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Enhanced Repair of a Cisplatin-Damaged Reporter Chloramphenicol-O-Acetyltransferase Gene and Altered Activities of DNA Polymerases α and β, and DNA Ligase in Cells of a Human Malignant Glioma Following In Vivo Cisplatin Therapy

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Abstract Current evidence suggest an important role for increased repair of drug-induced DNA damage as one of the major mechanisms involved in tumor cell resistance to cis-DDP. In this study, we examined the DNA repair capacity and the activities of three DNA repair related proteins, namely, DNA polymerases α and β, and total DNA ligase in cells of a malignant oligodendroglioma obtained from a patient before therapy and compared it with those of a specimen of the tumor acquired after the patient had failed cis-DDP therapy. DNA repair capacity was quantitated as the extent of reactivation of the chloramphenicol-O-acetyltransferase (CAT) gene in a eukaryotic expression vector that had been damaged and inactivated by prior treatment with cis-DDP and then transfected into the tumor cells. The extent of DNA-platinum adduct formation in the expression vector was determined by flameless atomic absorption spectrometry. The level of cis-DDP resistance of cells of the two tumors was determined with the capillary tumor stem cell assay. We observed a 2.8-fold increased capacity to repair Pt-DNA adducts and reactivate the CAT gene in cells of the tumor obtained after cis-DDP therapy, compared to cells of the untreated tumor. This was associated with increases of 9.4-fold and a 2.3-fold, respectively, in DNA polymerase β and total DNA ligase activities in cells of the treated tumor. At 5 μM cis-DDP, there was a 5.9-fold increase in the in vitro cis-DDP resistance of post-therapy tumor cells relative to cells of the untreated tumor. No significant difference in DNA polymerase α activity was observed between the two tumors. These data suggest that the enhanced ability to repair cis-DDP induced DNA damage, mediated, in part, by increased tumor DNA polymerase β and DNA ligase activities, plays an important role in the in vivo acquisition of cis-DDP resistance in human malignant gliomas, and that these proteins and/or their encoding genes may represent critical targets for strategies to overcome such resistance clinically.

Key words: drug-induced DNA damage, cis-DDP, malignant oligodendroglioma, CAT, eukaryotic expression vector

Cisplatin (cis-DDP) is clinically active against many human tumors, including those of the brain [Loeher and Einhorn, 1984; Spence et al., 1992]; however, as with most anticancer agents, the acquisition of resistance to this agent by tumor cells often prevents sustained therapeutic response. The enhanced repair of drug-induced DNA damage has now been identified as a major part of the complex cellular and molecular mechanisms underlying human tumor resistance to cis-DDP [Reed et al., 1986; Poirier et al., 1987; Behrens et al., 1987; Eastman and Schulte, 1988; Lai et al., 1988, 1989; Fichtinger-Schepman et al., 1988, 1990; Reed et al., 1989; Sheibani et al., 1989]. In a previous report, we demonstrated, using cell lines, that the ability to repair total genomic DNA interstrand crosslinks induced by cis-DDP correlates with de novo cis-DDP resistance in human malignant astrocytoma cells [Sriram et al., 1990]. To determine the role that DNA repair might play in the acquisition of cis-DDP resistance after in vivo therapy, we have, in the present study, extended these earlier observations and examined the

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DNA repair capacity of cells of paired biopsies of a malignant oligodendroglioma obtained from a patient before therapy and after failure to cisplatin therapy. DNA repair was quantitated as the extent to which the tumor cells were capable of reactivating a transfected chloramphenicol-O-acetyl-transferase reporter gene, previously damaged by cis-DDP treatment. This assay does not require exposure of the tumor cells to cis-DDP, and consequently, eliminates potential perturbations of the inherent DNA repair ability of the cells that may result from cis-DDP interactions with cellular components, such as proteins and DNA. Although the exact mechanisms involved in cellular DNA interstrand crosslink repair are still only poorly understood, a large body of evidence have defined a critical role for DNA polymerases and DNA ligases in the repair process, both directly in excision repair pathways, and/or as part of a recombinational repair process [Hannawalt et al., 1982; Friedberg et al., 1987; Sancar and Sancar, 1988; Perrino and Loeb, 1990]. DNA polymerases catalyze the synthesis of oligonucleotides for patching of gaps generated at sites of DNA damage by the action of endo- and exonucleases. Ligation of the newly synthesized DNA to the parent DNA strand then proceeds under the catalytic action of DNA ligases. Consequently, in this study, we examined the activities of DNA polymerases α and β, and total DNA ligase to determine whether alterations in the activities of these key DNA repair associated proteins were related to the observed changes in DNA repair capacity and the in vivo acquisition of cis-DDP resistance of the tumor cells following cis-DDP therapy.

MATERIALS AND METHODS

Biochemicals and Other Reagents

Cis-DDP (Bristol-Myers-Squibb, Wallingford, CT) was obtained commercially as aqueous solutions. ^{14}C-acetylcoenzyme A and γ-^{32}P-ATP and ^{3}H-dTTP were obtained from New England Nuclear, Boston, MA. Chloramphenicol, diethylaminoethyl (DEAE)-Dextran, ethidium bromide, and other reagents, unless otherwise stated, were purchased from Sigma Chemical Co., (St. Louis, MO).

Tumors

Tumor specimens were acquired incidental to surgery and on an institutionally approved protocol. The first specimen (JO 1) was obtained at time of initial surgery and prior to any chemotherapy. The second specimen (JO 2) was obtained after the patient had been placed on a high dose cis-DDP protocol and had relapsed to this therapy. The tumor at both times was classified as a malignant oligodendroglioma. Tumor cells not used immediately were stored in liquid nitrogen until required.

In Vitro cis-DDP Sensitivity Assay

Tumor cell cis-DDP sensitivity was assayed using the capillary tumor clonogenic cell assay [Ali-Osman and Beltz, 1988; Ali-Osman, 1991]. Briefly, triplicate plating mixtures containing 2.5×10^5 cells/ml and 0.2% agarose in enriched CMRL 1066 medium were prepared and cis-DDP stock solution added to achieve final concentrations in the range of 0–5 μM. The mixtures were vortexed and 50 μl drawn into triplicate sterile round glass microcapillary tubes (92 mm in length and 1.38 mm in diameter). After the agarose had gelled on a cold surface, the capillary tubes were incubated at maximum humidity, 5% CO_2 and 37°C for 2 weeks. Colonies of 50 μm or greater in diameter were counted and surviving fractions (S.Fs.) of cis-DDP-treated cultures relative to nondrug-treated controls were computed and plotted against the cis-DDP concentrations.

Preparation and Platination of pCAT Vector

The eukaryotic expression vector, pCAT (Promega Corporation, Madison, WI) contains the CAT gene under the regulatory control of SV40 promoter and enhancer sequences. Stock vector for these studies was prepared by transformation in competent cells and grown to the required amount. The plasmid released by alkaline lysis was ethanol precipitated, and purified by ethidium bromide/CsCl ultracentrifugation. Each plasmid preparation was monitored electrophoretically before use in the studies. Inactivation of the vector was achieved by adding appropriate volumes of 100× stock aqueous cis-DDP solutions to the vector in TE buffer (pH 7.2; total volume of 1 ml) to achieve final concentrations of 25 μM and 50 μM cis-DDP, respectively. After 3 h, the DNA was ethanol-precipitated, washed twice in 70% ethanol, and redissolved in TE buffer (pH 7.2).

Quantitation of DNA-Pt Adducts in pCAT Vector by Flameless Atomic Absorption

These studies were designed to estimate the level of Pt-DNA adducts induced in the pCAT vector which was being introduced in the tumor cells for repair. Triplicate samples of the pCAT vector were platinated and precipitated, as described above. The DNA was redissolved in Tris buffer (pH 7.2). Platinum content was analyzed by flameless atomic absorption spectroscopy (Varian model 30; Varian, Sugarland, TX), as we previously described [Ishikawa and Ali-Osman, in press]. The amount of DNA-Pt adducts formed after treating the vector with 25 and 50 μM cis-DDP was expressed as pmol Pt per μg pCAT DNA.

CAT Gene Transfection

This was performed as we previously described [Ali-Osman et al., 1990]. A total of 10^5 tumor cells were plated per T25 tissue culture flask in DMEM containing 15% FCS. After 24 h, the cultures were rinsed twice with tris-buffered saline (TBS) and once with serum-free DMEM. Ten micrograms purified vector DNA in 4 ml of a 2 mg/ml DEAE-Dextran solution were then added, and the flasks incubated for 3 h at 37°C. After washing twice in TBS, the cultures were refed with DMEM supplemented with 15% FCS and incubated at 37°C. Over 4 days, cells from triplicate flasks were harvested at 24-h intervals and used for CAT enzyme activity measurement as described below.

Assay of CAT Enzyme Activity

CAT enzyme activity was assayed using a modification of a previously described method [Sheibani et al., 1989]. Harvested cells were pelleted and resuspended in 50–150 μl (depending on cell number) Tris-HCl, pH 7.8, and freeze-thawed four times by immersion in liquid nitrogen followed by warming and vortexing at 37°C in a water bath. The mixture was centrifuged at 10,000*g* for 10 min, and the supernatant was removed and heated at 65°C for 15 min to inactivate any contaminating deacetylases. The resulting cell extract was then centrifuged for 10 min at 10,000*g* and the supernatant was used for the CAT activity measurement. The reaction mixture (total volume 250 μl for the CAT enzyme assay consisted of 1.25 μM chloramphenicol, 0.2 μCi ^{14}C-acetyl-CoA, 80 μM cold acetyl-CoA, 20 μl Tris buffer (pH 7.8), and 25 μl (40 μg protein) cell extract prepared as described above). The mixture, in a plastic scintillation vial, was carefully overlaid with 1.5 ml nonaqueous scintillation fluid (Econofluor, DuPont, KS). Radioactivity, resulting from the ^{14}C-acetylated chloramphenicol, was counted immediately, and after 1, 3, 12, and 24 h. CAT activity increased linearly over this time period and was determined as the increase in radioactivity over 24 h, over background. The protein content of the cell extracts was determined using the Lowry method [Lowry et al., 1951], and CAT activity was normalized per mg protein. Controls were run simultaneously and consisted of a) cell extracts of non-transfected cells, b) reaction mixture without cell extract, and c) purified CAT enzyme. 1 unit CAT activity was defined as the amount of enzyme that catalyzed the transfer of 1 umole of ^{14}C-acetyl group to chloramphenicol.

Kinetics of CAT Gene Expression

To determine the optimum post-transfection time required for maximum expression of the CAT gene in host tumor cells, triplicate sets of cultures of the tumor cells were set up at 10^5 cells per T25 flasks, and transfected with 10 μg of purified non-drug-treated CAT vector. Every 24 h, over 4 days, the cultures were harvested, cell extracts prepared, and CAT enzyme activity determined, as described above. CAT activity (units/mg protein) was then plotted against time.

DNA Polymerases α and β Activities

The activities of DNA polymerases α and β in the tumors were determined essentially as previously described [Yamaguchi et al., 1980; Ono et al., 1979], with some modifications in the salt concentration, as determined in optimization studies in our laboratory [Ali-Osman and Rairkar, 1992]. Tumor cells were homogenized by sonication in 200–500 μl of ice-cold Tris buffer (pH 7.6) containing 100 μM EDTA, 200 μM dithiothreitol, 500 μM KCl, 10% glycerol, and 0.5% Triton X-100. After centrifugation at 15,000*g* for 30 min at 4°C, the crude cell extract was centrifuged at 30,000*g* for 90 min and the supernatant used for the DNA pol assays. DNA polymerase α was assayed in a 25 μl reaction mixture consisting of 50 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 8 mM $MgCl_2$, 80 μg/ml activated DNA, 100 μM dCTP, dGTP, dATP, and ^{3}H-labeled dTTP (approx. 60 cpm/pmol), 15% glycerol, 400 μg/ml BSA, 20–40 mM KCl,

and 20 μl of cell extract. After incubation for 60 min at 37°C, aliquots of the reaction mixture containing radioactive DNA was collected on a DEAE cellulose filter and counted by liquid scintillation. Protein concentration of the cell extracts was determined with the Lowry et al. method [1951]. DNA polymerase β was similarly quantified except that the reaction mixture consisted of 50 mM Tris-HCl (pH 8.8), 1 mM dithiothreitol, 500 μM $MnCl_2$, 40 μg/ml Poly (dA), 40 μg/ml poly(dT)12-18, 100 μM 3H-dTTP, 400 μg/ml BSA, 100 mM KCl, 15% glycerol, and 5–10 μl cell extract. One unit of DNA polymerase α or β was defined as the amount of enzyme that catalyzed the incorporation of 1 pmole of ^{3}H-dTTP into newly synthesized DNA in 1 h. DNA polymerase activity was determined over a range of protein concentrations by adjusting the concentration of the original cell extract such that the 20 μl test volume contained 25, 50, and 100 μg protein, respectively.

DNA Ligase Assay

DNA ligase activity in tumor cell extracts was determined, as we previously described [Rairkar and Ali-Osman, 1992], by quantitating the extent to which the ligation of 15-mer and 17-mer adjacent M13 oligodeoxynucleotides, annealed to purified single stranded M13MP18 DNA (+ strand) template. The reaction mixture consisted of 50 mM Tris-HCl (pH 7.5), containing 10 mM $MgCl_2$, 2 mM ATP, 2 mM DDT and 15- and 17-mer (5′ end-labeled with ^{32}P-ATP and T4 polynucleotide kinase) M13 oligonucleotides. After 3 min at 25°C, the reaction mix was heat-denatured at 100°C to release the ^{32}P-labeled 32-mer ligation product from the M13 template. Following urea-polyacrylamide gel electrophoresis and autoradiography, the 32-mer band was excised, solubilized, and the radioactivity counted by beta-scintillation. The amount of 32-mer product was computed based on the amount of radioactivity in the band. One unit of DNA ligase was defined as the amount of enzyme required to produce 1 pmole of 32-oligomer at 25°C in h. The activity of DNA ligase was determined over a protein concentration range of 0–100 μg protein per reaction, as described for the polymerase assays.

RESULTS

In Vitro cis-DDP Sensitivity

The cis-DDP dose-response curves of tumor cells obtained before therapy (JO 1) and after therapy (JO 2) are shown in Figure 1. Over the entire concentration range of 0–5 μM cis-DDP, JO 2 cells were more resistant than JO 1 cells. At 5 μM cis-DDP, the surviving fractions of cells of JO 1 and JO 2 were decreased to 0.47 and 0.08, respectively, representing a 5.9-fold increase in cis-DDP resistance at that cis-DDP concentration.

Quantitation of DNA-Pt Adducts in pCAT Vector

The results of these studies are shown in Figure 2. Following a 3-h exposure of the pCAT vector to cis-DDP, there was a linear relationship between the amount of Pt bound to vector DNA and the concentration of cis-DDP to which the vector was exposed. Over the 3 h, exposure to 25 μM and 50 μM cis-DDP resulted in 3.9 and 6.5 pmol, respectively, of total platinum bound per μg of vector DNA. However, since the transfection efficiency under our experimental conditions was approximately 22–28%, the actual maximum levels of Pt-DNA adducts introduced into the cells were in range of 0.86–1 and 1.4–1.8 pmol Pt, respectively, per μg of pCAT vector treated with 25 mM and 50 mM cis-DDP.

Kinetics of CAT Gene Reactivation in Host Tumor Cells

The results of the CAT gene reactivation studies are summarized in Figure 3a,b. In both JO 1

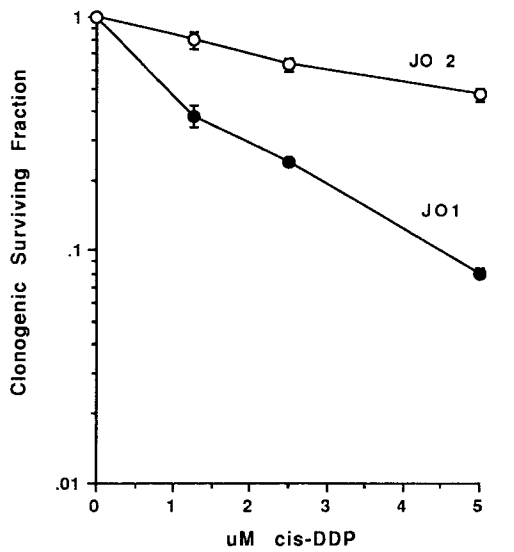

Fig. 1. Cis-DDP dose-response curve for malignant oligodendroglioma cells obtained from a patient before therapy (JO 1; open circles) and after failure to cis-DDP therapy (JO 2; closed circles). Cell survival was determined with the capillary tumor clonogenic cell assay.

and JO 2 cells transfected with undamaged pCAT, CAT enzyme activity increased linearly over time up to a maximum at approximately 48 h after transfection. Between 48 and 96 h, there was no significant increase in the CAT activity of control nondrug-treated cells. Overall, CAT gene expression was slightly higher in cells of JO 2 than in those of JO 1.

In cells transfected with cis-DDP-damaged pCAT vector, there was a significant difference between JO 1 and JO 2 cells in the extent of CAT gene reactivation over time. After transfection with vector that had been damaged with 25 mM cis-DDP, JO 1 cells showed a slow, time-dependent recovery in CAT gene expression and after 96 h had attained only 37% of control CAT activity. In contrast, the rate of CAT gene reactivation in JO 2 cells was relatively more rapid, and 96 h after transfection the cells had recovered approximately 93% of control CAT gene activity. Over the same period, no significant reactivation of the CAT gene in vector damaged with 50 μM cis-DDP was observed for cells of both tumors.

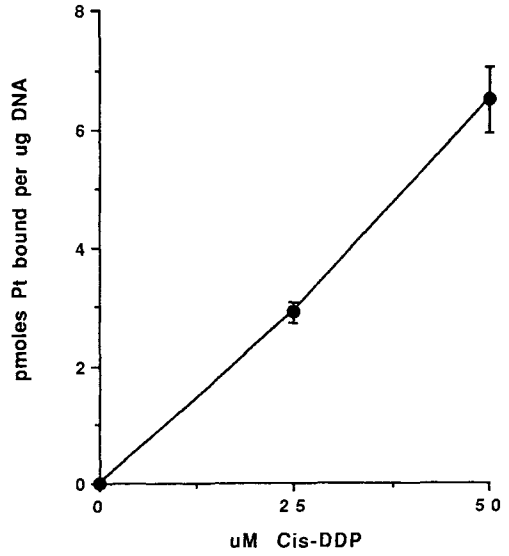

Fig. 2. Formation of Pt-DNA adducts in the pCAT eukaryotic expression vector after a 3-h exposure to 25 and 50 μM cis-DDP, as described under Materials and Methods.

Tumor DNA Polymerases α and β, and DNA Ligase Activities Before and After cis-DDP Therapy

As shown in Figure 3, the relationship between the activities of DNA polymerases α and β, and the protein concentration of the test cell extract was linear up to 50 μg protein. At 100 μg protein, linearity was less evident, particularly for DNA polymerase α. Consequently, all subsequent assays of both enzymes were performed at 50 μg protein. Table I shows the alterations in the activities of DNA polymerases α and β and of total DNA ligase in the tumor cells following cis-DDP therapy. DNA polymerase β activity in JO 2 tumor cells was 3.64 units/mg protein, compared with 0.81 units/mg protein in JO 1 cells. This represented a 4.49-fold increase in tumor DNA polymerase β activity following

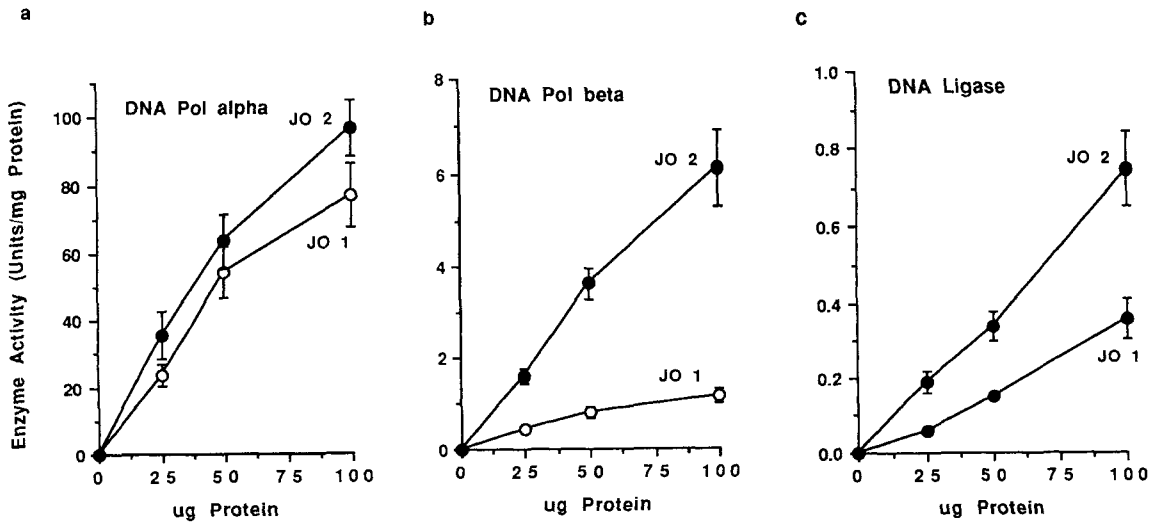

Fig. 3. Relationship between amount of tumor cell extract protein in assay and activities of DNA polymerases α (a) and β (b), and DNA ligase (c). Protein concentration of cell extracts were adjusted such that test volumes of 25 μl (DNA polymerase assays) and 10 μl (DNA ligase assays) contained the required protein amount (25, 50, and 100 μg).

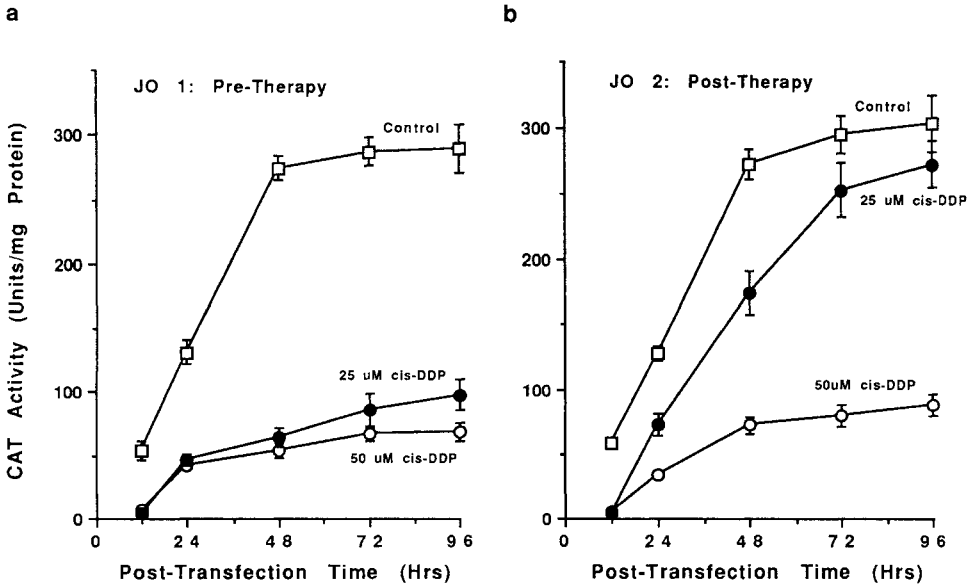

Fig. 4. Kinetics of reactivation of the CAT gene previously damaged with 25 μM (closed circles) and 50 μM (open circles) cis-DDP and in control undamaged vector (open squares) transfected into malignant oligodendroglioma cells obtained (a) before and (b) after cis-DDP therapy.

TABLE I. Alterations in DNA Repair-Associated Proteins in Human Malignant Oligodendroglioma Cells Obtained From a Patient Before and After Relapse to Cisplatin Therapy

	DNA pol α (pmol ^{3}H-dTTP incorporated/mg protein/h)	DNA pol β (pmol ^{3}H-dTTP incorporated/mg protein/h)	DNA ligase (fmol 32-oligomer ligated per mg protein/h)
JO 1	54.7 ± 7.7	0.81 ± 0.06	0.15 ± 0.7
JO 2	63.6 ± 4.3	3.64 ± 0.3	0.34 ± 1.5
JO 1:JO 2	1.16	9.4	2.3

therapy. The activities of DNA polymerase α were 54.7 units/mg protein and 63.6 units/mg protein in JO 1 and JO 2 cells, respectively. In contrast to the two DNA polymerases, total DNA ligase activities for both tumors increased linearly with increasing protein concentration up to 100 μg, the maximum amount of protein tested. There was a 2.3-fold increase in ligase activity, from 0.15 units/mg protein to 0.34 units/mg protein, in JO 1 cells relative to JO 2 cells. The changes in DNA polymerase β and DNA ligase activities were both statistically significant at a P value of 0.05.

DISCUSSION

Although, studies with tumor cell lines, primary tumor tissues, and peripheral blood lymphocytes have identified increased repair of drug-induced DNA damage as a major mechanism underlying cis-DDP resistance in human tumors [Reed et al., 1986, 1987, 1989; Poirier et al., 1987; Behrens et al., 1987; Lai et al., 1988; Fichtinger-Schepman et al., 1988, 1990; Sheibani et al., 1989; Lai et al., 1989; Ali-Osman et al., 1989, Ali-Osman et al., 1990a,b], relatively little is known about the relevance of this resistance mechanism in drug resistance that is acquired in vivo, i.e., subsequent to cis-DDP therapy. In an effort to understand the role of DNA repair in clinically acquired cis-DDP resistance in human malignant gliomas, we investigated the DNA repair status and its relationship with the in vivo acquired cis-DDP resistance in paired biopsies of a malignant oligodendroglioma obtained prior to and after the patient had failed cis-DDP therapy. The capacity of the tumor cells to reactivate a cis-DDP damaged eukaryotic expression vector carrying a reporter CAT gene was used as a measure of cellular DNA repair capability. The data showed that cells of the tumor that had failed cis-DDP therapy had acquired a 3-fold increased ability to repair cis-DDP damaged DNA. Simultaneously, a 4.5-fold and a 2-fold increase in DNA polymerase β and DNA ligase

activities, respectively, were measured in the post-therapy tumor cells. These changes were associated with an almost 6-fold increase in the in vitro cis-DDP resistance of the tumor cells following failed cis-DDP therapy. In contrast to DNA polymerase β and DNA ligase, we found no significant alteration in the activity of DNA polymerase α, the replicative DNA polymerase, in the tumor following cis-DDP therapy. The observed increase in DNA polymerase β was particularly interesting, in light of the now well-established role of DNA polymerase β in cellular DNA repair synthesis [Perrino and Loeb, 1990]. Elevation in DNA polymerase β had been previously reported in the P388 murine leukemia cell line that had been made resistant to cis-DDP [Kraker and Moore, 1988]. Additionally, other studies have shown that in chinese hamster ovary cells, the levels of DNA polymerase β RNA transcripts were significantly increased following exposure to three different types of DNA damaging agents, namely, N-methyl-N′-nitro-N-nitrosoguanidine, methyl methane sulfonate, and N-acetoxy-2-acetylaminofluorene [Fornace et al., 1989]. These in vitro data support a critical role for DNA polymerase β in the ability of tumor cells to process drug-damaged DNA, and ultimately in the resistance of the cells to such agents. Our present data suggest that increased DNA repair is a relevant mechanism in the acquisition of cis-DDP resistance in human gliomas in vivo, and the association of increased DNA polymerase β and DNA ligase with the increased DNA repair, imply that these proteins and their encoding genes could be potential targets for the development of novel strategies and agents directed at therapy-induced cis-DDP resistance in human gliomas.

The assay of cellular DNA repair capacity, using the CAT reporter gene, as described here, is uniquely suited to cells of human origin, since the CAT gene is absent in human and other eukaryotic cells. In bacteria, the CAT gene product catalyzes the transfer of the acetyl group from acetylcoenzyme A to chloramphenicol and thus confers resistance to chloramphenicol. Klocker et al. [1985] were among the first to exploit such a transient expression of a reporter CAT gene to study the ability of host human cells to repair DNA lesions induced by DNA-damaging agents. These authors showed that the ability of fibroblasts to repair both ultraviolet and N-methyl, N′-nitro-N-nitrosoguanidine damaged pSV-2 CAT plasmid, after transient transfection with the vector, was significantly lower in cells from xeroderma pigmentosum patients than in cells from normal subjects. Since these initial studies, the assay of DNA repair by this method has been successfully extended [Sheibani et al., 1989; Ali-Osman et al., 1989; Lehman and Oomen, 1985; Dean et al., 1988; Reed and Eastman, 1988] to study the repair of DNA damage induced in tumor cells by a variety of agents, including, bifunctionally active anticancer agents. Our data in the present study indicate that the level of reactivation of the CAT gene depends, not only on the repair capacity of the tumor cells, but also on the number of DNA-Pt adducts introduced into the vector, i.e., the extent of DNA damage. Thus, at an adduct level of 0.8 to 1 pmol per μg DNA (achieved in vector damaged with 25 mM cis-DDP), JO 2 tumor cells, with the higher repair capacity, could reactivate the CAT gene to almost control levels, whereas, at 1.4 to 1.8 pmol Pt-DNA adducts (vector treated with 50 mM cis-DDP), the extent of reactivation was approximately 28% that of controls.

We realize that the repair of a transiently transfected reporter gene may not completely or accurately reflect the complex process by which cells repair DNA damage in the cellular genome. In particular, it is likely that cis-DDP induced DNA intra-strand crosslinks, which constitute the predominant amount of DNA-bound Pt [Pinto and Lippard, 1985], will inactivate the vector carrying the reporter gene to the same degree as do DNA interstrand crosslinks, although the latter crosslinks are much more lethal to the cell. Furthermore, the accessibility of repair enzymes to damaged vector DNA will be expected to be significantly higher than that for intra-genomic DNA lesions. Despite these potential limitations, our present data and those in other reports [Eastman and Schulte, 1988; Sheibani et al., 1989] indicate that the reporter gene repair assay is a valid indicator of cellular DNA repair and that repair of cis-DDP damaged DNA assayed with this method correlates well with cellular resistance to cis-DDP.

Presently, we can only speculate as to the mechanisms underlying the enhanced capacity for DNA repair and the increased activities of DNA repair-associated proteins that we observed in the tumor cells following cis-DDP therapy. It is possible that these changes resulted from increased mutations induced in the tumor cells by the therapy, and/or, alterna-

tively, were a part of the natural progression of the tumor; both possibilities are consistent with the increased spontaneous mutation rates that have been associated with progressive malignancy and/or with exposure of cells to DNA damaging agents [Cairnes, 1975; Nowell, 1983; Schimke, 1984; Farber, 1984; Goldie and Coltman, 1979, 1984; Berger and Ali-Osman, 1990; Evans and Gralla, 1992]. We believe that the results of studies, such as we present here, on paired tumor specimens obtained from the same patient before and after therapy are important in attempts to understand the molecular basis of the acquisition of tumor drug resistance in the clinical setting and warrant a more detailed study with larger numbers of patients.

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