Correlation between Cell Killing by cis-Diaminedichloroplatinum(II) in Six Mammalian Cell Lines and Binding of a cis-Diaminedichloroplatinum(II)-DNA Antiserum

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ABSTRACT

The relationship between cell killing and the binding of the anticancer drug cis-diaminedichloroplatinum(II) (cis-DDP) to DNA was studied in six mammalian cell lines. Two of the human cell lines (COV413B) were of the same origin, comprising one sensitive to cis-DDP and the other with induced resistance to the drug. The four other lines, two rodent (RIF-1, Chinese hamster ovary) and two human (A2780, A1847), were unrelated. The cell lines differed in their sensitivity to cis-DDP, as tested in a clonogenic assay. cis-DDP-DNA binding was determined by quantitative immunocytochemistry using an antiserum against cis-DDP-modified DNA. The resistance factors relative to RIF-1, calculated from full survival curves for cis-DDP, were 3.8 ± 0.4 for Chinese hamster ovary cells and 8.8 ± 0.7 for both A2780 and A1847 lines. Using quantitative immunocytochemistry, the levels of the adduct-specific nuclear staining density compared with RIF-1 cells were 4.8 ± 0.2 for Chinese hamster ovary cells, 9.1 ± 0.2 for A2780, and 10.0 ± 0.1 for A1847 cells, i.e., in good agreement with the resistance factors. In studies with the COV413B cells and their cis-DDP-resistant counterpart COV413B-PrR, immunologically detected adduct levels again correlated closely with resistance factors (correlation coefficient = 0.97). The kinetics of cis-DDP-DNA adduct formation and loss was investigated in RIF-1, A2780, and A1847 cells by the immunocytochemistry technique. Adduct levels after a 1-h incubation with approximately equitoxic doses of cis-DDP increased by 18 to 32% (average, 27%) between 0 and 6.5 h after treatment and then declined. Adduct half-lives in this latter phase did not correlate with the sensitivities of the cells for cis-DDP. These results indicate that the initial level of cis-DDP-DNA binding measured by quantitative immunocytochemistry may be a reasonable predictor of sensitivity to this chemotherapeutic drug.

INTRODUCTION

cis-DDP (cisplatin) was introduced in clinical trials during the early 1970s and is now one of the clinically most important anticancer drugs (1). It is assumed that the covalent interaction of cis-DDP with DNA is instrumental in the cytotoxic action of the drug. This assumption is based on the prolonged inhibition of DNA replication after cis-DDP treatment (2), the high binding level of cis-DDP to DNA when compared with that to RNA or proteins (3), and the observation that DNA repair-deficient cells are extremely sensitive to cis-DDP (4). Many interaction products between cis-DDP and DNA have been studied (5–9), although the relationship between individual products and cell killing is not clear. Comparative studies in cultured cell lines have yielded differences in whether a correlation exists between the initial extent of cis-DDP-DNA binding and cell survival (10–16).

During the last few years, immunochemical techniques have become available to study the modification of DNA by a variety of drugs, such as cis-DDP (17–22), nitrogen mustard (23), L-phenylalanine mustard (23, 24), 1,3-bis(2-chloroethyl)-1-nitrosourea (23), cyclophosphamide (25), and procarbazine (26). These techniques have included flow cytometry, ELISA, and immunocytochemistry. Only a few of these proved to be specific and sensitive enough to measure DNA adducts after pharmacologically relevant drug doses. The use of antibodies to detect cis-DDP-DNA adducts in an ELISA has led to a 1000-fold more sensitive assay than the conventional measurement of platinum-DNA binding by atomic absorption spectroscopy (27). Such an assay can provide information complementary to that obtained by the alkaline elution technique (10).

We have recently reported on an immunocytochemical peroxidase assay for cis-DDP- and carboplatin-DNA binding using polyclonal antibodies raised against cis-DDP-modified calf thymus DNA (28–30). This immunostaining assay allows investigations of cis-DDP-DNA binding in different cell types within one population (e.g., tumor cells and stroma cells) and can detect cis-DDP-DNA adducts in samples of only a few hundred cells at clinically relevant drug doses. It was shown that the adduct-specific immunostaining could be quantitated by microdensitometry of individual nuclei (30). In this way, linear relationships could be established between the level of exposure to cis-DDP and the level of cis-DDP-DNA specific nuclear staining in animal tissues and tumors and in buccal mucosa cells from cancer patients who received cis-DDP- or carboplatin-based chemotherapy (28–31).

The availability of such an assay has led to suggestions for its use as a predictor of cis-DDP sensitivity in individual tumors in the clinic. In the present studies, we therefore wished to further investigate the quantitative relationship between cis-DDP-induced DNA modification and cell killing using this immunocytochemistry method. The goals were to test whether the initial or residual adducts detected by this antiserum in a number of cell lines correlated with survival and to therefore validate, or otherwise, its potential usefulness as a predictive assay. To accomplish this, four unrelated cell lines with different cis-DDP sensitivities were chosen for study, along with a further pair of cell lines, one of which had induced resistance to cis-DDP. The results show a close correlation between the cis-DDP-specific immunostaining signal and the level of cell killing, although they do not prove a general validity of the correlation.

MATERIALS AND METHODS

Cell Lines. The RIF-1 cell line, derived from a radiation-induced mouse RIF-1 fibrosarcoma, was provided by Dr. R. F. Kallman (Stan-
ford University, Stanford, CA). RIF-1 and CHO cells were grown as monolayer cultures in Ham's F-10 medium (Flow Laboratories, Irvine, United Kingdom) plus 10% FCS (Sera-Lab, Sussex, United Kingdom) under standard conditions (37°C and a humidified 5% CO₂:95% air mixture). RIF-1 cells were maintained by alternating passages in vitro and in vivo (32). CHO cells were passed weekly 10 times before returning to a frozen stock. Human ovarian carcinoma cell lines A2780 and A1847 (33), both derived from untreated patients and both growing as a monolayer in Dulbecco's modified Eagle's medium (Gibco, Paisley, United Kingdom), were obtained from Dr. R. F. Ozols (National Cancer Institute, Bethesda, MD). Human ovarian cell lines COV413B and COV413B-PtR were maintained in Dulbecco's modified Eagle's medium (Gibco) plus 8% FCS as described (34).

Drug Treatment and Cell Survival. Approximately 2 × 10⁶ cells were seeded into 60- x 15-mm dishes and treated 2 to 4 days later when in log phase of growth (0.5 to 2.0 × 10⁶ cells per dish). For the dose-response experiments, cells were treated for 1 h at 37°C with a graded series of cis-DDP doses (0 to 667 μM). They were then washed twice, cultured for 6 h in cis-DDP-free medium (COV413B and COV413B-PtR cells only), trypsinized, counted, and prepared for immunocytochemistry of cis-DDP-DNA binding.

In a second series of experiments, cells were incubated for 0, 1, 2, 4, or 6 h with either 10 μM (CHO) or 1.7 μM (RIF-1) cis-DDP at 37°C under standard conditions. The cells were washed twice and assayed for colony-forming ability and DNA adduct formation. In a third series of experiments, the persistence of cis-DDP-DNA binding was measured immunocytochemically. Cells were treated for 1 h with either 10 μM (RIF-1), 33 μM (CHO), or 100 μM (A2780 and A1847) cis-DDP at 37°C under standard conditions, washed twice, replenished with drug-free medium, cultured for 0, 6.5, 24, 48, or 72 h, harvested, counted, and prepared for immunocytochemistry.

Immunoreagents. The preparation of the rabbit antiserum NKI-A59 against cis-DDP-modified calf thymus DNA (platinum/nucleotide ratio of 6.7 × 10⁻⁵) has been described previously (17, 28). The NKI-A59 antiserum was applied without further purification. GAR was from Campbro Benelux, Elst, The Netherlands; PAP (rabbit) was from American Qualex, La Miranda, CA. Antisera were used in the following optimal dilutions: NKI-A59, 1:1800; GAR, 1:600; and PAP, 1:3000. All sera were diluted in phosphate buffer (10 mM KH₂PO₄:140 mM NaCl, pH 7.4) containing 10% FCS and 0.04% Triton X-100 (BDH, Poole, United Kingdom).

Immunocytochemical Assay. Cytospin preparations each containing about 10³ cells were made on glass slides coated with ovalbumin; 100 μl of 0.5% ovalbumin (Sigma, St. Louis, MO) in demineralized water was spread evenly on a 2.6- × 6-cm glass slide, quickly dried at 60°C, and baked for approximately 14 h at 120°C. Approximately 10³ cells were then centrifuged onto the slides, fixed by successive incubations in cold (−20°C) 100% methanol (10 min) and acetone (2 min), air-dried, wrapped in aluminum foil, stored at −20°C, and immunostained within 30 days. The immunocytochemical procedure was carried out as described previously (31). The general outline of the method was as follows. Slides were treated with H₂O₂ (to inactivate endogenous peroxidase), 1 mM KCl, 10 μg/ml of proteinase K, 42 mM NaOH in 80% methanol (to denature the DNA and increase accessibility), 10% FCS (to inhibit nonspecific antiserum binding), and antiserum NKI-A59. Antibodies bound in the final step were visualized by double PAP staining, i.e., by sequential incubation of the sections in GAR, PAP, GAR, and PAP. The peroxidase substrate for the staining reaction was 3,3′-diaminobenzidine·HCl (Sigma). The use of RNAses A and T₁ during preincubations and the use of wash buffer after each incubation (28, 29, 31) were omitted, since this did not change the extent or the quality of the immunostaining. All cytospin slides from one experimental series were stained simultaneously. Each sample was stained in duplicate in separate staining procedures.

Quantification of the Nuclear Staining Intensity. The staining intensity of individual nuclei was measured with a Knott (Munich, Federal Republic of Germany) light-microscopical device with a beam diameter of 0.5 μm, which was coupled to a Leitz Orthoplan microscope. Data were analyzed by an Atari ST computer (Sunnyvale, CA) programmed with a version of the Histochemical Data Acquisition System (Microscan, Leiden, The Netherlands) (30, 35). The integrated (i.e., total) absorbance of the whole nucleus was determined and expressed in arbitrary units. Two independently stained slides were analyzed; in each slide, the nuclear staining density of 15 to 30 randomly selected nuclei was measured. To simplify the comparison of the nuclear staining densities relative to RIF-1 (first four cell lines) or COV413B (last two cell lines) with the resistance factor, the reciprocal values of the relative nuclear staining density/μM cis-DDP were calculated. The relative nuclear staining density/μM cis-DDP is used to compare different cell lines; the values are expressed relative to either RIF-1 or COV413B cells and calculated from the mean of the nuclear staining density/μM cis-DDP from all dose points. To compare adduct levels of cells which had been treated with different concentrations of cis-DDP, the nuclear staining density was expressed per unit of concentration (μM).

Statistics. Levels of significance were calculated using analysis of variance followed by Scheffe's test; P < 0.05 was considered indicative of a significant difference between groups. The coefficient of correlation was calculated by linear regression analysis.

RESULTS

Survival and Initial cis-DDP-DNA Binding. Fig. 1 shows the survival curves of four cell lines for a 1-h incubation with cis-DDP. RIF-1 cells were highly sensitive to the drug, CHO cells showed an intermediate sensitivity, and the human ovarian cell lines A2780 and A1847 showed the greatest resistance. Resistance factors relative to RIF-1 varied slightly depending on the SF at which the estimate was made since all curves were not completely linear. RFs were 3.8 ± 0.4 (mean ± SE) for CHO.
and 8.8 ± 0.7 for A2780 and A1847 (Table 1). The data shown in Fig. 1 represent cells from the same experiments as used in the immunostaining tests. Other survival curve determinations for these cell lines made over a period of at least 3 yr always showed the same ranking order of sensitivity. The absolute sensitivities varied slightly over this period with no trend in a given direction (data not shown). The exception was the CHO cells which increased in sensitivity over two passages by a factor of approximately 2 and remained stable thereafter. All experiments were performed with the later passage CHO cells. Immunostaining and survival determinations were carried out within each experiment to avoid possible interexperiment variations.

Fig. 2 shows results of immunostaining the four cell lines for cis-DDP-DNA adducts immediately after drug exposure. The level of the nuclear staining density ranked as follows: RIF-1 > CHO > A2780 = A1847. Nuclear staining was absent when the NKI-A59 antiserum was substituted by preimmune rabbit serum. The nuclear staining density/μM cis-DDP (expressed as arbitrary units per μM platinum) from this experiment ranged from 9 to 11 for RIF-1, 1.8 to 2.6 for CHO, 0.6 to 1.5 for A2780, and 0.7 to 1.4 for A1847. To allow a comparison of the nuclear staining density of the cell lines relative to RIF-1 with the RF, the reciprocal value of the relative density/μM cis-DDP was calculated (Table 1). These values were 4.8 ± 0.2 for CHO, 9.1 ± 0.2 for A2780, and 10.0 ± 0.1 for A1847.

Table 1 Cell kill, adduct formation, and adduct loss after cis-DDP treatment in six tumor cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>RF*</th>
<th>Reciprocal of relative density/μM Pt</th>
<th>Adduct half-life (h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF-1</td>
<td>1.0</td>
<td>1.0</td>
<td>79</td>
</tr>
<tr>
<td>CHO</td>
<td>3.8 ± 0.4</td>
<td>4.8 ± 0.2</td>
<td>ND*</td>
</tr>
<tr>
<td>A2780</td>
<td>8.8 ± 0.7</td>
<td>9.1 ± 0.2</td>
<td>106</td>
</tr>
<tr>
<td>A1847</td>
<td>8.8 ± 0.7</td>
<td>10.0 ± 0.1</td>
<td>112</td>
</tr>
<tr>
<td>COV413B</td>
<td>1.0</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>COV413B-PIR</td>
<td>9.3 ± 0.2</td>
<td>7.5 ± 0.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

* RF, resistance factors relative to RIF-1 (first four cell lines) or COV413B (last two cell lines). RF = ratio of doses for a given surviving fraction.

° To simplify the comparison of nuclear staining densities relative to RIF-1 (first four cell lines) or COV413B (last two cell lines) with the RF, the reciprocal values of the density/μM cis-DDP are given in this table.

Half-lives of nuclear staining density from regression analysis of data between 6.5 and 72 h after treatment (Fig. 6).

Mean ± SE.

ND, not determined.

To test for a correlation between the two parameters, nuclear staining density was plotted against SF (Fig. 3). Since cis-DDP exposures greater than 20 μM gave no colonies of RIF-1 cells, only the data with less than this dose could be plotted in the figure. A good correlation was observed (correlation coefficient = 0.93; P < 0.001). This confirms that in the more resistant lines, higher survivals for a given drug dose were coupled with lower adduct levels, of the type detected by this antiserum. It also shows that not only did killing increase with adduct level within any one line, but that the relationship between killing and adduct level was approximately the same in all lines tested. Correlation coefficients between SF and nuclear staining density for the individual cell lines were 0.99 for RIF-1, 0.97 for CHO, 0.94 for A2780, and 0.99 for A1847 cells.

In addition to the unrelated cell lines described above, the parent cell line COV413B and its cis-DDP-resistant subline COV413B-PtR were studied. Fig. 4A shows that COV413B-PtR cells are 9.3 ± 0.2 times more resistant to cis-DDP than the parent cell line. This difference is paralleled by a difference in nuclear staining density (measured 6 h after exposure to cis-DDP) (Table 1; Fig. 4B). A plot of nuclear staining density against SF again (Fig. 4C) showed a good correlation between these parameters (correlation coefficient = 0.97; P < 0.001).

Prolonged Exposure to cis-DDP. As a further test of the relationship between antibody binding and drug-induced killing, the influence of prolonged exposure to cis-DDP in RIF-1 and CHO cells was studied. Cells were incubated for 1 to 6 h with approximately equitoxic doses of cis-DDP (RIF-1, 1.7 μM; CHO, 10 μM; based on cell killing after a 1-h incubation with cis-DDP). The survival of both RIF-1 and CHO cells decreased with increasing exposure time, exhibiting shouldered curves with RIF-1 cells showing a slightly greater response at the doses chosen (Fig. 5A). Binding of the NKI-A59 antibody showed a continuous increase during the 6-h incubation period, with little difference between the cell lines (Fig. 5B). The similar nuclear staining densities in RIF-1 and CHO cells under almost equitoxic conditions again indicates that the difference in sensitivity to cis-DDP between these cells can be largely explained at the level of cis-DDP binding to DNA. Correlation coefficients between SF and nuclear staining density for the individual cell lines were 0.88 for CHO and 0.97 for RIF-1. When data from

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**Fig. 2.** Nuclear staining density in arbitrary units measured by microdensitometry for cells incubated for 1 h with different concentrations of cis-DDP. Cytospin slides were prepared immediately after drug exposure for immunostaining. RIF-1 (■); CHO (○); A2780 (▲); A1847 (▲). Points, mean from two independently stained slides with 15 measured nuclei per slide; bars, SE.

**Fig. 3.** Correlation between the sensitivity to cis-DDP and the cis-DDP-DNA adduct-specific nuclear staining density. Survival data from Fig. 1 are plotted against nuclear staining densities from Fig. 2. RIF-1 (■); CHO (○); A2780 (▲); A1847 (▲). The best-fit line was calculated by linear regression analysis. The correlation coefficient was 0.93.
Fig. 4. Correlation between cell killing and nuclear staining density for cis-DDP-sensitive COV413B (C) and cis-DDP-resistant COV413B-PtR human ovarian carcinoma cells (O). Cells were harvested 6 h after a 1-h cis-DDP treatment for both determinations. A, dose-response curves for cell killing; B, dose-response curves for nuclear staining density (arbitrary units). Points, mean from two culture dishes; bars, SE. C, log SF versus nuclear staining density. The curve in C is a linear regression fit through the data (correlation coefficient = 0.97).

Fig. 5. Correlation of cell killing with nuclear staining density for RIF-1 and CHO cells treated for various times with cis-DDP. Drug doses were chosen to be approximately equitoxic: 10 μM for CHO ( ); 1.7 μM for RIF-1 ( ). Points, mean from two culture dishes; bars, SE. A, cell killing as a function of time; B, nuclear staining density (arbitrary units) as a function of time; C, cell killing versus nuclear staining density. The curve in C is a linear regression fit (correlation coefficient = 0.92).

Both cell lines were combined, the correlation coefficient was 0.92 (Fig. 5C; P < 0.01).

The nuclear staining density/μM of cis-DDP relative to RIF-1 after 1-h incubation was 6.5 in this experiment. This value is 1.3-fold higher than that in the earlier dose-response experiments, indicating some interexperimental variation.

Stability of cis-DDP-induced DNA Modification. To test whether initial or remaining adduct levels correlated better with killing, the time course of adduct formation and loss was followed for a period of 72 h. For these studies, an attempt was made to use cis-DDP doses giving the same initial adduct levels. Because of interexperiment variation, however, levels for the two human lines were slightly higher than that for RIF-1 cells. The nuclear staining density in all cell lines increased between 0 and 6.5 h (Fig. 6), but this increase was statistically significant only for RIF-1 (P < 0.05). There were significant decreases in adduct levels between 6.5 and 72 h for all cell lines, amounting to 47%, 39%, and 35% for RIF-1, A2780, and A1847 cells, respectively. This decline was statistically significant for all cell lines: P < 0.001 for RIF-1 and A2780; and P < 0.05 for A1847. From a regression analysis of nuclear staining density versus time, adduct half-lives were calculated to be 79, 106, and 112 h for RIF-1, A2780, and A1847, respectively. For these three cell lines, little or no increase in cell number was seen, suggesting DNA repair as the probable cause of the adduct decrease.

Immediately after incubation, the nuclear staining density/μM of cis-DDP of A2780 and A1847 was 7.6 times lower than RIF-1 cells (Fig. 6). This was in good agreement with the resistance factors from Fig. 1 (coefficient of correlation = 0.99, P < 0.01).

DISCUSSION

These studies addressed the question of whether cis-DDP-DNA binding, measured immunocytochemically, is a quantitative indicator of drug-induced cell killing. Three related lines of evidence suggest that this is the case. First, dose response experiments with four unrelated cell lines indicated a strong correlation (coefficient of correlation > 0.9) between the extent of cis-DDP-DNA adduct formation (measured as nuclear staining density) and cell kill (Figs. 1 to 3). Second, with two related lines, one sensitive and one with induced resistance to cis-DDP, a similarly strong correlation was observed (Fig. 4). Finally, experiments in which the exposure time was varied for a fixed drug concentration also yielded a good correlation between the two parameters (Fig. 5). For each cell line separately, a correlation would be expected, since both adduct numbers and cell killing increased with dose. The important finding, however, was that this relationship was approximately the same for all lines tested (slope of the logSF versus nuclear staining density curve). A reasonable estimate of killing could therefore be made from the immunocytochemistry measurements, independent of the absolute sensitivity of the line to cis-DDP. It is possible that differences in DNA content between the cell lines may influence the level of nuclear staining, since the whole nucleus is measured for staining density. The DNA indices of the cell lines (compared with human diploid cells) were 1.5 for RIF-1,
0.7 for CHO, 1.14 for A1847, and 0.9 for the sensitive and resistant COV413B cell lines. These relatively modest differences in DNA content can therefore not explain the differences in immunostaining signals. For example, the sensitive and resistant COV413B cell lines had the same DNA content (34) but differed in nuclear staining density by a factor of 7.5.

A linear relationship was found between the concentration of cis-DDP in the medium and the adduct-specific nuclear staining density (Fig. 2). Such a linear relationship has also been observed for the net uptake of cis-DDP in cultured cells as measured by atomic absorption spectroscopy (11, 15, 36–38), the binding to DNA in cultured tumor cells by cis-DDP (11, 14, 16, 34, 37), and the formation of cis-Pt(NH3)2(dpgpG) adducts in human leukocytes in vivo (39). Reports on a variety of cis-DDP-sensitive and cis-DDP-resistant cell lines also indicate that, for each individual cell line, cell kill increases with increasing levels of DNA adducts, measured either as interstrand cross-linking (10, 11) or intranstrand cross-linking (40). When different cell lines were compared within each of these studies, cis-DDP-resistant cell lines had lower adduct levels than did cis-DDP-sensitive cell lines.

In contrast to the above results, several authors have reported a less pronounced and occasionally even an inverse relationship between the sensitivity of cultured cell lines to cis-DDP and the initial extent of cis-DDP-induced DNA modification (12–16). In these cases, other factors, such as the capacity for DNA repair and/or the characteristics of the DNA replication machinery, might (also) be important factors. Different techniques from the one used here were used, and most studies investigated induced resistance rather than natural resistance. A direct comparison between our immunostaining signal and atomic absorption spectroscopy of total cis-DDP-DNA binding was not made for the presently studied six cell lines. It is likely that the different immunostaining signals of the COV413B and the COV413B-PtR cells reflect differences in cellular uptake of cis-DDP (34). It should be noted, however, that a lower sensitivity to cis-DDP is not always accompanied by a reduced platinum uptake (11). The good correlation between cell killing and immunostaining signals in the present set of 6 cell lines does not mean, of course, that the correlation is a general one, holding true not only for cultured cells but also for tumor cells in situ. Whether the latter correlation really exists, i.e., whether quantitative immunocytochemistry of cis-DDP-DNA adducts can be used to predict the response of individual human tumors, can only be investigated in a focused clinical study, which is now underway.

The time course of nuclear staining density changes throws some light on the type of adduct being detected. Adduct levels were already high directly after a 1-h exposure to cis-DDP, and a maximum was observed 6.5 h after cis-DDP treatment. Post-treatment increases in cis-DDP-DNA adducts have also been observed by others using different techniques; e.g., interstrand cross-link increases by up to a factor of 10 have been reported, maximum levels being reached between 6 and 24 h (4, 11, 12, 15, 16, 41). Since the initial increase in adduct-specific nuclear staining density in the present experiments was only 1.2-fold (Fig. 6; see also data from lung cancer lines from Ref. 42), this suggests that DNA-DNA interstrand cross-links probably do not significantly contribute to the recognition of plated DNA by the NKI-A59 antiserum. The specificity of the NKI-A59 antiserum in a competitive ELISA has recently been addressed.4 NKI-A59 showed a high affinity for DNA substituted with either cis-DDP or carboplatin; amounts as low as 15 fmol were found to inhibit antiserum binding by 50%. NKI-A59 also showed affinity to cis-DDP-modified poly(dG-C); poly [d(G-C)]; poly(dC) and poly(dG). No affinity was found for trans-DDP-modified DNA, enzymatically digested cis-DDP-DNA, poly(dA)-poly(dt), oligo(da)15-oligo(dt)15, oligo(dg)21, or oligo(dg)42. A marked difference between ELISA and immunocytochemical staining was observed; i.e., the antiserum efficiently recognized cis-DDP-modified DNA in the immunostaining assay, whereas the recognition of cis-DDP-modified DNA in the ELISA depended on the level of DNA modification. At modification levels occurring in patients (i.e., about 10 fmol of intranstrand adducts/μg of DNA; see Ref. 27), the antiserum showed no reactivity in an ELISA to plated DNA. In conclusion, the present evidence indicates that NKI-A59 recognizes G-linked cis-DDP in DNA. The relative recognition of the individual adducts [cis-Pt(Nh3)2(dpGpG), cis-Pt(NH3)2(d-pApG), cis-Pt(NH3)2(dGMP), and Pt(NH3)2dGMP] is not yet known.

A correlation between in vitro cell survival and the capacity to remove cis-DDP-DNA adducts has been previously reported for other cell lines (12, 40). It was clear from the present studies, however, that differences in adduct half-lives among the three cell lines did not parallel differences in sensitivity to cis-DDP. For example, the half-lives for the RIF-1 and the two human cell lines were not significantly different despite an almost 10-fold sensitivity difference. An explanation in terms of cell proliferation is unlikely, since no increase in cell numbers was seen over the 3-day period. It cannot be totally excluded, however, that a small fraction of resistant cells containing few adducts proliferated and replaced dying cells containing many adducts. In either case, a good correlation with cell killing was observed whether initial (within 6 h) or final (after 3 days) adduct levels were used.

It is not yet clear how important the extent of cis-DDP binding to tumor DNA is in the clinic (13, 43), although Reed et al. (43) have reported that cis-DDP-DNA adduct levels in leukocytes from cancer patients correlated with the reduction in tumor mass, supporting the clinical relevance of adduct determinations. The data reported here on cis-DDP-DNA binding, as measured by quantitative immunocytochemistry, indicate that the extent of DNA modification is a good indicator of survival in a series of tumor cell lines in vitro. This result suggests the potential usefulness of the immunostaining assay in the prediction of tumor cell kill in individual cancer patients. The advantage of this assay over others in a clinical setting is the small number of cells required and the ability to distinguish different cell types due to morphology preservation.

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REFERENCES

4. Plooy, A. C. M., Van Dijk, M., Berends, F., and Lohman, P. H. M. Formation and repair of DNA interstrand cross-links in relation to cytotoxicity and unscheduled DNA synthesis induced in control and mutant human cells
CISPLATIN-DNA BINDING AND CELL SURVIVAL


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