Kinetics of DNA Cross-Linking in Normal and Neoplastic Mouse Tissues following Treatment with cis-Diamminedichloroplatinum(II) in Vivo

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ABSTRACT

The formation and repair of cis-diamminedichloroplatinum(II) (cis-DDP)-induced DNA cross-links in cells from a number of different mouse tissues, both normal and neoplastic, were compared in three different populations of animals, tumor-free mice and mice bearing a transplanted fibrosarcoma (either FSa or NFSa) in their thighs. Groups of mice were given i.v. injections of 4–12-mg/kg doses of cis-DDP, and the amount of cis-DDP-induced DNA cross-linking was determined at different times after injection using an adaptation of the alkaline elution technique. The degree of cross-linking in each tissue was linearly related to the dose of cis-DDP at either 6 or 24 h after injection and varied significantly among the different tissues, with FSa, NFSa, kidney, and liver showing the highest level of cross-linking of the tissues studied. The relative contributions of DNA-interstrand and DNA-protein cross-links to the elution profiles were estimated by proteinase K (PK) digestion. At either 6 or 24 h after injection with cis-DDP, the rate of elution of the DNA was substantially increased by PK, indicating a large contribution of DNA-protein cross-links. This effect was observed in all tissues studied, although the proportion of PK-resistant lesions appeared to vary from tissue to tissue, liver and spleen showing a significantly lower proportion of DNA-interstrand to total cross-links than either of the tumors. For liver, virtually no interstrand cross-links could be detected after PK treatment.

The kinetics of the repair of cis-DDP-induced DNA cross-linking in these tissues were also compared. In cells from tumor-free animals, the amount of total DNA-interstrand plus DNA-protein cross-linking increased gradually, reaching a maximum after about 6 h; however, little evidence of repair of these lesions was observed in any of these normal tissues. In fact, the degree of cross-linking tended to increase somewhat between 6 and 24 h after injection. The kinetics of cross-linking in cells isolated from the FSa tumor were very different; while there was an initial increase in cross-linking up to 6 h, these lesions were subsequently repaired, although at a somewhat slower rate than has been reported for cultured mammalian cells. In contrast, NFSa tumor cells behaved more like the normal tissues, with little evidence of any cross-link repair in the first 24 h. For each of the tissues studied, the proportion of DNA-interstrand to total cross-links remained constant between 6 and 24 h. The data also suggest that the presence of the FSa tumor in an animal may have some effect on the kinetics of drug-induced lesions in the normal tissues of that animal, the tissues from FSa-bearing animals generally showing some small capacity to remove cross-links from their DNA. Investigations of this type may provide an easily characterizable and relevant pharmacological end point (DNA lesions) to indicate the distribution and modulation of chemotherapy agents that have DNA as their target.

INTRODUCTION

cis-DDP has received considerable attention as an antineoplastic agent (19) since the discovery of the cytotoxic effects of platinum compounds in 1985 (18). The cytotoxicity of cis-DDP has generally been attributed to its ability to interact with cellular DNA, causing a variety of lesions such as monofunctional adducts, bifunctional binding to a single base moiety, and DNA cross-links of several types including intrastrand, DNA-interstrand, or DNA-protein cross-links (e.g., see Ref. 24). There has been considerable disagreement as to the relative contributions of these different lesions to the cytotoxicity of the drug, although it appears that monofunctional adducts are unlikely to be toxic, suggesting that one or more of the bifunctional adducts are responsible for cell inactivation (24). In a comparative study using cis- and trans-DDP in L1210 mouse leukemia cells, Zwelling et al. (25) suggested that DNA-interstrand cross-links were probably the lethal lesions, since these correlated with cytotoxicity while DNA-protein cross-links did not.

What is perhaps better established is that two aspects of the interaction between cis-DDP and DNA appear to be critical in determining the ultimate cytotoxicity, namely the initial amount of damage produced and the ability of subsequent cellular DNA repair processes to enzymatically remove these lesions from the genome. Several studies have demonstrated the importance of such DNA repair processes in modifying cis-DDP cytotoxicity (7, 15), and we have shown that a mutant strain of CHO cells that was unable to remove cis-DDP-induced DNA cross-links was supersensitive to the drug (12). Thus, many investigations conducted in in vitro systems have established important relationships between the interactions of cis-DDP at the molecular level and its cytotoxicity. However, it is not clear whether these same relationships would hold in vivo, where many cells, particularly in normal tissues, are in a nondividing state.

In an attempt to address this question, we examined the kinetics of cis-DDP-induced DNA damage in vivo, and in particular we compared and contrasted the rate of cross-link formation and removal in different normal and neoplastic tissues of mice treated in vivo. Alkaline elution was used to determine levels of DNA lesions at various times up to 24 h after i.v. injection with...
therapeutically relevant doses of cis-DDP. The following specific questions were addressed: (a) are the characteristics of the DNA lesions (i.e., the qualitative nature and kinetics of formation and removal) produced by cis-DDP in mouse tissues similar to those observed in cultured mammalian cells; and (b) do any of these characteristics of DNA damage vary among different tissues and in particular between normal and neoplastic tissues? The results suggest that, although the kinetics of formation of DNA cross-links in mouse tissues is similar to that in cultured mammalian cells, normal tissues from tumor-free animals showed little capacity to repair this damage within 24 h. On the other hand, for one of the two tumors studied (FSAs), the capacity to repair DNA damage was comparable to that found in cultured cells.

MATERIALS AND METHODS

Mice, Cell Suspensions, and Drug Treatments. Details of the C3H mice, the methylcholanthrene-induced FSAs tumor (21), and the spontaneous NFSa tumor (1) have been published previously. Tumors were grown from an s.c. injection of 5 x 10^5 tumor cells into the hind legs of mice and were used for experiments when they reached 10–12 mm in diameter. Animals were sacrificed at various times after i.v. injection with cis-DDP and the various normal or tumor tissues were excised and quickly immersed in ice-cold Puck's saline A containing 5 mm EDTA. Single-cell suspensions were subsequently prepared from these tissues; details of these procedures have been described elsewhere (13). CHO cells were maintained in exponential monolayer culture at 37°C in a humidified 5% CO2/95% air atmosphere in McCoy's medium 5A (Hsu's modification; Grand Island Biological Co., Grand Island, NY) supplemented with 15% fetal bovine serum (Irvine Scientific, Santa Ana, CA). Cells were labeled overnight with [3H]thymidine (0.01 µCi/ml; 50 µCi/ mmol; Schwarz/Mann, Orangeburg, NY), chased for 8 h with label-free medium, and then treated with various doses of cis- or trans-DDP in serum-free medium for 1 h at 37°C. After treatment, the drug was replaced with complete growth medium, and the cells incubated at 37°C for varying periods up to 24 h.

Alkaline Elution. The alkaline elution technique originally devised by Kohn et al. for use with in vitro systems (10, 11) has been adapted for measuring DNA damage in cells from mouse tissues in vivo (13, 14). Since the majority of cells in animal tissues are nonproliferating and therefore do not take up radiolabeled DNA precursors, we have of necessity used a microfluorimetric assay for DNA. Briefly, approximately 6 x 10^6 cells were layered onto 47-mm-diameter, 2-µm-pore size PC filters (Nucleopore Corp., Pleasanton, CA), washed with ice-cold Puck's saline A, lysed with 10 ml of a lysis solution (2 M NaCl/0.04 M tetrasodium EDTA/0.2% Sarkosyl, final pH 10.0, either with or without PK; see below), and then washed twice with 0.02 M EDTA. The DNA was subsequently eluted in the dark with 0.1 M tetrapropylammonium hydroxide containing 0.02 M EDTA (free acid), pH 12.1, at a constant flow rate of 0.04 ml/min. Fractions were collected every 90 min for 15 h. The DNA in each fraction, as well as that remaining on the filter, was assayed using the fluorescent dye Hoechst 33258 (13, 14). For experiments with CHO cells, the DNA was also measured by liquid scintillation counting (12).

The choice of PC over PVC filters for use in combination with the fluorometric assay is based on several factors, including the fact that PVC filters tend to be highly variable from batch to batch and are frequently contaminated to the extent that DNA molecules may be desorbed from them during the elution that may interfere with the fluorescence assay. Such problems do not arise with PC filters. Sarkosyl lysis has been used since sodium dodecyl sulfate also interferes with the Hoechst 33258 assay.

Since the rate of elution of DNA from cis-DDP-treated cells may be influenced by both DNA-interstrand and DNA-protein cross-links, the contribution of the latter to the overall cross-linking was estimated by digestion of the samples with PK. The overall procedure is the same as that described above except that PK (0.5 mg/ml) was added to the lysis solution, and the lysis was retained for 0.5 h on the filter before rinsing. This treatment greatly reduces the influence of DNA-linked protein molecules on the elution profiles; the lesions resistant to digestion are presumed to be predominantly DNA-interstrand cross-links (11).

Detection of DNA Cross-Linking. In order to detect cis-DDP-induced DNA cross-links, the tissue or CHO cell suspensions were irradiated on ice with 5 Gy of X-rays to introduce a known frequency of strand breaks into the DNA; these 5-Gy-irradiated samples consequently eluted faster in the alkaline elution assay than did their nonirradiated controls. Treatment of mice or CHO cells with cis-DDP produced cross-links in the DNA; these cross-links resulted in a slower rate of elution than for the 5-Gy control with no drug treatment. Tissue and CHO cell suspensions were maintained on ice at all times between the completion of the drug treatment and the onset of the elution experiment to prevent repair of both the X-ray-induced strand breaks and the cis-DDP-induced cross-links. The CLF was determined from

\[ CLF = \log (f_0/f_0)/\log (f_0/f_a) \]

where \(f_0\), \(f_s\), and \(f_a\) are, respectively, the fraction of uneluted DNA for the nonirradiated control, the 5-Gy control, and the 5-Gy cis-DDP-treated sample, after a volume of 25 ml had been eluted. A CLF of 1.0 thus represents no cross-linking. All data are the average of three or more separate experiments. Unless otherwise stated, error bars refer to the SE of the data.

cis-DDP. cis-DDP was obtained from two sources: as Platino!, from Bristol Laboratories, Syracuse, NY; and, for some of the initial experiments, from the NCI (NSC 119875, Lot 37-3). The preparation from Bristol Laboratories contained 10 mg cis-DDP, 100 mg mannitol, and 90 mg NaCl and was dissolved in 10 ml distilled water; the NCI compound was dissolved in saline-mannitol (1% mannitol solution containing 9 mg NaCl/ml). These two samples of cis-DDP showed no detectable qualitative differences (i.e., kinetics and relative intertissue levels of cross-linking) in their interaction with cellular DNA; however, the NCI drug produced on the order of twice the DNA cross-linking activity on a per weight basis in each tissue. Throughout the remaining text, the source of the drug for a particular series of experiments will therefore be clearly indicated. trans-DDP was purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

The alkaline elution procedure used in the present investigation has been adapted by us for the measurement of DNA damage in tissue samples and deviates somewhat from the standard protocols described by Kohn et al. (11). It was therefore necessary to validate these modifications using cultured cells treated in vitro as a model system to demonstrate that the technique was capable of detecting both DNA-interstrand and DNA-protein cross-links in such a test system and also that the DNA elution profiles were not significantly dependent upon the type of DNA assay used (i.e., fluorometric or radioactive). We therefore used the modified protocol described earlier in "Materials and Methods" (i.e., PC filters, Sarkosyl lysis, and Hoechst DNA assay) to determine DNA cross-linking in [3H]thymidine-labeled CHO cells treated as exponentially growing monolayers with various doses of cis-DDP (10–20 µg/ml for 1 h). The two assays (fluorometric versus radioactive) were then performed simultaneously on the same elution fractions and generated essentially identical elution profiles (Chart 1). The CLF showed a linear dependence on the dose of cis-DDP at either 6 or 24 h after treatment, both with or
cis-PLATINUM-INDUCED DNA CROSS-LINKING IN VIVO

A major objective of the in vivo study was to measure and compare the amount of DNA damage produced in various tissues of mice treated with a single dose of cis-DDP of a magnitude (about 100 mg/m²) similar to those used clinically (3). In preliminary experiments, mice were treated with graded doses of cis-DDP in a range comparable to the clinical dose to establish suitable dosage protocols and to characterize the sensitivity of the assay procedure. Typical elution profiles for DNA isolated from FSa tumor cells 24 h after treatment with various doses of cis-DDP (4–12 mg/kg; Bristol) are shown in Chart 2. PK digestion was not included in these initial measurements, i.e., these profiles represent the total (DNA-interstrand and DNA-protein) cross-linking effect. Qualitatively similar profiles were obtained for all other tissues examined. In each tissue, increasing the dose of cis-DDP resulted in a progressively slower rate of elution of DNA from the filter relative to that for control mice receiving no drug, indicating a dose-dependent cross-linking of the DNA. The relative degree of cross-linking was linearly related to the dose of cis-DDP in each tissue at either 6 or 24 h after injection, within the dose range studied and the precision of the measurements; examples of such dose-response curves will be shown later. Measurable cross-linking was produced at doses as low as 4 mg/kg, satisfying the first requirement for this study, i.e., adequate experimental sensitivity.

A second objective of this study was to estimate the relative contributions of DNA-interstrand and DNA-protein cross-links to these alkaline elution profiles obtained from tissues treated with cis-DDP in vivo. The results for one such experiment for FSa and for liver (tumor-free mouse) cells are shown in Chart 3. At

Without PK digestion (data not shown). Furthermore, the relative proportion of DNA-interstrand to DNA-protein cross-links, as well as the cross-link repair kinetics, were similar when measured by the two assays and were comparable to the results obtained by Zwelling et al. (25) using 14C-labeled L1210 cells and PVC filters. Immediately after the 1-h drug treatment, relatively few cross-links could be detected, and virtually all of these were PK sensitive, suggesting that the majority of the initial cross-links are DNA-protein in nature. By 6 h after removal of the drug, the degree of cross-linking had increased substantially, some 20–30% of these cross-links now being resistant to PK. After 24 h, some 80 ± 5% of the total cross-links had been repaired; however, the proportion of these lesions resistant to PK digestion was still 20–30%, suggesting that the DNA-interstrand and DNA-protein cross-links were repaired with similar kinetics.

These in vitro control experiments were repeated using trans-DDP. In this case, considerable cross-linking was detected immediately after the 1-h drug treatment. The fluorometric and radioactive DNA assays again produced very similar DNA elution profiles, the degree of cross-linking showing a linear dose dependence in each case (data not shown). One important difference between the results with cis- or trans-DDP was that, for the trans isomer, only about 3–4% of the total cross-linking effect was PK resistant, again confirming the report by Zwelling et al. (25).

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of the DNA from each tissue was substantially increased following incubation with PK, indicating a large contribution from DNA-protein cross-linking. This effect was observed in all tissues studied, although the proportion of PK-resistant lesions varied somewhat from tissue to tissue (Table 1); in particular, the two normal tissues (liver and spleen) showed a significantly lower proportion of DNA-interstrand to total cross-links than did either of the tumors. For liver virtually no residual (i.e., interstrand) cross-links could be detected after PK treatment, while for the tumors the CLF measured after PK digestion was about 40% of the cross-links detected in FSa 6 h after injection had been removed by 24 h. As indicated in Chart 3, these data indicate that the proportion of PK-resistant to total cross-linking for FSa was the same (within experimental resolution) at either 6 or 24 h after injection, suggesting that the interstrand and DNA-protein cross-links were repaired with similar kinetics during this period. It should also be noted from Chart 4 that the level of total cross-linking varied substantially among the different tissues, with liver and FSa showing the highest levels, followed by kidney, while bone marrow and spleen displayed relatively low levels of damage.

A further objective of this study was to compare the time course of the DNA cross-linking effect in different mouse tissues. Typical elution profiles from an experiment in which mice were given a single dose of cis-DDP (8 mg/kg; Bristol) and sacrificed at various times after treatment are shown in Chart 3A and for liver cells from tumor-free mice in Chart 3B. In both tissues, the maximum level of total DNA cross-linking had been achieved by 6 h after injection. However, in the subsequent period up to 24 h, while little change was observed in the liver cells, the cross-linking in FSa tumor had decreased considerably compared to the 6-h levels, presumably reflecting the repair of DNA cross-links by FSa cells. Analysis of the unirradiated control elution profiles (data not shown) showed that cis-DDP caused no detectable DNA strand breaks in either FSa or any other tissue at any of the doses used at either 1, 6, or 24 h after injection, eliminating the possibility that an accumulation of DNA strand breaks with time may contribute to the observed kinetics. It is therefore likely that these data for FSa (Chart 3A) demonstrate true repair of cross-links.

The time course of the formation and disappearance of total DNA cross-linking (i.e., interstrand plus DNA-protein) after treatment with a single dose of cis-DDP was followed in several normal tissues from tumor-free animals. CLFs were calculated from DNA elution profiles such as those shown in Chart 3. The kinetics for spleen, bone marrow, liver, and kidney (cis-DDP, 4 mg/kg; NCI) is shown in Chart 4A. For each tissue, little cross-linking was detected after 1 h; however, when the animals were sacrificed at various times after injection of the drug, cross-links were gradually formed, reaching near-maximal levels by 6 h after injection. No subsequent repair of the cross-links was seen during the 6- to 24-h period in any of the normal tissues. In fact, in some tissues the CLF tended to decrease somewhat during this time. Based on the PK digestion data in Table 1, these data, at least for liver and spleen, largely represent DNA-protein cross-linking.

The kinetics of the total cross-linking effect was also measured in the FSa and NFSa fibrosarcomas. The data for FSa (cis-DDP, 4 mg/kg; NCI) are shown in Chart 4B. The kinetics for both the NFSa (cis-DDP, 8 mg/kg) and FSa tumor (cis-DDP, 12 mg/kg) after treatment with cis-DDP from Bristol are shown in Chart 5 for comparative purposes. In both tumors, cross-linking was again relatively low at 1 h but increased thereafter, reaching near-maximal levels by 6 h after injection. While the kinetics of cross-link formation were similar in both tumors, the subsequent response of the tumors to these lesions was very different. NFSa (Chart 5) resembled the normal tissues (Chart 4A), showing little if any cross-link repair within the first 24 h. On the other hand, as discussed earlier, FSa (Charts 4B and 5) exhibited significant repair activity irrespective of the source of the drug; more than 40% of the cross-links detected in FSa 6 h after injection had been removed by 24 h. As indicated in Chart 3, these data indicate that the proportion of PK-resistant to total cross-linking for FSa was the same (within experimental resolution) at either 6 or 24 h after injection, suggesting that the interstrand and DNA-protein cross-links were repaired with similar kinetics during this period. It should also be noted from Chart 4 that the level of total cross-linking varied substantially among the different tissues, with liver and FSa showing the highest levels, followed by kidney, while bone marrow and spleen displayed relatively low levels of damage.

The kinetics of cross-linking in selected normal tissues from both NFSa- and FSa-bearing animals was also examined to determine whether the presence of a particular tumor might have any effect on the kinetics of DNA damage in other tissues. In order to establish more accurately both the maximum levels of cross-linking and the degree of repair (or lack thereof) in the three different situations (tumor-free, NFSa-bearing, and FSa-bearing animals), the drug dose dependence of the total cis-DDP-induced cross-linking in the FSa and NFSa tumors, and also in the spleen and liver from each group of animals, was determined. Complete dose-response curves were generated at 6 h after injection to measure maximal levels of damage and at 24 h after injection to allow sufficient time for appreciable repair to occur in cases where repair was active. In all cases, the cis-DDP from Bristol was used. Examples of such dose-response curves are shown in Chart 6A for FSa tumor and in Chart 6B for the spleen from NFSa-bearing mice. All dose responses for each tissue were linear within the precision of the measurements. The slopes of these dose responses (determined by linear regression analysis) are presented in Table 2; in each case, a minimum of

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time (h)</th>
<th>CLF (-PK)</th>
<th>CLF (+PK)</th>
<th>% of PK-resistant cross-link factor*</th>
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<tr>
<td>Spleen</td>
<td>6</td>
<td>1.94 ± 0.12²</td>
<td>1.05 ± 0.04</td>
<td>7 ± 4</td>
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<tr>
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<td>24</td>
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<td>1.09 ± 0.04</td>
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<td>1.05 ± 0.03</td>
<td>3 ± 2</td>
</tr>
<tr>
<td></td>
<td>24</td>
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<td>1.11 ± 0.03</td>
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<tr>
<td>NFSa</td>
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<td>19 ± 1</td>
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<tr>
<td></td>
<td>24</td>
<td>3.14 ± 0.12</td>
<td>1.34 ± 0.06</td>
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</tr>
<tr>
<td>FSa</td>
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<td>3.61 ± 0.45</td>
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<tr>
<td></td>
<td>24</td>
<td>3.42 ± 1.05</td>
<td>1.69 ± 0.50</td>
<td>20 ± 8</td>
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* Calculated from the ratio

(CLIF-1) with PK

(CLIF-1) without PK × 100%

b Data from tumor-free mice.

c Mean ± SE.
nine data points (three different doses, three or more points per dose) were used in the analysis. Also shown in Table 2 are the percentage changes in cross-linking occurring during the 6- to 24-h time period.

An examination of the data in Table 2 confirms several of the observations referred to above that were based on more limited data. In each group of animals, the level of damage in liver was always more than twice that measured in spleen, with cross-linking in both tumors being slightly lower than in the liver. DNA damage in tissues from tumor-free or from NFSa-bearing mice either remained constant or increased slightly between 6 and 24 h. On the other hand, 51% of the cross-links detected at 6 h in the FSa tumor were removed by 24 h. The data from FSa-bearing mice also revealed a rather surprising effect in that the kinetics of DNA damage in normal tissues from this group of animals appeared to be somewhat altered relative to that for the respective tissues from tumor-free or NFSa-bearing mice; both the liver and spleen from FSa-bearing animals showed some capacity to remove cross-links (24 and 22%, respectively) during this time period.

DISCUSSION

The importance of the interaction between cis-DDP and DNA to its cytotoxic action in vitro (and thus presumably to its antitumor activity) has been demonstrated in several studies (e.g., Refs. 12, 15, 16, and 24). As was discussed earlier, these in vitro studies have suggested that both the initial amount of cross-linking produced and the subsequent ability of cells to remove these lesions from their DNA appear to be important factors correlating with cytotoxicity. In the present study, we examined the kinetics of cis-DDP-induced DNA cross-linking in mouse tissues in vivo; both marked similarities and significant differences between these in vivo data and previous in vitro results were apparent.

The kinetics of the formation of cis-DDP-induced total (DNA-interstrand plus DNA-protein) cross-linking observed here for...
mouse tissues in vivo are quite similar to those reported previously for L1210 cells (23, 25) and for CHO cells (12) in vitro, with cross-linking increasing over the first few hours and reaching near-maximum levels after about 6 h (Charts 4 and 5). This suggests that the same molecular interactions thought to be involved in the formation of DNA cross-links in vitro are also operative in the in vivo drug response. Thus, the increase in cross-linking up to 6 h after injection in each tissue is presumably due to the same slow formation of the second arm of the cross-link originally described by Zwelling et al. (23) for L1210 cells, although there will inevitably be some additional contribution from pharmacokinetic effects in vivo.

A further similarity between the present in vivo data (Chart 3; Table 1) and previous in vitro studies (12, 15–17, 25) is the large proportion of cross-links that were sensitive to digestion by PK, suggesting a substantial DNA-protein cross-linking effect in addition to DNA-interstrand cross-linking. The magnitude of the PK effect (i.e., in the ratio of PK-resistant to total cross-links) was similar at either 6 or 24 h after injection (Table 1), even in the case of FSa where appreciable repair was apparent. These observations are consistent with the study of Zwelling et al. (25) with cultured L1210 cells, which showed that the ratio of DNA-interstrand to total cross-linking remained relatively constant once the interstrand cross-links had been formed, indicating that both classes of cross-link are repaired at a similar rate, even though their rate of formation may be different. What is perhaps unusual is that the actual proportion of PK-resistant cross-links appeared to vary among the different tissues (Table 1). In particular, the tumors appeared to have a somewhat higher ratio of DNA-interstrand to DNA-protein cross-links than did the two normal tissues studied; the relative proportion of the two types of lesion in the two tumors was in fact similar to that which we observed in cis-DDP-treated CHO cells when the DNA cross-linking was simultaneously measured using the microfluorometric and radioactive DNA assays (Chart 1) and also comparable to the results obtained by Zwelling et al. (25) with L1210 cells. The two normal tissues, on the other hand, had unusually low levels of DNA-interstrand cross-links.

These results, together with the data in Chart 4 and Table 2 showing that the total levels of cross-linking vary substantially among the different tissues treated in vivo, suggest that important factors may modulate both the quantity and quality of the cis-DDP-induced lesions in vivo. If such factors were pharmacological in origin, they would not be amenable to study in vitro. One factor that has been investigated in some detail is the role of thiols in protecting against the cytotoxicity of cis-DDP, both in vitro by agents such as cysteamine (20) and thiourea (26) and in vivo by WR2721 (22) and diethyldithiocarbamate (2). However, modification of cis-DDP-induced DNA cross-linking by thiols cannot explain the present findings since we have shown previously that the liver from C3H mice has a very high GSH content, while FSa and spleen have relatively low levels (14). Thus, on the basis of GSH levels alone, one would predict relative cross-linking levels opposite to those shown in Chart 4 and Table 2 for liver and spleen.

The subsequent response of the cells in vivo in terms of removing cis-DDP cross-links from their DNA was less pronounced than has been reported for cultured mammalian cells. Of all the tissues studied, only the FSa tumor repaired cross-links with an efficiency approaching that of cultured cells, albeit somewhat slower (Charts 4–6; Table 2). None of the tissues examined from tumor-free mice or from NFSa-bearing animals (Table 2) showed any capacity to remove cross-links from their DNA during the first 24 h. The lack of an efficient repair pathway for cis-DDP-induced DNA damage by normal tissues when compared to cultured mammalian cells in exponential growth is perhaps not surprising since the population of cells in these tissues is made up largely of nonproliferative, terminally differentiated cells. It has been well established in several experimental systems (5, 8, 9) that differentiation of mammalian cells is accompanied by a reduced ability to remove chemically induced DNA damage in vitro. What is perhaps more surprising is that only one of the two tumors, FSa, appeared to repair cis-DDP cross-links; NFSa apparently has no capacity to remove these lesions. However, this deficiency may be related to the fact that NFSa is composed largely of normal host cells, particularly macrophages (about 80% of the total population), which are themselves specialized differentiated cells.

The alterations in the kinetics of drug damage in normal tissues from the FSa-bearing animals (Table 2), although not dramatic, were reproducible and therefore require further comment. This effect appears to be dependent on the tumor burden in that, when we repeated these measurements with larger (>15 mm) FSa tumors, the capacity of the normal tissues to repair cis-DDP cross-links; NFSa apparently has no capacity to remove these lesions. However, this deficiency may be related to the fact that NFSa is composed largely of normal host cells, particularly macrophages (about 80% of the total population), which are themselves specialized differentiated cells.

The alterations in the kinetics of drug damage in normal tissues from the FSa-bearing animals (Table 2), although not dramatic, were reproducible and therefore require further comment. This effect appears to be dependent on the tumor burden in that, when we repeated these measurements with larger (>15 mm) FSa tumors, the capacity of the normal tissues to repair cis-DDP cross-links; NFSa apparently has no capacity to remove these lesions. However, this deficiency may be related to the fact that NFSa is composed largely of normal host cells, particularly macrophages (about 80% of the total population), which are themselves specialized differentiated cells.
(26) where the thioarene appeared to prevent the conversion of monoadducts to actual cross-links. The role of thioles in the removal of cis-DDP cross-links, whether direct or indirect, obviously requires further investigation.

In conclusion, the data in this study have revealed that, while there are some basic similarities between the effects of cis-DDP in both the in vivo and in vitro situations, some important differences are also apparent. These differences relate to both the quantity and the quality of the lesions produced in the DNA of the different tissues and to the general absence of repair of these lesions in normal tissues on a time scale similar to that seen in vitro. Thus, the relationship between cis-DDP cross-links and their repair established with cultured cells cannot be easily extended to the in vivo situation, perhaps because the majority of the cells in mammalian tissues are nonproliferative, differentiated cells. While these results will have to be compared to other indicators of cellular toxicity in animals, investigations such as those presented here may at least provide an easily characterizable and relevant pharmacological end point (DNA lesions) to indicate the distribution and modulation of the antitumor effect of chemotherapy agents that have DNA as their target.

REFERENCES

Kinetics of DNA Cross-Linking in Normal and Neoplastic Mouse Tissues following Treatment with \textit{cis}\textsuperscript{-}Diamminedichloroplatinum(II) \textit{in Vivo}

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