Cellular basis for differential sensitivity to cisplatin in human germ cell tumour and colon carcinoma cell lines

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Summary Cisplatin (CDDP) resistance mechanisms were studied in a model of three germ cell tumour and three colon carcinoma cell lines representing intrinsically CDDP-sensitive and -resistant tumours respectively. The CDDP sensitivity of the cell lines mimicked the clinical situation. The glutathione levels of the cell lines correlated with CDDP concentrations inhibiting cell survival by 50% (IC₅₀): total cellular sulphhydryl content (TSH) was unexpectedly inversely correlated with IC₅₀. IC₅₀ correlated neither with glutathione S-transferase (GST) nor with GST's expression, topoisomerase I or II activity. Immediately after 4 h incubation with CDDP, platinum (Pt) accumulation and Pt bound to DNA were not correlated, but after another 24 h drug-free culture, Pt binding to DNA in germ cell tumour but not in colon carcinoma cell lines correlated with IC₅₀. With the exception of in vitro sensitivity and TSH, none of the parameters studied discriminated between the two groups of cell lines. Correlation of IC₅₀ with Pt accumulation did not influence statistical correlations. Analysis of variance revealed a correlation between IC₅₀ and the combination of glutathione, GST activity and Pt bound to DNA. But at other CDDP cytotoxicity levels sensitivity was also correlated with Pt accumulation, topoisomerase II activity and TSH in various combinations. This model of intrinsic CDDP resistance showed that multiple parameters ought to be studied to explain CDDP resistance, but did not elucidate the cause of the unique sensitivity of germ cell carcinoma, although the unexpected values of TSH deserve further attention.

Keywords: intrinsic resistance; Pt-DNA damage; sulphhydryl; glutathione; topoisomerase

The antineoplastic drug cis-diaminedichloroplatinum(II) (cisplatin, CDDP) is an extremely active drug in the treatment of patients with disseminated testicular germ cell tumours. Over 80% of these patients can be cured with a CDDP-containing drug regimen (Loehrer and Einhorn, 1984). In contrast, no cures can be obtained with this compound in patients with colon cancer and most other solid tumours (Loehrer and Einhorn, 1984). Understanding of CDDP sensitivity and resistance has predominantly come from the study of cell lines selected for CDDP resistance by in vitro drug incubations (for review see Andrews and Howell, 1990). These studies revealed several causes for CDDP resistance. Relevant mechanisms are decreased cellular accumulation of CDDP, increased detoxification, by either the glutathione (GSH) or the metallothionein system, and enhanced DNA repair, often resulting in decreased DNA platination (for review see Andrews and Howell, 1990). In most cases of in vitro acquired CDDP resistance, a combination of these factors is found. Unselected cell lines may provide a cellular model of drug resistance that is more representative of the clinical situation. Bedford et al. (1988) previously used germ cell and bladder tumour cell lines as the CDDP-sensitive and -resistant representatives of a model. In that study, a correlation of CDDP sensitivity with platinum (Pt) accumulation and deficient DNA damage repair in one of the two germ cell lines was found. In general, for germ cell tumour cell lines a greater sensitivity to CDDP has been reported compared with cell lines derived from other solid tumour types (Oosterhuis et al., 1984; Pera et al., 1987; Walker et al., 1987; Bedford et al., 1988; Parris et al., 1990; Kelland et al., 1992; Masters et al., 1993; Hill et al., 1994). In the present study parameters potentially relevant to CDDP sensitivity were examined in three germ cell tumour and three colon carcinoma cell lines, as their original tumours reflect extremely sensitive and resistant tumour types. Cellular detoxification mechanisms, Pt accumulation, DNA platination and repair and the nuclear enzymes DNA topoisomerase (topo) I and II were compared as they may contribute to cellular CDDP sensitivity. In order to achieve a good definition of the model, basic cellular characteristics of the cell lines were also determined.

Materials and methods CDDP was obtained from Bristol-Myers (Weesp, The Netherlands) and RPMI-1640 medium, Leibovitz L15 medium and fetal calf serum (FCS) from Life Technologies (Paisley, UK), GSH, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, sodium pyruvate, protease XXIV and propidium iodide were purchased from Sigma (St Louis, MO, USA). 5-Bromo-2'-deoxyuridine was obtained from Serva (Heidelberg, Germany), rabbit anti-mouse immunoglobulin [F(ab)² fragments, fluorescein conjugated] from Dakopatts (Glostrup, Denmark), glutamine from Flow Laboratories (Irvine, UK) and diaminobenzoic acid from Fluka (Buchs, Germany). Nucleo PI and DNase I were purchased from Boehringer Mannheim (Almere, The Netherlands).

For the cell lines used in this study, origin and pretreatment were as described in Table I. The subclone Ntera2/D1 (Tera; Timmer-Bosscha et al., 1993) of Tera-2 (Fogh, 1978), 833 KE (Bronson et al., 1980) and Scha (Andrews et al., 1987) were used as germ cell tumour cell lines and Colo 320 (Quin et al., 1979), SW 948 (Leibovitz et al., 1976), and Caco-2 (Fogh et al., 1977) as colon carcinoma cell lines. All cell lines, except SW 948, were cultured in RPMI-1640 with 10% heat-inactivated (30 min, 56°C) FCS. SW 948 was cultured in Leibovitz L15–RPMI-1640 (1:1) enriched with 12.5% FCS, 0.05 m pyruvate, 0.1 m glutamine and 0.025% (v/v) β-mercaptoethanol. Tera, 833 KE, SW 948 and Caco-2 were grown as monolayers, Colo 320 grew loosely attached and Scha grew attached and partly in suspension. Firmly attached cells were harvested by scraping or treatment of

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Received 3 August 1994; revised 18 November 1994; accepted 21 November 1994.
Table 1. Origin and previous treatment of the three germ cell tumour and the three colon carcinoma cell lines

| Cell line   | Tumour of origin | Site     | Previous treatment          | Reference       |
|-------------|------------------|----------|-----------------------------|-----------------|
| Tera-2, clone Ntera2/D1 | Embryonal carcinoma | Lung metastasis | Radio- and chemotherapy (no CDDP) | Fogh (1978) |
| 833 KE     | Embryonal carcinoma, teratoma, choriocarcinoma, seminoma | Abdominal metastasis | Chemotherapy (no CDDP) | Bronson et al. (1980) |
| Scha       | Embryonal carcinoma, seminoma | Bone metastasis | Radiotherapy | Andrews et al. (1987) |
| Colo 320   | Adenocarcinoma    | Colon    | No radio- or chemotherapy   | Quin et al. (1979) |
| SW 948     | Adenocarcinoma    | Colon    | No radio- or chemotherapy   | Leibovitz et al. (1976) |
| Caco-2     | Adenocarcinoma    | Colon    | No radio- or chemotherapy   | Fogh et al. (1977) |

Cultures with protease XXIV for 10 min. Scha and Colo 320 were harvested by gentle shaking. All cell lines were cultured at 37°C in a humidified atmosphere with 5% carbon dioxide.

CDDP sensitivity was analysed with the microculture tetrazolium assay as described previously (Timmer-Bosscha et al., 1989). Before the assays were performed, the linear relationship of cell number to formazan crystal formation was checked and cell growth studies were performed. Each cell line was seeded at optimum density in order to test survival after at least two or three cell divisions had taken place in the control cells. This was day 4 for Tera, 833 KE, Scha and Colo 320 and day 6 for SW 948 and Caco-2. For Tera, 833 KE and Scha 1 × 10⁴, 1.5 × 10⁴ and 3 × 10⁴ cells, respectively, were incubated continuously with a range of CDDP concentrations, in a total volume of 0.2 ml of culture medium in 96-well plates. For Colo 320, SW 948 and Caco-2, 2.5 × 10⁴, 5 × 10⁴ and 1 × 10⁴ cells, respectively were incubated continuously in a total volume of 0.1 ml. A minimum of three experiments per cell line was performed, each in quadruplicate.

Population doubling time during log phase was determined by cell count in a haemocytometer with trypan blue dye exclusion as a measure of viability. Experiments were repeated three times for each cell line. Cell cycle distribution was determined according to Preiser et al. (1992). In brief, exponentially growing cells were incubated with 10 μM 5-bromo-2-deoxyuridine for 30 min, washed with phosphate-buffed saline (PBS; pH 7.4), fixed in 70% ethanol and hybridized with hydrochloric acid of a molarity that gave optimal results (Tera, 833 KE, Scha and Colo 320, all 2.5 M; SW 948 and Caco-2, 3 M), subsequently incubated with anti-5-bromo-2-deoxyuridine monoclonal antibody, a second antibody [rabbit anti-mouse/F(ab')₂, fluorescein conjugated] and washed with PBS. Final propidium iodide 20 μg ml⁻¹ was added and fluorescence was analysed on a flow cytometer (FACS 440, Becton Dickinson, Sunnyvale, CA, USA). Calculations and statistical analysis of cell cycle distribution were performed with the analysis program Consort 30 version. G12/88 (Becton Dickinson). Reported values are the mean of three separate measurements. Relative cell volume was determined on a flow cytometer (FACStar, Becton Dickinson, Sunnyvale, CA, USA) scanning the forward scatter of unstained viable cells.

Cellular protein was determined according to Lowry et al. (1951). For nuclear protein determination cell nuclei were isolated as described by De Jong et al. (1990) and total nuclear protein contents were determined according to Bradford (1976). For both protein determinations three or more separate experiments were performed for each cell line. DNA content was measured in 0.5 × 10⁴ cells by the diaminobenzidine assay (Kissane and Robins, 1958). In each assay, a standard curve with salmon sperm DNA in 1 M ammonium hydroxide was included. A minimum of three separate experiments for each cell line were performed.

The conditions and measurements for GSH, total sulphhydryl content (TSH) and glutathione S-transferase (GST) activity in the cell lines were as described previously (Hospers et al., 1988) Reported values are the mean of three independent experiments. The amount of GSTπ isoenzyme was determined in all lines using sodium dodecyl sulphate-polyacrylamide gel electrophoresis followed by Western blotting and subsequent incubation with a monoclonal antibody raised against GSTπ (Peters et al., 1989, 1992). For each line the expression was determined in three independent cell protein extracts.

The topo I and II catalytic activities were determined in 0.35 M sodium chloride nuclear extracts of cells in the logarithmic phase of growth by relaxation of supercoiled PBR 322 (topo I) and the decatenation assay (topo II), with 270, 90, 30, 10, 3, 1 and 0.3 ng of protein, as described previously (De Jong et al., 1990). Experiments were performed in triplicate, while the small-cell lung cancer cell line, GLC4, was included as a reference (De Jong et al., 1990).

For determination of cellular Pt content, 5 × 10⁶ cells of each cell line were incubated for 4 h at 37°C with CDDP concentrations ranging from 33 to 100 μM. After washing three times with PBS (0°C) pellets were dissolved in 0.5 ml 65% nitric acid in an oven at 70°C for 2 h. Thereafter the Pt content was determined by atomic absorption spectrophotometry (AAS) as described previously (Hospers et al., 1988). At each CDDP concentration experiments were performed in triplicate or quadruplicate. For measurements of Pt bound to DNA a total of 5 × 10⁶ cells of each cell line was treated with CDDP concentrations ranging from 33 to 100 μM for 4 h at 37°C. Cells were washed three times with PBS (0°C). DNA was isolated and the amounts of DNA and Pt were measured as described previously (Hospers et al., 1988). At each CDDP concentration experiments were performed in quadruplicate. The kinetics of Pt bound to total nuclear DNA were determined after a 4 h incubation of 10⁶ cells with 16.5 μM CDDP in germ cell tumour cell lines. After incubation with 16.5 μM Pt bound to DNA in the Tera cells was at detection limit of the AAS. The main drawback of this dose was that it was 7–24 times the IC₅₀ of the germ cell tumour cell lines. In order not to favour the colon carcinoma cell lines in this respect, the colon carcinoma cell lines were incubated with 33 μM CDDP (4–8 times their IC₅₀). Incubation of the colon carcinoma cell lines with a higher CDDP concentration was avoided as it would lead to too high a level of Pt binding to DNA and therefore would invalidate the comparison. Immediately after incubation (t = 0) one part of each sample was washed with PBS (0°C, three times); in the other part medium was refreshed and cells were kept at 37°C for 24 h and then washed with PBS (t = 24) as above. Both parts were further processed for measurement of Pt bound to DNA. In order to decrease sample viscosity for Pt and DNA measurements, DNA was digested by nuclease P1 (7.8 μg ml⁻¹ cells) and DNAase I (8.4 U 10⁻⁶ cells) in a buffer containing 10 mM Tris, 4 mM magnesium chloride, 0.1 mM EDTA and 0.24 mM zinc sulphate. Pt amount in these samples was measured as described previously (Hospers et al., 1988) with in addition Zeeman background correction.

The addition of this equipment to the AAS apparatus allowed the differential correction of the background absorp-
tion over the temperature range used to atomise the Pt; this in contrast to the fixed background value, established at one temperature, obtained without the use of Zeeman correction. Absolute values of Pt bound to DNA after 24 h were corrected for DNA synthesis over this time course, by determination of the dilution of the specific activity of DNA of cells incubated with \(^3\)Hthymidine before CDDP incubation (Hospers et al., 1988). The dilution factor was calculated as: specific activity at \(t = 24\) divided by specific activity at \(t = 0\). Immediately after all CDDP incubations and at \(t = 24\) in the repair samples, cell viability was tested by trypan blue dye exclusion.

Differences between the group of germ cell tumour and the group of colon carcinoma cell lines were analysed with the Student's \(t\)-test for unpaired samples. Correlation coefficients were determined by Spearman rank analyses. Analyses of variance were performed using the SPSS program for medical statistics (SPSS, Chicago, MI, USA). Only \(P\)-values \(< 0.05\) were considered significant.

**Results**

The survival curves of the cell lines after continuous CDDP incubation are shown in Figure 1. The CDDP concentrations inhibiting survival by 50\% (IC\(_{50}\)) ranged from 0.69 to 7.90 \(\mu\)M, thus a maximum 11.4-fold difference in sensitivity between the cell lines was found. The germ cell tumour cell lines are the most sensitive lines with IC\(_{50}\) values ranging from 0.69 to 2.42 \(\mu\)M, while the IC\(_{50}\) values of the colon carcinoma cell lines ranged from 4.16 to 7.90 \(\mu\)M. There was a significant difference between the mean IC\(_{50}\) of the germ cell tumour and the colon carcinoma cell lines \((P < 0.01)\).

In Table II the basic phenotypical parameters, namely cell doubling time, relative cell volume, cellular protein, nuclear protein, cellular DNA content and percentage of cells in S-phase, are shown for all cell lines. For these characteristics up to 4-fold differences were observed. Only relative cell volume was significantly larger in the group of germ cell tumour than in the group of colon carcinoma cell lines \((P < 0.025)\). No correlations with CDDP sensitivity were found as far as these basic cellular characteristics were concerned.

Mean cellular GSH level (Table III) was not significantly lower in the germ cell tumour than in the colon carcinoma cell lines. But GSH levels of the six cell lines were positively correlated with CDDP IC\(_{50}\) \((r = 0.90, P < 0.05)\). Surprisingly, TSH levels showed an inverse correlation with CDDP IC\(_{50}\) \((r = -0.94, P < 0.05)\). There was also a significant difference between both groups of cell lines with respect to TSH \((P < 0.025)\) (Table III). GST activity and the amount of GST\(_x\) (Table III) were not significantly different between both groups of tumours, nor was either parameter statistically related to CDDP sensitivity. There was no relation between GST activity and the amount of GST\(_x\) in the cell lines.

No difference was found between the topo I activities in the nuclear protein extracts of all cell lines, and activities were similar to the topo I activity of GLC4 (for GLC4 a mean of 1 \pm 1 ng nuclear protein was needed to observe complete relaxation of plasmid DNA). When topo II activity was expressed relative to GLC4 (for GLC4, with a mean of 10 \pm 4 ng nuclear protein or less decatenation was no longer observed), five of the cell lines had higher topo II activities. The mean topo II activity was not different between the group of germ cell tumour and the group of colon carcinoma cell lines. Within the panel of cell lines tested, one germ cell tumour cell line had a higher topo II (833 KE) and one colon carcinoma cell line a lower topo II (Caco 320). There was no correlation between topo II activity and the amount of nuclear protein, DNA per cell or percentage of cells in S-phase.

Cellular Pt content in the various cell lines is shown in Figure 2. Immediately after 4 h incubation with CDDP no loss of cell viability was observed (data not shown). Neither a difference in accumulation between both groups nor a relation with CDDP sensitivity was found. Correlation of cellular Pt content for cellular protein or relative cell volume did not reveal a more obvious statistical relation with CDDP sensitivity. In the most sensitive germ cell tumour cell line, Tera, a low cellular Pt level was found, while in Scha cellular Pt levels were generally high compared with other cell lines. Although in two of the three germ cell carcinoma cell lines a relatively high amount of Pt bound to DNA was found (833 KE, Scha) neither a difference between the groups of tumour cell lines nor a correlation with CDDP sensitivity was found (Figure 3). Calculation of the amount of Pt bound to DNA per 10\(^6\) cells, thus correcting for the large differences in DNA content per cell, did not improve the relation between Pt bound to DNA and CDDP sensitivity. Measurement of Pt bound to DNA 24 h after a 4 h CDDP incubation revealed an increase, compared with \(t = 0\), in one germ cell tumour and one colon carcinoma cell line, no alteration over this period in another cell line of both cell types and a decrease of Pt bound to DNA in the third cell line of both cell types.

**Figure 1** Survival of Tera (○), 833 KE (□), Scha (Δ), Colo 320 (●), SW 948 (■) and Caco-2 (▲) after continuous incubation with CDDP measured by microculture tetrazolium assay. The mean IC\(_{50}\) values of the group of germ cell tumour and colon carcinoma cell lines were significantly different \((P < 0.01; n = 3)\).

| Table II | Cellular characteristics of the three germ cell tumour (Tera, 833 KE, Scha) and the three colon carcinoma (Colo 320, SW 948, Caco-2) cell lines expressed as indicated |

| Cell line   | Doubling time \(\mu\)d \(n = 3\) | Relative cell volume \(\%\) | Cellular protein \((\mu g 10^{-12} cells)\) | Nuclear protein \((\mu g 10^{-14} nuclei)\) | DNA content \((\mu g 10^{-12} cells)\) | Percentage of cells in S-phase |
|-------------|---------------------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|
| Tera        | 14.0 \± 0.6 \(n = 3\)          | 116.0                       | 233 \± 62                       | 29.1 \± 5.2                     | 15.8 \± 4.5                     | 46.7 \± 2.2                     |
| 833 KE      | 25.3 \± 2.1                     | 110.0 \± 5.8                | 372 \± 43                       | 49.4 \± 8.4                     | 19.8 \± 3.1                     | 55.1 \± 2.9                     |
| Scha        | 35.6 \± 11.6                    | 99.6 \± 3.6                 | 165 \± 18                       | 52.5 \± 16.1                    | 15.7 \± 0.8                     | 24.6 \± 1.4                     |
| Colo 320    | 15.2 \± 0.6                     | 84.2 \± 1.6                 | 235 \± 12                       | 26.9 \± 3.7                     | 7.6 \± 0.8                      | 42.7 \± 2.3                     |
| SW 948      | 19.2 \± 1.2                     | 76.8 \± 2.4                 | 356 \± 45                       | 30.8 \± 7.4                     | 25.7 \± 2.4                     | 56.7 \± 3.2                     |
| Caco-2      | 30.3 \± 11.4                    | 91.0 \± 1.2                 | 330 \± 30                       | 95.6 \± 10.2                    | 31.8 \± 4.7                     | 31.0 \± 10.0                    |

\*Significant difference between the group of germ cell and the group of colon carcinomas \((P < 0.025)\). \*Mean \± s.d. \((n \geq 3)\).
Table III  CDDP sensitivity-related parameters of the three germ cell tumour (TERA, 833 KE, Scha) and the three colon carcinoma (Colo 320, SW 948, Caco-2) cell lines expressed per mg of cellular protein

|        | TSH (µg TSHP) | GSH (µg GSHP) | GST activity (nmol CDNBF min⁻¹) | GST-µ (µg GST-µ) | Topo II′ |
|--------|---------------|---------------|----------------------------------|------------------|-----------|
| Tera   | 65.7 ± 10.1"  | 1.9 ± 0.2     | 95 ± 8                           | 6.3 ± 1.8        | 3         |
| 833 KE | 52.5 ± 16.9   | 5.6 ± 1.9     | 90 ± 12                          | 3.9 ± 0.6        | 10        |
| Scha   | 38.6 ± 4.0    | 9.8 ± 1.6     | 36 ± 9                           | 6.9 ± 1.2        | 3         |
| Colo 320 | 25.7 ± 8.3   | 7.7 ± 0.8     | 75 ± 40                          | 4.2 ± 0.4        | 1         |
| SW 948 | 26.5 ± 9.9    | 9.8 ± 2.9     | 68 ± 31                          | 4.3 ± 0.5        | 3         |
| Caco-2 | 21.4 ± 6.0    | 12.3 ± 1.8    | 255 ± 25                         | 7.2 ± 0.9        | 3         |

*Spearman rank analysis of TSH vs IC₉₀, r = -0.94, P<0.05. *Spearman rank analysis of GSH vs IC₉₀, r = 0.90, P<0.05. *1-Chloro-2,4-dinitrobenzene. *Topo II activity relative to the cell line GLC4 (de Jong et al., 1990). Topo II activity for GLC4 was no longer seen when 10 ± 4 ng protein per lane or less was used. *Mean ± s.d., (n ≥ 3).

Figure 2  Cellular Pt accumulation after 4 h CDDP incubation determined with AAS of Tera (1), 833 KE (2), Scha (3), Colo 320 (4), SW 948 (5) and Caco-2 (6) (n = 3 - 4). Neither a significant difference between both groups of cell lines nor a correlation of Pt accumulation with CDDP sensitivity was found.

Figure 3  Amount of Pt bound to DNA after 4 h CDDP incubation determined with AAS of Tera (1), 833 KE (2), Scha (3), Colo 320 (4), SW 948 (5) and Caco-2 (6) (n = 4). Neither a significant difference between both groups of cell lines nor a correlation of Pt bound to DNA with CDDP sensitivity was found.

(Table IV). Values shown in Table IV are corrected for DNA synthesis. DNA specific activity decreased; dilution factors varied from 0.46 to 0.74 in the six cell lines and were not different between the group of germ cell tumour and the group of colon carcinoma cell lines. Although within the group of germ cell tumour cell lines the correlation between Pt bound to DNA at t = 24 and IC₉₀ was found, no correlation was found within the group of colon carcinoma cell lines.

Statistical processing of data by analysis of variance was performed to detect whether it was possible to relate combinations of parameters to sensitivity. The sensitivity of the cell lines, defined as CDDP IC₉₀, was correlated with GSH in combination with Pt bound to DNA (100 µM CDDP incubation) and GST activity (r = 0.997, P = 0.010). Any other combination of parameters did not reveal significant correlations with IC₉₀. However, even for a combination of parameters, no difference between the group of germ cell tumour and the group of colon carcinoma cell lines was found at the IC₉₀ level. Analyses of variance were also performed in relation to a level of a CDDP concentration inhibiting survival by 10% (IC₁₀) and a concentration inhibiting survival by 90% (IC₉₀). IC₉₀ correlated with GSH combined with cellular Pt accumulation (67 µM CDDP incubation) and topo II activity (r = 0.994, P = 0.018). When IC₉₀ was related to the combination of GSH/Pt accumulation there was also a significant difference between both groups of cell lines. When IC₉₀ values were correlated with Pt bound to DNA (100 µM CDDP incubation) and TSH, a difference between the groups of cell lines was found as well as correlation with sensitivity at this high cytotoxicity level.

Discussion

In this study the survival curves of the cell lines after CDDP incubation show that the model with germ cell tumour and colon carcinoma cell lines as representatives of CDDP-
Table IV  Values of Pt bound to DNA immediately (t = 0) and 24 h (t = 24) after a 4 h incubation with 16.5 μM CDDP for the three germ cell tumour (Tera, 833 KE, Sch) and 33 μM for the three colon carcinoma (Caco 320, SW 948, Caco-2) cell lines

| Cell line | Pt bound to DNA at t = 0 | Pt bound to DNA at t = 24 h |
|-----------|--------------------------|-----------------------------|
| Tera      | 28.2 ± 19.7 mg Pt/mg DNA | 61.3 ± 43.3 mg Pt/mg DNA |
| 833 KE    | 32.1 ± 6.5 mg Pt/mg DNA  | 35.2 ± 23.3 mg Pt/mg DNA  |
| Scha      | 26.9 ± 4.2 mg Pt/mg DNA  | 16.6 ± 3.2 mg Pt/mg DNA   |
| Colo 320  | 48.4 ± 10.2 mg Pt/mg DNA | 32.2 ± 15.7 mg Pt/mg DNA  |
| SW 948    | 46.3 ± 12.8 mg Pt/mg DNA | 61.7 ± 26.0 mg Pt/mg DNA  |
| Caco-2    | 48.7 ± 3.3 mg Pt/mg DNA  | 44.0 ± 8.1 mg Pt/mg DNA   |

*Mean ± s.d. (n = 3–4).

germ cell tumour cell lines than in the colon carcinoma lines. In view of the data of Koropatnick and Pearson (1993), this should imply that the metallothioneins in the present model behaved like the transfected mouse metallothioneins and not like those induced by heavy metal incubation of the cells. Another possible explanation might be the finding that rat testicular metal-binding proteins were not, in contrast to the metal-binding proteins in other tissues, metallothioneins (Waalkes and Perantoni, 1986). Compared with metallothionein, these testicular proteins had a similar molecular weight but a different amino acid composition; most striking was the small amount of cysteine residues present. Analogously, it could be that the sulphydryl-containing proteins in germ cell tumours were different from those in colon carcinoma. Based on the inverse correlation between TSII and IC50, it could even be speculated that, if these proteins do bind Pt, a toxic complex might be formed in the germ cell tumour cell lines. Increased GST activity has been found in several cell lines with acquired CDDP resistance (for review see Andrews and Howell, 1990). However, in our study no direct correlation of GST activity with CDDP sensitivity was observed. In the analysis of variance, GST activity in combination with GSH level and Pt bound to DNA showed a correlation with CDDP sensitivity at IC50. This is compatible with a role of GST in cellular detoxification in these cell lines. There was no difference between both groups of cell lines with respect to GST activity of amount of GSTα. However, the GST activity in colon carcinomas is generally reported to be increased (Moscow et al., 1989; Peters et al., 1992), while in germ cell tumours GST activity is decreased compared with normal adjacent tissue (Strohmeyer et al., 1992). Probably normal germ cells depend on a high GST level for cellular detoxification and, therefore, the lower GST in the germ cell tumour cell lines may be one of the causes of their CDDP sensitivity.

Topo I activity was similar in all cell lines. Giaccone et al. (1992) also described only small differences in topo I RNA expression in in vitro untreated lung carcinoma cell lines, while in these lines CDDP sensitivity varied as well as sensitivity to drugs for which topo I is the target. Also, in an ovarian carcinoma cell line with in vitro acquired CDDP resistance, cross-resistance for a topo I-directed drug in the absence of a difference in topo I levels was found (Niimi et al., 1992). So, although it cannot be stated on the basis of these results that topo I does not play a role in CDDP resistance, its activity is not a predictive factor for CDDP sensitivity in vitro. Fry et al. (1991) found a higher topo II expression in germ cell tumour cell lines than in bladder tumour cell lines, and in a panel of lung cancer cell lines high topo II RNA expression correlated with sensitivity for multiple drugs, including cisplatin (Giaccone et al., 1992). In cell lines with acquired CDDP resistance decreased (Yang and Douple, 1991) as well as increased (De Jong et al., 1991) topo II activities have been found compared with their sensitive parental lines. In our cell lines we found no correlation between topo II activity and CDDP sensitivity and no difference between the two groups of tumour types. The high topo II activity in 833 KE was in agreement with the high levels in this cell line described by Fry et al. (1991). But a high topo II level was not found in the two other germ cell tumour cell lines. Combined with the varying results that have been found in acquired CDDP resistance, these data exclude a direct, common role for topo II in CDDP resistance.

Decrease of cellular Pt uptake in cells with in vitro acquired resistance is a frequent mechanism of resistance (for review see Gately and Howell, 1993), and the amount of Pt bound to DNA in tumour cells in vitro is often correlated with CDDP sensitivity (Sherman and Lippard, 1987). In a report by Bedford et al. (1988), accumulation of Pt in two germ cell tumour and one bladder tumour cell line correlated with CDDP sensitivity. The amount of initial DNA platinisation after 1 h incubation with CDDP was correlated with the CDDP sensitivity of these lines. However, the amount of Pt bound to DNA after 1 h incubation with untreated tumour cells.
CDDP followed by 24 h culture offered a good correlation (Bedford et al., 1988). In contrast, Hill et al. (1994) found the lowest DNA platination in the most sensitive lines and the highest in the most resistant lines, using a panel of four germ cell tumour cell lines and an incubation and post-incubation scheme according to Bedford et al. (1988). In our model cellular Pt uptake did not correlate with sensitivity. The germ cell tumour cell line Scha, for example, had a high cellular Pt level, while the most sensitive germ cell tumour cell line, Tera, had a low cellular Pt content. Pt bound to DNA did not correlate with sensitivity unless combined with parameters of cellular detoxification. This is surprising as Pt binding to DNA is usually considered to be the mechanism of toxicity of CDDP. Correlation with Pt bound to DNA at t = 24 h was found in the group of germ cell tumour cell lines, but not in the group of colon carcinoma cell lines. An increase in Pt–DNA levels during the 24-h post-incubation period was also found in two of the germ cell tumour cell lines described by Hill et al. (1994). As that study used an immunochemical technique whereas the present study used AAS to measure Pt–DNA, it is unlikely that this increase is due to an artifact of the technique used. The fact that we also found increases in Pt bound to DNA in one colon carcinoma and no repair in another seemed contrary to the results reported by Bedford et al. (1988). In that study the resistant cell line was incubated with a concentration of CDDP close to its own IC50. In our study the resistant cell lines were incubated with CDDP concentrations exceeding their IC50 by 4- to 8-fold. In these circumstances a lack of repair capacity is also found in the colon carcinoma cell lines. This suggested that the lack of repair capacity described for germ cell tumour cell lines (Bedford et al., 1988; Kelland et al., 1992; Hill et al., 1994) might be at least partly due to the high incubation concentrations relative to their IC50 values in the studies described, and not only to a unique phenomenon in germ cell tumour cell lines.

Analysis of variance was used to correlate combinations of parameters with sensitivity. It showed a good correlation between IC50, the parameter normally used to indicate drug sensitivity, and Pt bound to DNA in combination with GSH and GST activity. However, at other cytotoxicity levels (IC20 and IC50) also, combined correlations with other parameters such as topo II activity, cellular Pt content and TSH were found. The diversity of correlations might indicate that in these cell lines different mechanisms may be important at different levels of sensitivity, although since only a few cell lines were analysed the results can only be used as a guideline. The fact that at all cytotoxicity levels on more components of the detoxifying system were involved is a strong indication for the relevance of this system in intrinsic tumour cell CDDP sensitivity. On the other hand, the contribution of a certain parameter in the various cell lines might vary widely. For instance, in the germ cell tumour cell line Scha, the GSH level was similar to that in the colon carcinoma cell line SW 948. But the high Pt accumulation in Scha seemed to overwhelm its GSH pool, leading to a higher Pt–DNA binding (r = 0) in Scha than in SW 948 after incubation with the same CDDP concentration. The colon carcinoma cell line Caco-2 exhibited a high degree of Pt accumulation but a low level of Pt–DNA binding. This might be caused by a high efficacy of its detoxifying system, as GSH levels and GST activity were the highest in this cell line.

Based on our model of unselected cell lines it can be concluded that multiple parameters must be analysed to explain CDDP resistance in vitro. However, other parameters that we did not include in this study (for review see Kelland, 1994) might explain the sensitivity of germ cell tumour and the insensitivity of colon carcinoma cell lines. Thus the sensitivity of germ cell tumours to a range of drugs inducing DNA damage (Masters et al., 1993) and the possible role of DNA damage recognition proteins (McLaughlin et al., 1993) are intriguing.

In conclusion, the study described here did not reveal a cause of the unique sensitivity of germ cell tumours, but the unexpected relation between IC20 and TSH should be studied further.

Abbreviations: AAS, atomic absorption spectrophotometry; CDDP, cisplatin, cis-diaminedichloroplatinum(II); CDNB, 1-chloro-2,4-dinitrobenzene; FCS, fetal calf serum; GSH, glutathione; GST, glutathione S-transferase; IC50, drug concentration inhibiting survival by 10%; IC50, drug concentration inhibiting survival by 50%; IC50 cell, drug concentration inhibiting survival by 50%; PBS, phosphate-buffered saline (0.14 M sodium chloride, 2.7 mM potassium chloride, 6.4 mM disodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, pH 7.4); Pt, platinum; topo, DNA topoisomerase; TSH, total sulphhydryl content.

Acknowledgements

The authors would like to thank Tineke van der Sluis for technical assistance. This study was supported by a grant from the Dutch Cancer Foundation (GUC 90–18).

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