DNA Repair in Cells Sensitive and Resistant to *cis*-Diamminedichloroplatinum(II): Host Cell Reactivation of Damaged Plasmid DNA†

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ABSTRACT: *cis*-Diamminedichloroplatinum(II) (*cis*-DDP) has a broad clinical application as an effective anticancer drug. However, development of resistance to the cytotoxic effects is a limiting factor. In an attempt to understand the mechanism of resistance, we have employed a host cell reactivation assay of DNA repair using a *cis*-DDP-damaged plasmid vector. The efficiency of DNA repair was assayed by measuring the activity of an enzyme coded for by the plasmid vector. The plasmid expression vector pRSVcat contains the bacterial gene coding for chloramphenicol acetyltransferase (CAT) in a configuration which permits expression in mammalian cells. The plasmid was transfected into repair-proficient and -deficient Chinese hamster ovary cells, and CAT activity was subsequently measured in cell lysates. In the repair-deficient cells, one *cis*-DDP adduct per *cat* gene was sufficient to eliminate expression. An equivalent inhibition of CAT expression in the repair-proficient cells did not occur until about 8 times the amount of damage was introduced into the plasmid. These results implicate DNA intrastrand cross-links as the lesions responsible for the inhibition of CAT expression. This assay was used to investigate the potential role of DNA repair in mediating *cis*-DDP resistance in murine leukemia L1210 cells. The parent cell line L1210/0 resembled repair-deficient cells in that about one adduct per *cat* gene eliminated expression. In three resistant L1210 cell lines, 3–6-fold higher levels of damage were required to produce an equivalent inhibition. This did not correlate with the degree of resistance as these cells varied from 10- to 100-fold resistant. This is considered a limitation of the assay in that at high levels of DNA damage, the repair of one adduct is blocked by the presence of other adducts. However, the assay readily detects the presence or absence of repair and confirms that these resistant L1210 cells have an enhanced capacity for repair of *cis*-DDP-induced intrastrand cross-links.

Cellular resistance to *cis*-diamminedichloroplatinum(II) (*cis*-DDP),[1] as with other cancer chemotherapeutic agents, is a major clinical limitation. Many experimental systems have been developed to investigate the mechanisms of this resistance [reviewed in Eastman et al. (1988)]. Various alterations in resistant cells have been reported, e.g., decreased drug accumulation and increased glutathione concentration, and the majority of papers have described resistance as being due to multiple mechanisms. It is likely that some of these changes are not primarily responsible for the resistance.

Investigations from this laboratory have focused on murine leukemia L1210 cells that are preferentially resistant to *cis*-DDP (L1210/DDP). These cells show a slight reduction in drug accumulation, but the major contribution to resistance occurred after the critical target, DNA, had been platinated (Richon et al., 1987). An analysis of the formation and removal of specific DNA damage led to the conclusion that these cells possessed an enhanced DNA repair capacity (Eastman & Schulte, 1988). The sensitivity for those studies was made possible by the synthesis of a radiolabeled analogue. In the current paper, we have investigated this phenomenon further using a different assay for repair that does not require radiolabeled drug and can therefore be applied to the analysis of any DNA-damaging agent. The assay also provides information on cellular responses to DNA damage without confounding effects of damage to other cellular molecules.

The assay involves host cell reactivation of a *cis*-DDP-damaged transient expression vector. This vector, the plasmid pRSVcat, contains the bacterial *cat* gene coding for chloramphenicol acetyltransferase (CAT) in a configuration that permits expression in mammalian cells. It also contains pBR322 sequences allowing for replication in bacteria and ampicillin resistance. Lesions introduced into the pRSVcat vector will diminish or abolish *cat* gene expression after transfection. Repair of these lesions by the recipient cells will restore expression, thereby providing an efficient system to study DNA repair. A similar system has been used to show that cells from xeroderma pigmentosum patients, which were unable to excise pyrimidine dimers, also failed to restore expression of UV-irradiated transfected DNA (Lehmann & Oomen, 1985; Protic-Sabljic & Kraemer, 1985; Klocker et al., 1985). Normal human fibroblasts repaired damaged plasmid and expressed much higher levels of CAT activity.

Materials and Methods

Drugs. For cytotoxicity experiments, *cis*-DDP (Platinol, Bristol Laboratories; Syracuse, NY) was dissolved according to the manufacturer's recommendations. For platination of plasmid DNA, *cis*-DDP (Developmental Therapeutics Program, Division of Cancer Treatment, NCI) was dissolved in

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[1] Abbreviations: CAT, chloramphenicol acetyltransferase (protein); *cat*, gene for CAT; CHO, Chinese hamster ovary; *cis*-DDP, *cis*-diamminedichloroplatinum(II); DEAE, diethylaminoethyl; UV, ultraviolet.

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0.02 M $NaClO_4$, pH 5.5, immediately prior to use.

Cells and Culture Conditions. The sensitive murine leukemia L1210 cells, L1210/0, and the *cis*-DDP-resistant sublines have previously been described (Eastman & Bresnick, 1981; Eastman & Richon, 1986). The resistant cell lines L1210/DDP_5 and L1210/DDP_{10} were developed by growth in stepwise increases in *cis*-DDP concentration over several years and are now maintained in 5 and 10 μg/mL, respectively. They exhibit approximately 50- and 100-fold resistance to *cis*-DDP. The L1210/PDD cell line was obtained from Dr. Burchenal, Memorial Sloan Kettering, New York, NY. These cells were originally selected for resistance in vivo (Burchenal et al., 1977). They had been maintained frozen since 1978 in the Vermont Regional Cancer Center. Suspension cultures of the cell lines were maintained in McCoy's 5a (modified) medium (GIBCO, Grand Island, NY) supplemented with penicillin (300 units/mL), streptomycin (300 units/mL), fungizone (1 μg/mL), and 15% calf serum.

Sensitivity of the L1210 cells to drug was routinely confirmed by measuring the inhibition of cell growth. Approximately 5×10^4 cells/mL were incubated in triplicate with varying concentrations of drug over a 3-day period. The resulting cell number was determined by using a Coulter Counter Model Z_f (Coulter Electronics, Hialeah, FL). Values were expressed as IC_{50}, which is the concentration effective in inhibiting cell growth by 50%. The properties of the cell lines were presented in Table I of Richon et al. (1987).

The CHO cell lines designated AA8, UV5, UV20, and UV41 (from Dr. Larry H. Thompson, Lawrence Livermore National Laboratory, Livermore, CA) were maintained in monolayer culture in α-Minimum Essential Medium (Gibco), supplemented with 2.5% fetal bovine serum, 2.5% horse serum, penicillin, and streptomycin. The UV5, UV20, and UV41 cell lines were derived by mutation of the AA8 cells and represent three different complementation groups, each deficient in a preincision step of excision repair (Thompson et al., 1981). For growth inhibition studies, 500 cells were plated in 60-mm tissue culture dishes for 18 h prior to a 2-h drug incubation. Following platination, the plates were washed twice with Hank's balanced salt solution (Gibco), and fresh medium was added. The colonies were fixed, stained with Giemsa, and counted at 7–10 days.

Plasmid Preparations. pRSVcat (5027 base pairs; kindly provided by Dr. B. Howard, National Institutes of Health) contains the bacterial *cat* gene inserted between the 3′ long terminal repeat (LTR) of Rous sarcoma virus (RSV) and SV40 polyadenylation sequences to make a gene transcriptional unit (Gorman et al., 1982). Plasmid DNA was prepared by alkaline lysis and cesium chloride/ethidum bromide ultracentrifugation (Maniatis et al., 1982). DNA samples were routinely electrophoresed in a 1% agarose gel to ensure more than 75% of plasmid DNA was in the supercoiled form. DNA solutions were stored in ethanol at −20 °C. For *cis*-DDP treatment, plasmid DNA was dissolved in 0.02 M $NaClO_4$, pH 5.5, and adjusted to 250 μg/mL (as assayed by A_{260}) by further addition of $NaClO_4$ and appropriate concentrations of *cis*-DDP. Incubation was for 8 h at 37 °C. Aliquots containing 10 μg of DNA were stored at −20 °C until transfection. Platination of DNA approaches 100% under these conditions (Eastman, 1983). In initial experiments, this was confirmed by flameless atomic absorption spectroscopy.

Transfection. Transfection was performed by using the DEAE-dextran technique modified from Sussman and Milman (1984) and Gopal (1985). L1210 cells were concentrated by centrifugation, washed, and resuspended at 10^7 cells/mL in McCoys medium (unsupplemented). Cell suspension (1 mL) was added to 15-mL conical centrifuge tubes. The DNA solution containing 10 μg of plasmid DNA, 50 μL of DEAE-dextran stock solution (5 mg/mL DEAE-dextran, M_r 500 000, in 1 M Tris, pH 7.4), and 100 μL of serum-free medium was added. After incubation at 37 °C for 2 h, 10 mL of complete medium was added, and the cells were transferred to 100-mm culture dishes and incubated at 37 °C for 48 h. In the case of L1210/PDD and L1210/DDP_{10} cells, after the 2-h incubation, 1 mL of 30% glycerol in phosphate-buffered saline was added, incubated for 2.5 min, and then diluted with 10 mL of medium. Cells were recovered by centrifugation, resuspended in 10 mL of complete medium, and plated for expression.

CHO cells were plated at 2.5×10^6 cells/100-mm culture dish 16 h prior to transfection. The medium was replaced with 1 mL of DNA solution containing 10 μg of plasmid, 250 μg/mL DEAE-dextran, and 50 mM Tris, pH 7.4, in complete α-Minimal Essential Medium. After 2-h incubation, 10 mL of medium was added and incubation continued for 48 h.

Transient Expression Assay. Cells were harvested 48 h after transfection, resuspended in phosphate-buffered saline, transferred to a microfuge tube, centrifuged, and resuspended in 200 μL of 0.25 M Tris-HCl, pH 7.8. The cells were lysed by freeze–thawing 3 times. The lysate was centrifuged (14000*g*) for 15 min at 4 °C, and the supernatant was heated at 65 °C for 7 min to destroy deacetylase activity (Mercola et al., 1985). The sample was chilled and spun at 4 °C for 5 min. The supernatant was assayed for CAT activity by the method of Neumann et al. (1987) as modified by Eastman (1987a). Briefly, the 250-μL incubation contained 100 mM Tris-HCl, pH 7.8, 1 mM chloramphenicol, 0.1 μCi of [^{3}H]-acetyl-coenzyme A, and up to 100 μL of heated cell lysate. The reaction was performed in a 5-mL plastic scintillation vial overlayed with 5 mL of immiscible scintillation cocktail (Econofluor, DuPont NEN Research Products). The vials were incubated at room temperature in a scintillation counter while they cycled for 2 h, counting for 1 min every 10 min. As the reaction proceeds, the [^{3}H]acetylchloramphenicol that is produced diffuses into the scintillation cocktail and is measured. Results are expressed as the amount of radioactivity that diffuses into the cocktail in 2 h. We have previously demonstrated linearity with enzyme concentration as long as less than 50% of the tritium entered the scintillation cocktail in 2 h (Eastman, 1987a).

Recovery and Analysis of Transfected Plasmid. L1210/0 and L1210/DDP_5 cells were transfected with platinated pRSVcat as described above. Cells were harvested 6 and 48 h following transfection, centrifuged, and resuspended in 5 mL of Hank's balanced salt solution containing deoxyribonuclease I (50 μg/mL) (Sigma Chemical Co., St. Louis, MO) and 10 mM $MgCl_2$. Samples were incubated at 37 °C for 30 min, centrifuged, and washed with 5 mL of PBS. This procedure removes DNA from the cell surface (Graham & Van Der Eb, 1973). Plasmid DNA was extracted from the cells by the method of Hirt (1967). The supernatant was extracted with phenol and chloroform, and the DNA was precipitated with ethanol. The pellet was resuspended in 100 μL of 10 mM Tris-HCl/1 mM EDTA, pH 8.0, and a 25-μL aliquot was digested with *Hin*dIII in the recommended buffer (BRL, Gaithersburg, MD). The DNA was electrophoresed on a 1% agarose gel, transferred to a nylon membrane, and hybridized to ^{32}P-pRSVcat (Maniatis et al., 1982).

Results

Optimization of Transient Expression Assay. These ex-

Table I: Survival and DNA Repair Capacity of Cell Lines

cell line	cell survival[a] (μg/mL)	adducts/plasmid inhibiting CAT act. by 63%	control act.[b] (%)
CHO-AA8	4.7	19.3	55
CHO-UV5	2.3	1.6	14
CHO-UV20	0.1	2.2	4
CHO-UV41	0.05	3.0	36
L1210/0	0.12	3.4	50
L1210/PDD	1.2	10	3
L1210/DDP_5	5.8	23	6
L1210/DDP_{10}	13.2	12	5

[a] For CHO cells, values represent the concentration of *cis*-DDP in a 2-h incubation that reduced colony-forming capacity by 50%. For L1210 cells, values represent the concentration of *cis*-DDP present continuously that reduced growth by 50% over 3 days. Exposure of L1210 cells for 2 h required about 5-fold higher drug concentration. [b] Approximate percent conversions of [^{3}H]acetyl-coenzyme A to [^{3}H]-acetylchloramphenicol in 2 h in lysates from cells transfected with undamaged plasmid.

periments required that plasmid be transfected into L1210 cells which grow in suspension. Accordingly, transfection efficiency was initially optimized in L1210/0 cells. Of several techniques tested, DEAE-dextran gave the best transfection. Several modifications were subsequently made for the other cell lines as detailed under Materials and Methods. This technique was also used for the CHO cells even though they were grown as monolayer cultures. With the exception of L1210/PDD and L1210/DDP_{10} cells, the use of glycerol shock was found to reduce the level of expression. For the L1210/0 cells, heating the cell lysate at 65 °C for 7 min prior to enzyme assay enhanced the activity by 50%. This destroys deacetylase activity present in the cell lysate (Mercola et al., 1985). The other cell lysates were similarly heated.

Each cell line exhibited a different capacity to express undamaged DNA. In L1210/0 cells, transfection of undamaged plasmid resulted in approximately 50% conversion of [^{3}H]-acetylcoenzyme A to acetylchloramphenicol during a 2-h incubation. The equivalent values for the other cell lines are presented in Table I. CAT activity derived from damaged plasmid was expressed as a percentage of these values. The inhibition curves produced were independent of the overall level of transfection into the cell line. This was confirmed in experiments in which damaged and undamaged DNA was intentionally transfected into L1210/0 cells under suboptimal conditions, i.e., less DEAE-dextran, with or without a glycerol shock. Under conditions in which unplatinated plasmid led to 5–50% of the [^{3}H]acetyl-coenzyme A being metabolized, the inhibition curves resulting from platinated DNA were identical (not shown).

Plasmid Reactivation Assay in CHO Cells. To confirm that this assay was measuring DNA repair, we investigated plasmid reactivation in CHO cells of known DNA repair capacity. An example of the assay is shown in Figure 1. DNA repair-proficient AA8 cells were transfected with plasmid damaged to different extents. Lysates were made from these cells 48 h after transfection and assayed for CAT activity. The amount of [^{3}H]acetylchloramphenicol diffusing into the scintillation cocktail with time was recorded directly by repeated scintillation counting. The amount of activity resulting from transfection with undamaged plasmid is defined as 100%. The inhibition of CAT activity with increasing damage can then be graphed as in Figure 2. These curves can be further described by the level of adducts that results in a 63% reduction in activity, i.e., the level of damage producing a "mean lethal hit". In the three repair-deficient cell lines, 1.6–3 adducts per plasmid were sufficient to produce a mean lethal hit whereas

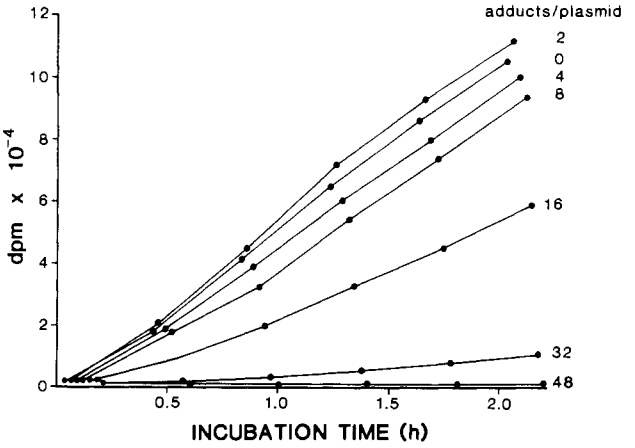

FIGURE 1: Kinetics of formation of [^{3}H]acetylchloramphenicol in lysates from DNA repair-proficient CHO-AA8 cells that had been transfected 48 h previously with undamaged or *cis*-DDP-damaged pRSVcat. Incubation conditions are described in the text. The curves were obtained by repeated scintillation counting as the product of the reaction diffused into the scintillation cocktail. The amount of reaction at 2 h was used to derive the inhibition curve for AA8 cells in Figure 2.

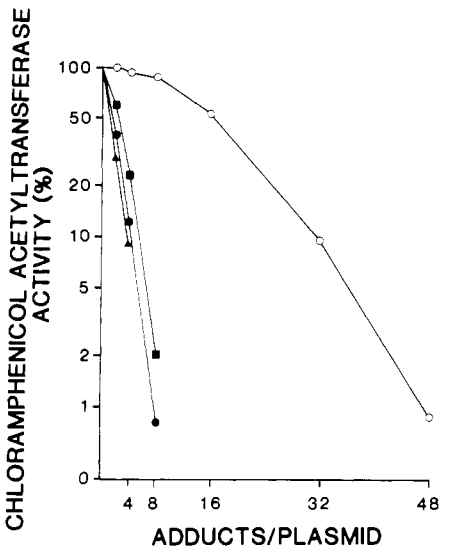

FIGURE 2: Expression of the *cat* gene in DNA repair-proficient (AA8) and repair-deficient (UV5, UV20, UV41) CHO cells transfected with *cis*-DDP-damaged plasmid. Each point represents the amount of [^{3}H]acetylchloramphenicol produced in a 2-h incubation and is expressed as a percent of that produced by lysates from cells transfected with undamaged plasmid. The symbols represent AA8 (○), UV5 (●), UV20 (▲), and UV41 (■) cell lines.

approximately 8 times as much damage was required in AA8 cells (Table I).

These results were consistent with the designation of repair deficiency accorded to the cell lines. In later studies, each new batch of platinated plasmid was transfected into UV41 cells as a control to confirm the level of DNA damage.

Expression of cis-DDP-Damaged Plasmid in L1210 Cells. Transfection of plasmid into L1210/0 cells demonstrated that about three adducts per plasmid were required to reduce CAT activity by 63% (Table I). Transfection into the resistant cells demonstrated that they all had the ability to express CAT activity at much higher levels of damage (Figure 3). The L1210/DDP_5 cells demonstrated activity at higher levels of damage than the other cell lines. However, the inhibition curves from this cell line consistently showed a slight increase in CAT activity at low levels of damage. We suggest this is due to an enhancement of transcription by low levels of dam-

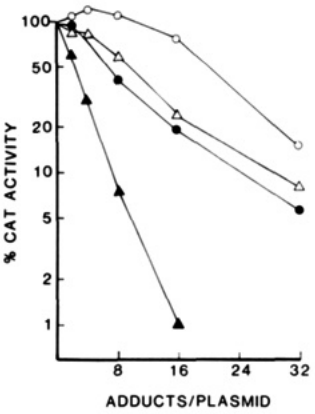

FIGURE 3: Expression of the *cat* gene in parent L1210/0 and three *cis*-DDP-resistant cell lines transfected with *cis*-DDP-damaged plasmid. The symbols represent L1210/0 (▲), L1210/PPD (●), L1210/DDP_5 (○), and L1210/DDP_{10} (△) cell lines.

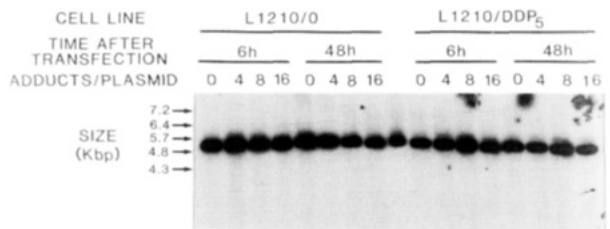

FIGURE 4: Recovery of plasmid DNA from L1210/0 and L1210/DDP_5 cells 6 and 48 h after transfection of undamaged and *cis*-DDP-damaged plasmid. The DNA was recovered in the Hirt supernatant, digested with *Hin*dIII, electrophoresed, Southern transferred, and hybridized to ^{32}P-pRSVcat.

age. Accordingly, the enhanced level of activity in these cells may not be due to a greater enhancement of repair than in the other resistant cell lines.

Analysis of Transfected Plasmid. An alternate explanation for the results observed here is that the platination of DNA affects the level of transfection or that platinated DNA is degraded differently in the various cell lines. Although these possibilities appeared unlikely given the agreement between the repair capacity and CAT activity in CHO cells, this was investigated by rescuing and analyzing the plasmid after transfection. Platinated DNA was transfected into L1210/0 and L1210/DDP_5 cells. After 6 and 48 h, plasmid was rescued into the Hirt supernatant, digested with a restriction endonuclease, and analyzed by gel electrophoresis, Southern transfer, and hybridization to ^{32}P-pRSVcat. The restriction enzyme digestion was included to avoid the detection of multiple bands that resulted from concatamerization of the plasmid. The results showed identical hybridization signals under all conditions (Figure 4). This demonstrated that the platination did not alter the level of transfection. The same level of plasmid was detected at both 6 and 48 h after transfection, demonstrating no differential degradation of the plasmid in either cell line.

Discussion

Many mammalian cells efficiently express genes that are introduced by transfection of recombinant plasmids. The transient expression of transfected DNA has been used to study the effect of DNA damage on transcription and to look at the ability of recipient cells to repair such lesions. Several investigators showed that damaging plasmid with ultraviolet light inhibited expression of the *cat* gene after transfection into normal human fibroblasts (Lehmann & Oomen, 1985; Protic-Sablijic & Kraemer, 1985; Klocker et al. 1985). The inactivation was much higher in cells from xeroderma pigmentosum patients who were deficient in repair of UV damage. DNA repair-deficient cells are also hypersensitive to *cis*-DDP. This is true for *Escherichia coli* uvr (Beck & Brubaker, 1973) as well as xeroderma pigmentosum cells (Fraval et al., 1982) and Chinese hamster ovary mutants (Meyn et al., 1978). This suggests that UV and *cis*-DDP damage is repaired at least in part by the same mechanism.

However, there do appear to be differences in cellular responses to UV and *cis*-DDP damage. The CHO mutants were selected for sensitivity to UV light, and each cell line had about 4–6-fold enhanced sensitivity. These cell lines demonstrate a 2–100-fold enhanced sensitivity toward *cis*-DDP (Table I). Each of these cell lines demonstrated the expected inability to repair *cis*-DDP-damaged plasmid. The results showed that the mean lethal hit (63% reduction in activity) occurred at a level of about three adducts per plasmid. The *cat* gene is 1640 base pairs, about one-third of the plasmid. It appears that one adduct per *cat* gene is sufficient to totally inhibit expression. This is a slight discrepancy from the reports of UV-damaged plasmid in which one adduct per transcribed strand of the gene was sufficient to produce the same level of transcription (Protic-Sabljic & Kraemer, 1985). This might be due to inaccuracy in assessing the level of damage or to the use of different damaging agents. Interstrand cross-links could inactivate both strands of the gene, but this cannot explain the results with *cis*-DDP because this type of lesion represents less than 1% of the total platination. Treatment of DNA in vitro with *cis*-DDP produces damage of which more than 90% is intrastrand cross-links (Eastman, 1987b). This strongly suggests the importance of DNA intrastrand cross-links to the observed toxicity of this drug.

The AA8 cell line is competent for repair and was capable of expressing equivalent CAT levels when up to 8 times the amount of damage had been introduced into the plasmid. This strongly supports the notion that this assay is able to measure DNA repair. It should be emphasized that the levels of DNA repair measured by this assay do not correlate with the differential toxicities of the various cell lines. In particular, the UV5 cells are reportedly deficient in the incision step for repair and are unable to repair damaged plasmid; however, their sensitivity to *cis*-DDP more closely resembles that of the AA8 cells than the hypersensitive cell lines. The reason for this is not presently apparent. It is possible that this represents alternate essential functions of the proteins involved or some other factors that are not necessarily linked to cell survival. An explanation will require a complete characterization of these proteins.

The plasmid reactivation assay was used to investigate the DNA repair capacity of L1210 cells. The sensitive L1210/0 cells exhibited activity very similar to that in the repair-deficient CHO cell lines; that is, they appeared to repair no damage, and about three adducts per plasmid were sufficient to produce a mean lethal hit. In our previous studies with a radiolabeled drug analogue, some repair was seen in these cells (Eastman & Schulte, 1988). They were able to repair about 20% of their adducts during the first 6 h after damaging the cells, but subsequently little if any repair was observed. It is necessary to emphasize that the L1210/0 cells are considered normal with regard to their response to *cis*-DDP; i.e., the level of DNA-bound adducts and the concentration of *cis*-DDP required to produce toxicity are similar to those for many other cell lines (Eastman & Schulte, 1988). After similar drug treatments, the L1210/0 cells are only about 5-fold more sensitive to *cis*-DDP than the CHO-AA8 cells. Therefore, the

L1210/DDP_{10} cells are markedly more resistant than even the CHO cells. The *cis*-DDP-resistant L1210 cell lines all exhibited the ability to express CAT from plasmid containing considerably higher levels of adducts. The L1210/PDD and L1210/DDP_{10} cells demonstrated very similar CAT activity even though they exhibited about 10-fold difference in drug sensitivity. The L1210/DDP_5 cells exhibited equivalent CAT activity at a somewhat higher level of adducts per plasmid. Characteristic of this line was the slightly increased activity seen at low levels of damage. We believe this is due to damage-induced enhanced transcription. A similar observation was made in studies transfecting UV-damaged plasmid into cells and selecting for cells that had integrated the plasmid into their genome (Spivak et al., 1984). In that case, the damage probably enhanced the integration frequency. The slope of the inhibition curve for the L1210/DDP_5 cells is similar to the slope for the other resistant cell lines, suggesting that these cell lines may all be repairing about the same amount of damage from the plasmid.

Alternate explanations for these results were sought. It was determined that the inhibition curves were independent of the different levels of expression of undamaged plasmid in the various cell lines. It was further demonstrated that platination of the plasmid did not affect the level of transfection and that there was no differential degradation of the plasmid after transfection. Perhaps of interest was the observation that no degradation appeared to occur between 6 and 48 h after transfection. The DNA recovered in these assays represents only intracellular plasmid because the cells were digested with deoxyribonuclease before lysis. However, this does not represent the nuclear plasmid which is only a small fraction of the total recovered. It is this small fraction whose repair and transcription are responsible for the observed gene expression.

These results support the hypothesis that enhanced DNA repair contributes to the resistance of L1210 cells to *cis*-DDP. The results do not demonstrate increasing repair with increasing resistance. We believe this is a limitation of the assay. The higher levels of modification produce a frequency of 1 adduct per 150 base pairs. The distance between some of these adducts will be much shorter such that overlapping repair patches will make adequate repair impossible. This level of modification far exceeds the level in the genomic DNA that causes toxicity to the cell. However, in the least resistant cells whose resistance had been developed during passage in vivo, a significant increase in DNA repair was seen. This implicates enhanced DNA repair as an early event in the development of resistance in these cells.

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Registry No. *cis*-DDP, 15663-27-1.

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