An Investigation of the Sequence-Specific Interaction of *cis*-Diamminedichloroplatinum(II) and Four Analogues, Including Two Acridine-Tethered Complexes, with DNA inside Human Cells†

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Received April 2, 1992; Revised Manuscript Received August 28, 1992

ABSTRACT: The sequence specificity of DNA damage caused by *cis*-diamminedichloroplatinum(II) (cisplatin) and four analogues in human (HeLa) cells was studied using *Taq* DNA polymerase and a linear amplification system. The primer extension is inhibited by the drug–DNA adducts, and hence the sites of these lesions can be analyzed on DNA sequencing gels. The repetitive alphoid DNA was used as the target DNA in human cells. A comparison was made between adduct formation in human cells and in purified DNA. The sequence-specific position and relative intensity of damage was similar in both systems for cisplatin, dichloro(ethylenediammine)platinum(II) ($PtenCl_2$), and *N*-[3-*N*-(ethylenediamino)propyl]acridine-4-carboxamidedichloroplatinum(II) ($4AcC_3PtenCl_2$). However, no DNA damage could be detected in cells for *trans*-diamminedichloroplatinum(II) (transPt) or *N*-[3-*N*-(ethylenediamino)propyl]acridine-2-carboxamidedichloroplatinum(II) ($2AcC_3PtenCl_2$) despite the ability of these latter analogues to damage purified DNA. Cisplatin, $PtenCl_2$, and $4AcC_3PtenCl_2$, which significantly damaged DNA inside cells, also show antitumor activity in mouse models. However, transPt and $2AcC_3PtenCl_2$, which did not detectably damage DNA inside cells, did not show such antitumor activity. This correlation between intracellular DNA damaging ability and in vivo antitumor activity indicates the potential use of the human cells/*Taq* DNA polymerase/linear amplification technique as a convenient method for screening new cisplatin analogues for useful chemotherapeutic activity.

cis-Diamminedichloroplatinum(II) (cisplatin)[1] is successfully used as a cancer chemotherapeutic agent (Loehrer & Einhorn, 1984). Its biological effectiveness is thought to be due to covalent modification of cellular DNA. Cisplatin forms both intrastrand and interstrand DNA cross-links as well as protein–DNA cross-links. These stable covalent adducts would be expected to inhibit DNA replication and transcription and are probably responsible for its antitumor activity.

The nature of the platinum covalent adducts formed with purified DNA have been investigated after hydrolysis to nucleotides (Eastman, 1983; Fichtinger-Schepman et al., 1985; Hansson & Wood, 1989; Bruhn et al., 1990). These studies have shown that the adducts consist of purines cross-linked via their N^7 atoms. The cross-linked lesions occur (in decreasing order of incidence) as intrastrand cross-links at the dinucleotides GG, AG, and GNG; interstrand cross-links between G bases; and monoadducts.

We have recently investigated the in vitro sequence specificity of DNA adducts formed by cisplatin and four analogues—the same five compounds examined in this paper (Murray et al., 1992a). Utilizing plasmid DNA and the *Taq* DNA polymerase linear amplification system, it was found that runs of two or more consecutive Gs were the major site of adduct formation for cisplatin and the four analogues. The sequences, GA, AG, and GC, were also damaged, but to a lesser extent. Ponti et al. (1991) have used a similar technique to investigate the sequence specificity for cisplatin and carboplatin adducts in plasmid DNA.

In this paper we describe the DNA sequence specificity of cisplatin adducts in human cells. The DNA sequence specificity of several agents has been determined in cells: UV light (Lippke et al., 1981); nitrogen mustard (Grunberg & Haseltine, 1980); bleomycin (Murray & Martin, 1985); dimethyl sulfate (Saluz & Jost, 1989; Church & Gilbert, 1984), and ^{125}I-labeled Hoechst 33258 (Murray & Martin, 1988). The DNA target used in this study was α-RI DNA, which is a 340-bp tandemly repeated sequence in human DNA constituting approximately 1% of the genome (Jorgensen et al., 1986). Alphoid DNA is sufficiently homogeneous to allow unambiguous assignment of the DNA sequence by Maxam–Gilbert sequencing (Wu & Manuelidis, 1980). The high copy number of this DNA sequence allows the straightforward determination of the sequence specificity of DNA damaging agents.

† H.M. was supported by the Australian Research Council, and P.R.E. and G.W. were supported by the National Health and Medical Research Council.

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[1] Abbreviations: cisplatin, *cis*-diamminedichloroplatinum(II); transPt, *trans*-diamminedichloroplatinum(II); $PtenCl_2$, dichloro(ethylenediammine)platinum(II); $2AcC_3PtenCl_2$, *N*-[3-*N*-(ethylenediamino)propyl]-acridine2-carboxamidedichloroplatinum(II); $4AcC_3PtenCl_2$, *N*-[3-*N*-(ethylenediamino)propyl]acridine-4-carboxamidedichloroplatinum-(II); bp, base pair; SDS, sodium dodecyl sulfate.

0006-2960/92/0431-11812$03.00/0

The linear amplification method employs the properties of DNA polymerase to investigate the sequence selectivity of the interaction of cisplatin with DNA. An oligonucleotide primer is hybridized to a defined sequence of DNA and is extended by *Taq* DNA polymerase up to the cisplatin lesion. Cisplatin adducts cause distortions in DNA which inhibit polymerization at the site of adduct formation. Thus an analysis on DNA sequencing gels allows the sequence selectivity of cisplatin to be determined by reference to dideoxy sequencing lanes. The use of *Taq* DNA polymerase permitted the use of thermal cycling to linearly amplify the products and, hence, increase the sensitivity of the system. The linear amplification procedure was originally developed for genomic sequencing (Saluz & Jost, 1989), but we have adapted it for the cisplatin experiments. Using this method, the DNA sequence selectivity of cisplatin can be determined in human cells. Other methods, for example, exonuclease III (Royer-Pokra et al., 1981), Klenow fragment of DNA polymerase (Pinto & Lippard, 1985; Gralla et al., 1987), and chemical modification (Schwartz et al., 1989), do not have the simplicity, sensitivity, or sufficiently low background to allow the DNA sequence specificity of cisplatin lesions to be easily determined in human cells. Thus the linear amplification procedure permits, for the first time, the determination of cisplatin damage in human cells.

Cisplatin and four analogues were examined in this study (for structures see Figure 1). The four analogues were *trans*-diamminedichloroplatinum(II) (transPt), dichloro(ethylenediammine)platinum(II) ($PtenCl_2$), *N*-[3-*N*-(ethylenediamino)-propyl]acridine-2-carboxamidedichloroplatinum(II) ($2AcC_3PtenCl_2$), and *N*-[3-*N*-(ethylenediamino)propyl]acridine-4-carboxamidedichloroplatinum(II) ($4AcC_3PtenCl_2$). The latter two analogues possess an attached intercalating chromophore and were originally designed in an attempt to modify the cisplatin sequence specificity of adduct formation (Lee et al., 1992). Cisplatin, $PtenCl_2$, and $4AcC_3PtenCl_2$ showed antitumor activity in the mouse P388 leukemia model whereas transPt and $2AcC_3PtenCl_2$ did not show such activity (Loehrer & Einhorn, 1984; Bruhn et al., 1990; Lee et al., 1992).

MATERIALS AND METHODS

Materials. Previously described procedures were used to synthesize cisplatin and transPt (Kaufman & Cowan, 1963); $PtenCl_2$ (Johnston, 1966); $2AcC_3PtenCl_2$ and $4AcC_3PtenCl_2$ (Lee et al., 1992). All compounds were dissolved in dimethylformamide to give a 1 mM stock solution. The [γ-^{32}P]-ATP was purchased from Amersham, Ampli *Taq* DNA polymerase was obtained from Perkin-Elmer-Cetus.

Cell Culture. HeLa cells were grown using conventional techniques in RPMI 1640 medium augmented with 2 mM glutamine, but no antibiotics were present.

Cisplatin Treatment. Approximately 10^6 HeLa cells in a total volume of 1 mL of medium were placed in a noncoated microtitre plate (1.5 cm diameter wells) with the appropriate concentration of cisplatin (or analogue). After an 18-h incubation at 37 °C, the cells were washed twice with phosphate-buffered saline. The DNA was purified from cells as previously described (Murray & Martin, 1985). This involved lysis with SDS, protein removal by proteinase K and phenol, and ethanol precipitation, and finally the DNA was dissolved in 30 μL of 10 mM Tris-HCl, pH 8.8. Two microliters were used for the linear amplification reaction.

Purified HeLa DNA was treated with cisplatin or analogue as described previously (Murray et al., 1992a). In this treatment the DNA was incubated for 18 h at 37 °C, ethanol precipitated, and dissolved, and a portion was subjected to the linear amplification procedure.

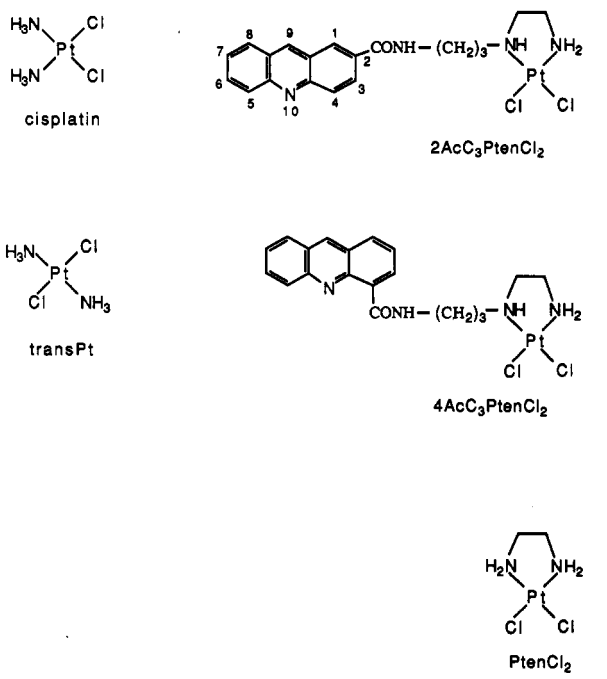

FIGURE 1: Structures of cisplatin and four analogues.

Linear Amplification Method. One picomole of primer oligonucleotide [the 18 base primer no. 5 5′GTGGAATTTG-CAAGTGGA3′ [bps 89–106 in αB3 (Murray & Martin, 1987)] or the 18 base primer no. 20 5′GAATTCTTCTCTCT-AGCA3′ (bps 345–328 in αB3) was 5′ labeled in a final volume of 10 μL with 60 mM Tris-HCl, pH 7.5, 9 mM $MgCl_2$, 10 mM dithiothreitol, 2 units of polynucleotide kinase, and 5 μCi of [γ-^{32}P]ATP at 37 °C for 30 min.

The linear amplification procedure was carried out as described previously (Murray et al., 1992a) except that no bovine serum albumin was present in the amplification mix. Unless otherwise stated the 18 base primer no. 5 was used in the linear amplifications. Two microliters of damaged DNA and controls were added to (final concentrations) 16.6 mM $NH_4(SO_4)_2$, 67 mM Tris-HCl, pH 8.8, 6.7 mM $MgCl_2$, 300 μM each of dATP, dGTP, dCTP, and dTTP, 0.05 pmol of 5′-^{32}P-labeled oligonucleotide, and 0.25 unit ampli*Taq* DNA polymerase (Perkin-Elmer-Cetus) in a final volume of 5 μL and overlaid with 50 μL of mineral oil. The DNA sequence standards were dideoxy double-stranded DNA sequencing lanes which were performed using the same primer oligonucleotide and either the plasmid αB3 or purified human DNA (Murray, 1989). Linear amplification by thermal cycling was performed at 95 °C for 30 s (time at the temperature), 50 °C for 60 s, 72 °C for 90 s for 25 cycles in a Perkin-Elmer-Cetus DNA Thermal Cycler 480. Two microliters of the reaction were loaded onto a 6% polyacrylamide–urea DNA sequencing gel. The gel was dried down on 3MM paper before autoradiography.

DNA concentrations were determined by UV absorbance at 260 nm. Pt concentrations were measured by flameless atomic absorption spectrophotometry.

Densitometry. A Tracktel Vision Systems densitometer was used to quantitate the intensity of the bands on the sequencing gel autoradiographs. Experiments carried out on two occasions were examined in detail by the densitometer. The peak heights were determined, and the blank (untreated control) was subtracted. Ten damage sites were analyzed by

the densitometer. The relative intensity of damage was calculated relative to an average of the intensity at bps 148, 150, 176, and 186 for oligonucleotide no. 5. The relative intensity of damage was then compared in purified DNA and cells. Experiments with cisplatin and the four analogues were examined by the densitometer.

RESULTS

Cisplatin Experiments. In these experiments, human cells were treated with cisplatin (or an analogue); the DNA was extracted from the cells; using *Taq* DNA polymerase and a linear amplification procedure, the sequence specificity of damage was determined on DNA sequencing gels.

The results of the treatment of HeLa cells with cisplatin can be seen in Figure 2. With primer no. 5, the sites of cisplatin adduct formation are shown in Figure 2 (lanes 7 and 8). The bands in these lanes correspond to positions where *Taq* DNA polymerase has been halted by cisplatin lesions. The intensity of bands in lanes 7 and 8 is significantly higher than in the control lanes 1 and 2, which are derived from untreated cells. The sequence specificity of the cellular cisplatin adducts was determined by comparison to the dideoxy sequencing lanes G, A, T, and C, which used the same primer oligonucleotide no. 5.

The DNA sequences, which suffered more intense damage by cisplatin in human cells, were as follows [the underlined base (numbered) indicates the peak of damage intensity, and the bases in capital letters are the presumed site of covalent modification; also the sequences are shown in decreasing order of intensity as determined by the densitometer]: 5′AG<u>G</u>A3′ (186); AGG<u>A</u> (150); A<u>G</u>A (176); A<u>G</u>A (148); G<u>A</u> (126); AG<u>t</u> (183); A<u>G</u>t (160); G<u>G</u>c (114); and AG<u>c</u> (117). Note that the sequences shown are from the consensus sequence and the *Taq* DNA polymerase approaches from the left of the sequences shown. Similar results were obtained with primer oligonucleotide no. 20, which reveals information about the opposite strand to primer no. 5.

The cisplatin concentrations used in the experiment depicted in Figure 2, 25 and 50 μM, were relatively similar in the degree of DNA damage produced. Other experiments have shown that cisplatin damage can be detected at 5 μM. Concentrations above 50 μM do not seem to produce appreciably greater levels of damage.

The quantity of Pt adducts attached to DNA was determined by atomic absorption spectrophotometry. Treatment of cells with 10 μM cisplatin produced 2.5 Pt bound per 10 000 bp; 25 μM, 8.8 Pt bound per 10 000 bp; and 50 μM, 29 Pt bound per 10 000 bp. These results indicate that cisplatin damage could be detected in our assay when 2.5 Pt are bound per 10 000 bp. (Note that these atomic absorption spectrophotometry results are from a different experiment than that shown in Figures 2 and 3.)

Figure 3 shows a comparison between cisplatin adduct formation in HeLa cells and in purified HeLa DNA. A 10-fold greater concentration of cisplatin was needed in cells (50 μM) to achieve a similar level of damage as in purified DNA (5 μM). However, the sequences damaged in the two environments were very similar to each another. In addition, the intensity at each damage site as determined by densitometry was also similar in the two environments. The relative intensity of damage at each site was calculated, and a purified DNA/cells ratio was determined. The ratios ranged from 0.62 to 1.35 with the standard deviation being ±0.25. The damage sites with the greatest variation from a ratio of 1 occurred at sites with a low intensity of damage and hence were able to be measured less accurately. The ratios for the five most intense damage sites ranged from 0.91 to 1.13. Thus, this low variation between the two environments is probably within experimental error.

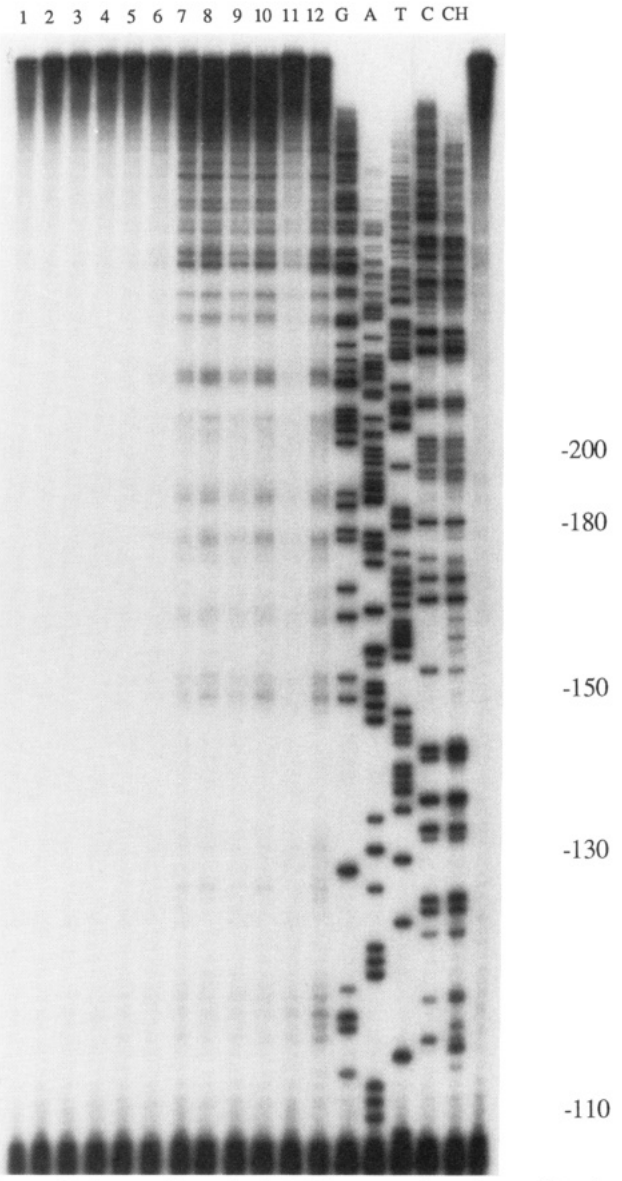

FIGURE 2: Autoradiograph of a DNA sequencing gel containing the linear amplification products of DNA extracted from HeLa cells treated with cisplatin and four analogues, using oligonucleotide no. 5 as primer. Lanes 1 and 2 are untreated cellular controls. The HeLa cells were treated with transPt in lane 3 (25 μM) and lane 4 (50 μM), with $2AcC_3PtenCl_2$ in lane 5 (25 μM) and lane 6 (50 μM), with cisplatin in lane 7 (25 μM) and lane 8 (50 μM), with $PtenCl_2$ in lane 9 (25 μM) and lane 10 (50 μM), with $4AcC_3PtenCl_2$ in lane 11 (25 μM) and lane 12 (50 μM). The dideoxy sequencing was performed on pBR325 αB3 (lanes G, A, T, and C) and HeLa DNA (lane CH). Note that the dideoxy sequencing lanes labeled as G, A, T, C, and CH give the sequence on the template strand.

Experiments with transPt, $PtenCl_2$, and Two Acridine-Tethered Complexes. Figure 2 reveals the effects of four cisplatin analogues on DNA inside human cells. It can be seen that cisplatin (lanes 7 and 8), $PtenCl_2$ (lanes 9 and 10), and $4AcC_3PtenCl_2$ (lanes 11 and 12) produce significant levels of DNA damage above background. However, transPt (lanes 3 and 4) and $2AcC_3PtenCl_2$ (lanes 5 and 6) do not give rise to any significant levels of DNA damage and are indistinguishable from the controls (lanes 1 and 2) by densitometer analysis. Experiments treating HeLa cells with transPt and

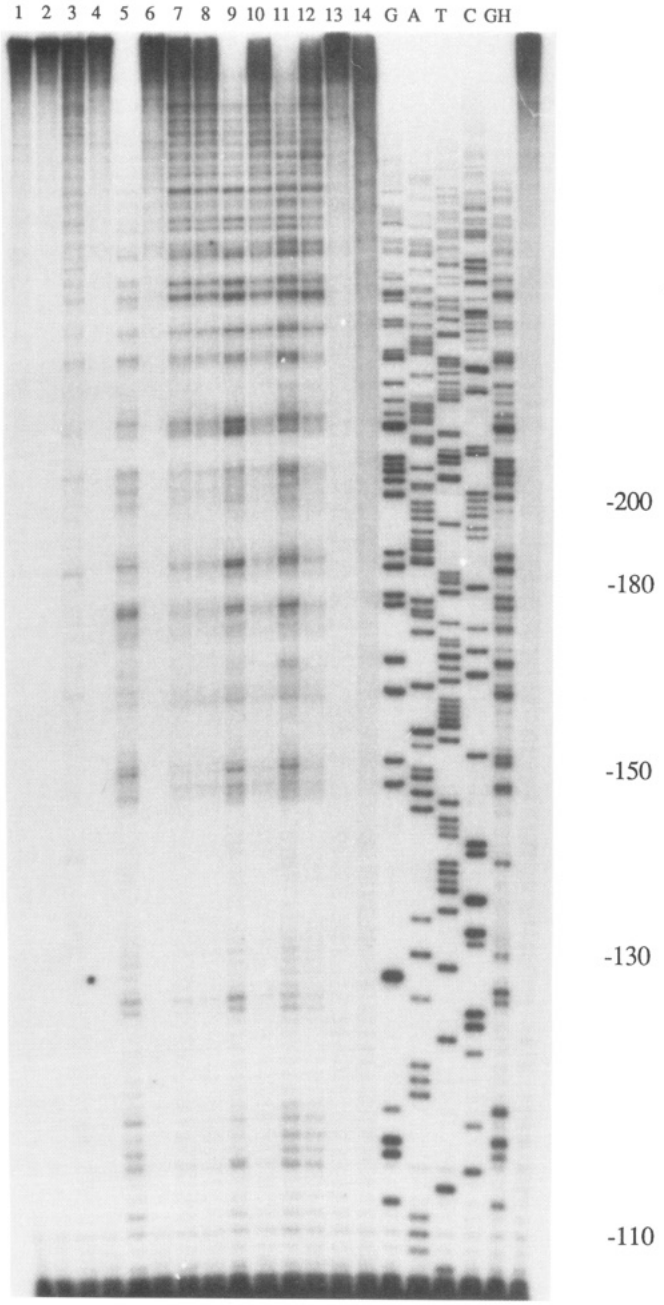

FIGURE 3: Autoradiograph of a DNA sequencing gel comparing the effect of cisplatin and four analogues in HeLa cells with that in purified HeLa DNA. The oligonucleotide no. 5 was used as primer in the linear amplification reactions. Lanes 1, 2, 4, 6, 8, 10, and 12 contain DNA derived from treated HeLa cells; and lanes 3, 5, 7, 9, 11, 13, and 14 are from treated purified DNA. Lanes 1, 2, 13, and 14 are untreated cellular controls. The purified DNA or cells were treated with transPt in lane 3 (50 μM) and lane 4 (50 μM), with $2AcC_3PtenCl_2$ in lane 5 (5 μM) and lane 6 (50 μM), with cisplatin in lane 7 (5 μM) and lane 8 (50 μM), with $PtenCl_2$ in lane 9 (5 μM) and lane 10 (50 μM), with $4AcC_3PtenCl_2$ in lane 11 (5 μM) and lane 12 (50 μM). The dideoxy sequencing was performed on pBR325 αB3 (lanes G, A, T, and C) and HeLa DNA (lane GH). Note that the dideoxy sequencing lanes labeled as G, A, T, C, and GH give the sequence on the template strand.

$2AcC_3PtenCl_2$ have been carried out on six occasions, and no significant DNA damage has ever been detected with these compounds.

In contrast transPt (Figure 3, lane 3) and $2AcC_3PtenCl_2$ (lane 5) can damage DNA in vitro (Murray et al., 1992a). Figure 3 reveals that $2AcC_3PtenCl_2$ (lane 5) damaged purified human DNA to the same extent as $4AcC_3PtenCl_2$ (lane 11). However, a higher concentration of transPt (50 μM) (lane 3) was needed to damage purified DNA to the same extent as cisplatin (5 μM) (lane 7).

With $4AcC_3PtenCl_2$ (Figure 2) there was a significant increase in the cellular cisplatin DNA damage from 25 (lane 11) to 50 μM (lane 12) which was not observed for cisplatin or $PtenCl_2$. The sequence-specific pattern of DNA adduct formation caused by $4AcC_3PtenCl_2$ in human cells was different than that caused by cisplatin and $PtenCl_2$ (these latter two compounds gave similar results). This was also found with purified DNA (Figure 3) and with the plasmid pUC8 (Murray et al., 1992a).

Cisplatin, $PtenCl_2$, and $4AcC_3PtenCl_2$ all showed a very similar sequence specificity of adduct formation in cells and in purified DNA. Also densitometer measurements indicate (as for cisplatin) that the relative intensity of damage at each site is very similar. The purified DNA/cells ratio was determined for $PtenCl_2$ and ranged from 0.83 to 1.31 with the standard deviation being $\pm$0.14; and for $4AcC_3PtenCl_2$ the range was 0.66–1.42 with the standard deviation being $\pm$0.22. As with cisplatin, the differences between the two environments are probably due to random experimental variation.

DISCUSSION

Cisplatin Damage in Human Cells. In this study we have compared the DNA sequence specificity of cisplatin adduct formation in human cells with that in purified DNA. This study represents the first occasion to our knowledge that the sequence specificity of cisplatin has been determined in human cells. This was accomplished utilizing *Taq* DNA polymerase and the linear amplification procedure.

We have found that the sequence selectivity of cisplatin is very similar in cells and purified DNA for both position and intensity of damage. (The main difference is that higher concentrations of cisplatin are needed to achieve the same level of damage as purified DNA.) This result has also been found for all agents that have used alphoid DNA as target DNA (Lippke et al., 1981; Grunberg & Haseltine, 1980; Murray & Martin, 1985, 1988). It might be expected that the complex environment of DNA in chromatin, where it is packaged into nucleosomes and other higher order structures, would greatly affect the sequence-specific interaction of a damaging agent with DNA. However, for bleomycin, Murray and Martin (1985) have explained this effect as being due to randomly phased nucleosomes on the tandemly repeated target sequence, alphoid DNA. Bleomycin cleaves in the linker region of nucleosomes. But since bleomycin cleavage is very similar in cells and in purified DNA, then alphoid DNA must consist of a series of overlapping linker regions with respect to the alphoid DNA sequence. This results in a cleavage pattern for cells that is very similar to that of purified DNA. The same explanation can also be invoked to explain the present results with cisplatin, since it seems likely that the N^7 of guanine would be protected to some extent by the nucleosome core from attack by these compounds. There is indirect evidence that cisplatin preferentially damages the linker region of nucleosomes (Hayes & Scovell, 1991).

Extension from oligonucleotide/primer no. 5 gives rise to a particular DNA sequence. We have recently defined the DNA sequences extended by oligonucleotides no. 5, no. 20, and two other oligonucleotides in human DNA (Murray et al., 1992b). These oligonucleotide give rise to a sequence that is very close to that of 340-bp consensus α-RI DNA but dissimilar to 170-bp consensus alphoid DNA sequence. The 340-bp α RI DNA repeat consists of approximately 100 000 copies per cell and is a subset of the 170-bp repeat. The α-RI

DNA repeat is not perfectly homogeneous but contains random base substitutions which are 7% on average for the 340-bp repeat (Jorgensen et al., 1986; Murray & Martin, 1987). Because of this microheterogeneity, the sequence specificity is difficult to define absolutely. The cellular DNA sequence selectivity of cisplatin appears to be very similar to that previously found for plasmid sequences (Murray et al., 1992a); i.e., the major sites of damage are predominantly runs of two (or more) Gs and at GA and AG.

Despite the problem of absolutely defining the DNA sequence of alphoid DNA, it can be assumed that, since the sequence specificity of cisplatin is the same in purified DNA as in cells, the sequence selectivity of cisplatin in cells is the same as with purified plasmid DNA (Murray et al., 1992a).

Since the pattern of DNA damage for cisplatin was the same in cells and in purified DNA, this gives confidence that no preferential repair of a Pt cross-link is occurring, for example, GA intrastrand cross-links could be preferentially repaired.

In the quantitation of cisplatin adducts, it is assumed that *Taq* DNA polymerase is affected to a similar extent by each adduct. This assumption might be invalid in certain sequence contexts and in circumstances where the DNA has a high level of bound Pt. Also certain cisplatin lesions might be very inefficient at halting *Taq* DNA polymerase, e.g., monofunctional adducts (Murray et al., 1992).

Using atomic absorption spectrophotometry, it was possible in our experiments to determine that a 50 μM cisplatin treatment of cells produced 29 Pt bound per 10 000 bp. Cisplatin adducts could be detected in our *Taq* DNA polymerase/alphoid DNA/cells assay when the cells were treated with 10 μM cisplatin—in this latter case there are 2.5 Pt bound per 10 000 bp. Recently, a PCR procedure has been described where the presence of a single cisplatin lesion inhibits the PCR process (Jennerwein & Eastman, 1991). This sensitive assay has been used to detect the presence of cisplatin lesions in mouse cells. Utilizing the PCR method, Jennerwein and Eastman (1991) could detect one Pt per 10 000 bases. This PCR method is more sensitive than our method by a factor of 2.5. However, this latter method can only provide information about the presence or absence of a cisplatin adduct in a sequence of DNA, while the method described in this paper allows the position and intensity of each cisplatin adduct in a DNA sequence to be determined.

transPt DNA Damage inside Cells. transPt (see Figure 1) is a stereoisomer of cisplatin which is inactive as an antitumor agent. No damage could be detected with transPt in human cells. There are several plausible explanations for this occurrence, among them: (i) the formation of adducts which are not efficient at inhibiting DNA polymerase; (ii) more efficient repair of lesions; (iii) preferential reaction with cellular components or deactivation rather than reaction with DNA; and (iv) inefficient entry of the analogue into cells. Although it is difficult to distinguish between these possibilities, both i and ii are likely to be significant since it has been observed (Murray et al., 1992a) that transPt–DNA adducts are not very efficient at halting DNA polymerase. Likewise, there is evidence that transPt lesions are more efficiently repaired than those of cisplatin (Bruhn et al., 1990; Heiger-Bernays et al., 1991).

Cellular DNA-Adduct Formation by Acridine-Tethered Analogues. $PtenCl_2$ is the parent compound of $2AcC_3PtenCl_2$ and $4AcC_3PtenCl_2$. The analogues $2AcC_3PtenCl_2$ and $4AcC_3PtenCl_2$ are similar in structure, since they contain the same chemical moieties and only differ in the position of attachment of the linker arm to the acridine chromophore (Figure 1).

The analogues $2AcC_3PtenCl_2$ and $4AcC_3PtenCl_2$ were designed to specifically target the reactive platinum to DNA by attachment to the DNA-intercalating acridine moiety (Lee et al., 1992). These compounds have the potential to modify the nature of the interaction of the platinum moiety with DNA—an important property given that the antitumor activity of cisplatin is considered to be due to its ability to cross-link DNA. Thus, a more efficient overall reaction with nucleophilic sites on DNA might lead to higher potency, while specific alterations in the pattern of drug/DNA lesions might alter the biological profile of the compounds. An additional consequence of preferentially targeting the reactive platinum to DNA is the reduction in reaction with other cellular nucleophiles such as thiols. Moreover, the platinum–DNA adducts formed may be sufficiently novel to escape the normal repair process. Interaction with cellular thiols and enhanced repair of platinum–DNA adducts, are among the mechanisms causing cellular resistance to simple platinum complexes (Bruhn et al., 1990). The DNA-targeting strategy has been shown to be effective with aniline mustard DNA alkylators, where DNA-targeted compounds are both more potent cytotoxins (Gourdie et al., 1990) and possess quite different patterns of DNA alkylation (Prakash et al., 1990) than do the parent compounds.

The compounds $PtenCl_2$ and $4AcC_3PtenCl_2$ gave rise to significant damage in human cells. The cellular DNA sequence specificity for both position and relative intensity of damage was very similar for each of these compounds when compared with purified DNA.

However, no significant damage was detected with $2AcC_3PtenCl_2$ in human cells. As for transPt, there are four plausible explanations (see above). Explanation i is not likely because $2AcC_3PtenCl_2$ is just as efficient as $4AcC_3PtenCl_2$ at causing damage to purified DNA as measured by the *Taq* DNA polymerase/linear amplification procedure. The difference between $2AcC_3PtenCl_2$ and $4AcC_3PtenCl_2$ may be due to the three-dimensional structure of the compounds. In $2AcC_3PtenCl_2$, the presence of the side chain at the 2-position means that the acridine will lie perpendicular to the bases (i.e., it will "spear" the DNA helix) whereas for $4AcC_3PtenCl_2$ the acridine will lie parallel to the bases in the maximum overlap position.

The compounds transPt and $2AcC_3PtenCl_2$ did not have antitumor activity whereas $PtenCl_2$ and $4AcC_3PtenCl_2$ did have antitumor activity. A mouse model was used to evaluate the antitumor activity using transplanted tumors and a life-extension assay (Loehrer & Einhorn, 1984; Bruhn et al., 1990; Lee et al., 1991). Thus, a very interesting aspect of these results is the apparent correlation of the detection of damage in cells and antitumor activity (as measured by an increase in the lifespan of tumor-bearing mice and in other systems). This indicates that the alphoid DNA/human cells/*Taq* DNA polymerase system may have value as an assay to predict whether a new cisplatin analogue has antitumor activity.

ACKNOWLEDGMENT

We would like to thank Brian McDonald for use of the Tracktel Densitometer.

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