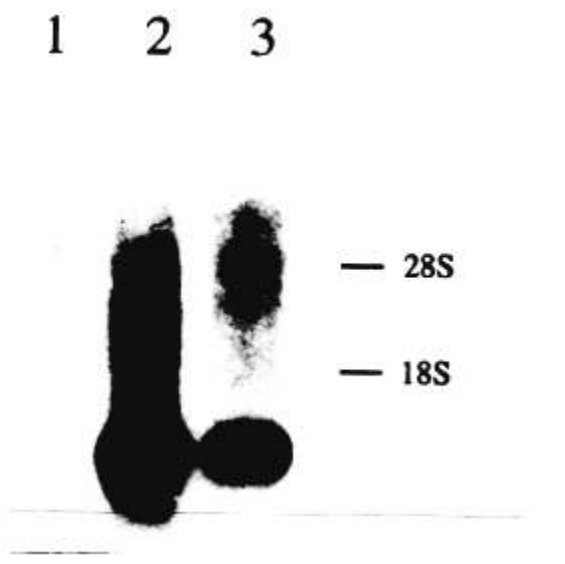

Figure 2. Expression of MT gene. Lane 1, A549 cells; lane 2, SW1271 cells; lane 3, SW900 cells (10 μg RNA per lane). After normalization to 28S values, relative densitometry readings for MT RNA levels were 0.87, 21.00 and 9.04, respectively.

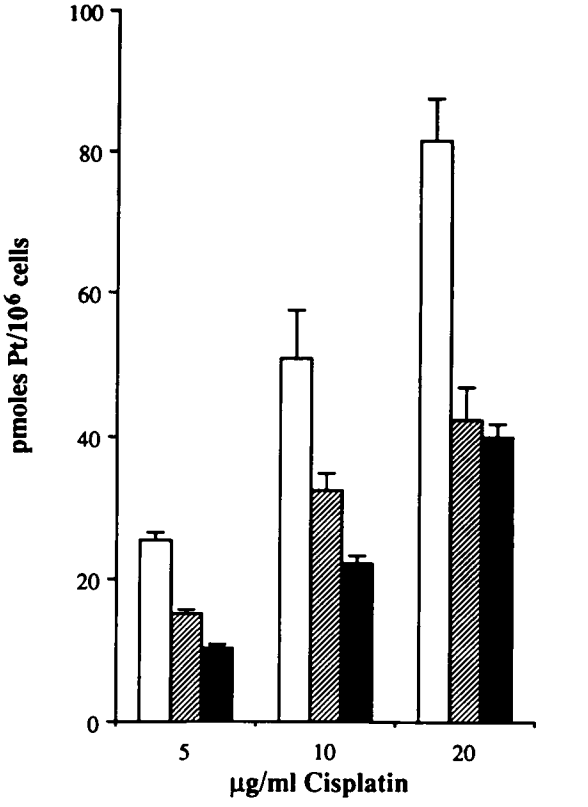

Figure 3. Uptake of cisplatin equated for cellular volume by monolayer cultures of SW900 (□), SW1271 (▨) or A549 cells (▩) following a 1 h drug incubation at 37°C. The values have been normalized for differences in cellular volume (see Table 1), ascribing unity to the larger A549 cells and represent the mean ± SE of two experiments analyzing duplicate samples.

Cellular uptake of cisplatin

Dose-dependent uptake of cisplatin by each of the three cell lines was established (see Figure 3) and the results indicate that uptake was greatest by the most cisplatin sensitive SW900 cells. Values for cisplatin uptake, normalized to an extracellular drug concentration of 1 μg/ml per unit cellular volume, for the SW900, A549 and the SW1271 cells were 1.33 ± 0.18, 0.60 ± 0.04, 0.81 ± 0.13 pmol $Pt/10^6$

cells/μg/ml cisplatin/unit of cell volume ± SE, respectively. The SW900 cells accumulated significantly more platinum than either the SW1271 or A549 cells ($p < 0.001$).

Induction and removal of Pt–DNA adducts

The specific activity of radiolabeled DNA decreased in the two SCLC cell lines during the 18 h repair period. Calculated dilution factors were 0.83 ± 0.07 for SW1271 cells and 0.77 ± 0.02 for SW900 cells, but was 1.00 ± 0.02 for line A549. Total DNA platination levels, calculated as the sum of the four specific adducts measured (by competitive ELISA), are listed in Table 4. In these studies, equimolar rather than equitoxic concentrations were used, since these were considered more clinically relevant since patients receive standard cisplatin doses. Furthermore, a direct relationship between total adduct formation and cisplatin concentration used has been described.[31,32] The highest level of DNA-platination immediately after a 1 h exposure to cisplatin (10 μg/ml) was identified in the most cisplatin sensitive cell line, SW900, with approximately 2.2- to 2.8-fold lower levels appearing characteristic of the two more resistant lines. Following an 18 h 'repair' period, A549 cells appeared most proficient in adduct removal. In contrast, the more cisplatin-resistant cell line, SW1271, was apparently unable to remove any of the adducts during this post-treatment incubation period. Histograms of specific adduct formation and removal are shown in Figure 4. In terms of the distribution of adducts as a percentage of the total platination, in all three cell lines the major adduct formed was Pt–GG, comprising 40–50% of the total platination immediately after drug treatment and rising marginally to 48–57% 18 h after drug removal. This suggests that additional bifunctional adducts may be formed during the 'repair' period, since the monofunctional adduct Pt–GMP comprised 25–32% of the total platination at zero time but by 18 h the levels in all cell lines had fallen to between 14 and 19%, probably indicative of adduct rearrangement rather than DNA repair of this

Table 4. Total platination of DNA measured by competitive ELISA immediately and 18 h after exposure to 10 μg/ml cisplatin for 1 h

	Amount in fmol Pt/μg DNA[a]		
	SW900 cells	SW1271 cells	A549 cells
0 h	40.9 ± 2.4	14.5 ± 2.4	19.3 ± 1.0
18 h	31.3 ± 3.0	17.5 ± 1.6	11.7 ± 1.0
Removal rate[b]	24%	0%	39%

[a] Total binding was calculated by adding together the amounts of the four individual adducts (see Figure 3). Values are the mean ± SE of two different ELISAs each performed on four dilutions in duplicate wells. Values at 18 h were corrected for dilution by DNA synthesis.
[b] Percentage remaining after 18 h post-treatment incubation period.

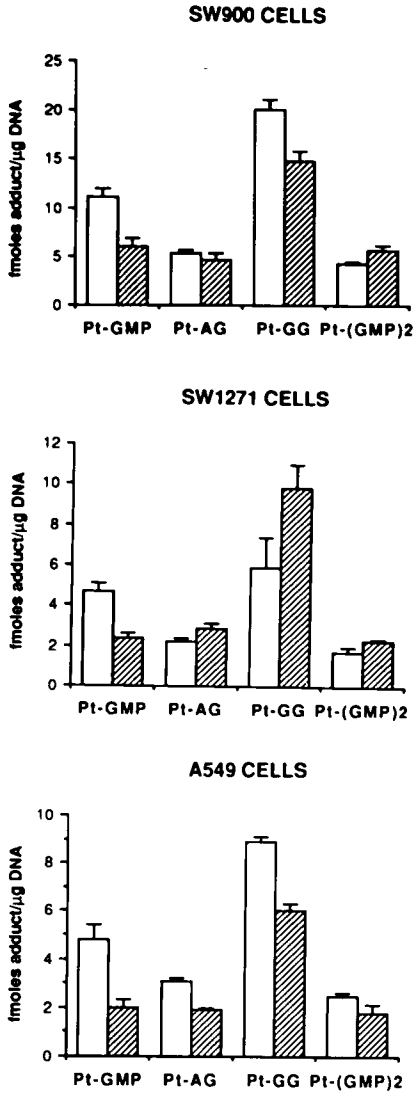

Figure 4. Induction and removal of Pt–GMP, Pt–AG, Pt–GG, and Pt–$(GMP)_2$ in SW900, SW1271 or A549 cells. The number of adducts were determined immediately after a 1 h exposure to 10 μg/ml cisplatin (□) and following an 18 h post-treatment incubation period (▨). Values are the mean ± SE of two different ELISAs performed in four dilutions in duplicate wells. Values at 18 h were correlated for dilution by DNA synthesis as described in Materials and methods.

lesion. The data in Figure 4 indicate that the A549 cells appeared proficient in the removal of all four adducts, although there was only modest removal of the bifunctional Pt–$(GMP)_2$ adduct. In contrast, both SCLC cell lines SW1271 and SW900 appeared deficient in removal of certain bifunctional Pt–DNA adducts. The most sensitive line SW900 appeared to remove only the major Pt–GG adducts formed, whilst SW1271 cells proved generally deficient in removal of Pt–AG, Pt–GG and Pt–$(GMP)_2$ adducts. In attempting to correlate adduct formation and removal with cisplatin sensitivities, it can be noted that significantly more lesions were formed in the most sensitive SW900 cells and since neither the Pt–AG nor the Pt–$(GMP)_2$ adducts were efficiently removed, these may constitute the lethal lesions. Arguing against this, however, is our documentation of the lack of removal of any of the major adducts by the more resistant SW1271 cells. It is noticeable though, that in the more resistant cell lines less adducts were formed immediately after drug treatment. In the A549 cells, a significant proportion of all these adducts were 'repaired' during the 18 h post-treatment incubation period, consistent with their increased survival. However, increased survival was also characteristic of the SW1271 cells which apparently were generally 'repair' deficient, as judged by lack of adduct removal, indicating perhaps an increased capacity to tolerate this drug-induced DNA damage.

Discussion

Lung cancer exhibits an interesting spectrum of resistance to drugs and to X-irradiation both clinically and experimentally.[33] In this study we have selected an inherently cisplatin-sensitive SCLC cell line (SW900) and compared its properties with those of two intrinsically cisplatin-resistant cell lines, one a NSCLC line derived from an adenocarcinoma (A549) and another from a SCLC (SW1271). The overall range of cisplatin sensitivities amongst these three lines was only 5-fold. This figure is comparable with the 4-fold range reported for six SCLC cell lines by Sasaki *et al.*,[34] but less than the $\geq$8-fold difference reported between lines established from adenocarcinomas as opposed to SCLCs. Researchers specifically selecting for cisplatin-resistant lung cancer cell lines have also frequently worked with sublines expressing only modest levels of resistance (i.e. 3- to 7-fold),[11,32,35,38] which are considered clinically relevant, although others have used lines with much higher orders of resistance (11- to 45-fold).[11,35,37,39,41]

Our observation that the two cisplatin-resistant cell lines (A549 and SW1271) showed increased sensitivity to both ADR and VP-16 when compared with SW900 is in agreement with an earlier report comparing two other SCLC lines[42] and with that from other groups who reported some collateral sensitivity to VP-16 in an 11-fold resistant SCLC subline H69/cisplatin,[37] a 28-fold resistant NSCLC PC-9/cisplatin subline[11] and in an 11.4-fold resistant squamous carcinoma lung subline PC10-B3.[43] However, more generally a correlation between sensitivity to VP-16, ADR and cisplatin has been reported, e.g. in eight lung cancer lines not selected for *in vitro* resistance[41] and in the majority of eight drug-selected sublines.[35] The relative resistance of SW900 cells to VP-16 reported here confirms data from an earlier publication by Long *et al.*[13]

A549 and SW1271 cells also proved to be relatively resistant to MMC and cadmium chloride. The SW1271 cells showed the highest level of resistance to MMC (9-fold) relative to SW900 cells. These cells had only minimal DT-D activity suggesting a lack of activation of MMC to its cytotoxic species which would be in agreement with the earlier report of decreased sensitivity to MMC in a DT-D deficient human colon carcinoma cell line.[44] However, in the A549 cells resistance to MMC was associated with elevated DT-D activity, which may rule out a role for the enzyme in metabolic activation of MMC in this cell line. This 'atypical' response of A549 cells, showing resistance to MMC yet elevated DT-D activity, was also noted when these cells were studied as xenografts.[45] In the overall series of NSCLC lines evaluated by this group, however, the first indication of a relationship between DT-D content and MMC sensitivity was described. This contrasts with the lack of correlation reported by Robertson *et al.*[46] in a study of 15 human cell lines including four SCLC and seven NSCLC lines. Although, since we have previously described increased activity of DT-D in cisplatin-resistant human ovarian carcinoma sublines,[28,47] the possibility exists that this enzyme may mediate the cytotoxicity of other antitumor agents. Cross-resistance to cadmium chloride has been described in two independently derived cisplatin-resistant human lung cancer lines.[37,39] However, whilst in the H69 cisplatin cells this resistance was associated with increased MT content and overexpression of MT mRNA,[37] in the PC-9 cisplatin cells both parameters were decreased.[39] In this study MT may be implicated in

the relative resistance of the SW1271 versus the SW900 SCLC cell lines, but the very low MT content and expression of the A549 cells appears to rule out any involvement in the more resistant adenocarcinoma line. Thus, the role of MT in cisplatin resistance in lung cancer, as in other tumor types,[5,48] remains controversial.

Detoxification mechanisms have generally been considered to play a significant role in cisplatin resistance. In this study, elevated levels of GSH were identified in both of the more cisplatin-resistant cells and depletion by BSO significantly potentiated cisplatin cytotoxicity in these two lines, yet was without effect on the most sensitive SW900 cells. Increased GSH levels have been described previously in more cisplatin-resistant adenocarcinoma lines compared with more sensitive SCLC cell lines[38,49] and in various cisplatin-selected resistant lung cancer lines,[39,50,51] although this is again not an invariable finding.[37] GSH-related enzymes, especially the GSTs, have been considered important factors in mediating cisplatin resistance. For example, Nakagawa *et al.*[9] reported that expression of GST-pi mRNA in three SCLC and three NSCLC cell lines inversely correlated with their sensitivities to cisplatin. However, Kasahara *et al.*[37] concluded that the GSTs played only a minor role in the human SCLC cell lines they examined and Hospers *et al.*[32] reported unaltered GST and GR levels in their GLC_4-cisplatin cell line. In our study no consistent alterations were identified in GR, GP or GST activities. Also, it is noticeable that the increased resistance to ADR and VP-16 in the SW900 cells was not associated with enhanced activity of the detoxifying enzyme GP or of catalase. This would appear to contrast with the increased catalase activity associated with *in vivo* acquired drug resistance in three cell lines derived from one patient with SCLC during clinical follow-up and treatment.[10]

Decreased cisplatin accumulation has been a consistent, but not invariable, finding in many cisplatin-selected resistant cell lines from a variety of species, as reviewed by Andrews and Howell[5] and has been reported in both SCLC-[36] and NSCLC-[39,43,52] resistant cell lines. Our observation of reduced platinum accumulation in the cells inherently resistant to cisplatin is consistent with these findings, although it contrasts with the lack of significant differences noted in other resistant SCLC sublines.[32,37]

In this study reduced platinum accumulation by the more cisplatin-resistant A549 and SW1271 cells was also reflected at the DNA level by a similarly reduced level of induction of total Pt–DNA adducts, compared with the levels achieved in the more sensitive SW900 cells. This contrasts with our previous data obtained comparing two ovarian[29] and two teratoma[30] cell lines with inherently differing responses to cisplatin or when evaluating three selected cisplatin-resistant cell lines,[28,47,53] where the most sensitive line had either a comparable level of adduct formation or a significantly reduced total platination level. However, Hospers *et al.*[40] reported a decreased level of total Pt–DNA binding in their 11-fold resistant GLC_4-cisplatin cell line, as well as decreased formation of interstrand cross-links. The percent distribution of adducts formed was very similar in all three of the lung cell lines tested here, with Pt–GG proving the major adduct, comparable with the pattern reported in other cell types.[29–31] However, the extent of adduct removal during the 18 h post-treatment incubation period was markedly different in the three cell lines. The most cisplatin-sensitive line SW900 showed some removal of Pt–GG lesions, but the extent was less than that noted in earlier investigations of TR175 ovarian[29] and RT112 bladder[30] cell lines which were considered repair proficient lines. Furthermore, negligible removal of Pt-AG or of the bifunctional $Pt–(GMP)_2$ lesions was noted in these SW900 cells. In contrast, the resistant adenocarcinoma A549 cell line showed a general proficiency in removal of all the adducts. This appears to be the first description of a lung cancer cell line in which altered repair of cisplatin-induced DNA damage appears to operate as a significant resistance mechanism, since repair of the major Pt–GG adducts was not significantly modified in the GLC_4-cisplatin-resistant line of Hospers *et al.*[40] and repair of interstrand cross-links was similar in the H69 parental and H69/cisplatin-resistant lines.[37] However, in the resistant SCLC cell line SW1271, effectively no repair of drug-induced DNA damage was detected. Our interpretation of this finding is that SW1271 cells appear to tolerate these lesions and replicate on this damaged template. This apparent deficiency in removing the major Pt–GG adduct has been identified in some of our earlier studies comparing ovarian tumor cell lines with differing inherent sensitivities to cisplatin[29,54] and also in an ovarian tumor cell line selected for cisplatin resistance.[47] The precise mechanism(s) associated with increased damage tolerance, which include alterations in DNA replication and post-replication repair,[55,56] remain to be defined. When attempting to interpret these data, it is also important to remember that the analytical

methods used here only measure DNA damage and its repair in the overall genome, so heterogeneity of drug-induced DNA damage and repair in specific genes, as reported recently in relation to cisplatin,[57,58] cannot be ruled out.

In summary, these data show that the lung cancer cell lines which were selected on the basis of their differential sensitivities to cisplatin, differ in multiple parameters. Altered repair of cisplatin-induced DNA damage appears implicated in the more cisplatin-resistant NSCLC line A549, although this is apparently adduct specific. However, the general lack of repair in the more resistant SCLC cell line SW1271 indicates that an alternative resistance mechanism, yet to be identified, is operating whereby these cells are able to tolerate the platination and replicate on this damaged template. Therefore, these human lung cancer cell lines provide useful models for elucidating mechanisms responsible for differential repair and tolerance of cisplatin-induced DNA damage.

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