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Repair synthesis by human cell extracts in cisplatin-damaged DNA is preferentially determined by minor adducts

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ABSTRACT

During reaction of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) with DNA, a number of adducts are formed which may be discriminated by the excision-repair system. An *in vitro* excision-repair assay with human cell-free extracts has been used to assess the relative repair extent of monofunctional adducts, intrastrand and interstrand cross-links of *cis*-DDP on plasmid DNA. Preferential removal of *cis*-DDP 1,2-intrastrand diadducts occurred in the presence of cyanide ions. In conditions where cyanide treatment removed 85% of total platinum adducts while ~70% of interstrand cross-links remained in plasmid DNA, no significant variation in repair synthesis by human cell extracts was observed. Then, we constructed three types of plasmid DNA substrates containing mainly either monoadducts, 1,2-intrastrand cross-links or interstrand cross-links lesions. The three plasmid species were modified in order to obtain the same extent of total platinum DNA adducts per plasmid. No DNA repair synthesis was detected with monofunctional adducts during incubation with human whole cell extracts. However, a two-fold increase in repair synthesis was found when the proportion of interstrand cross-links in plasmid DNA was increased by 2–3 fold. These findings suggest that (i) *cis*-DDP 1,2-intrastrand diadducts are poorly repaired by human cell extracts *in vitro* , (ii) among other minor lesions potentially cyanide-resistant, *cis*-DDP interstrand cross-links represent a major lesion contributing to the repair synthesis signal in the *in vitro* assay. These results could account for the drug efficiency *in vivo*.

INTRODUCTION

Among various DNA repair processes, nucleotide excision repair plays a major role in cell survival by removing numerous types of DNA damage. The current view of this mechanism in eukaryotes largely stems upon the model established in *Escherichia coli* (1, 2). However, despite recent progress (3), understanding of this repair pathway still lacks a complete knowledge of the proteins involved and their precise functions. Moreover, full details on the types of DNA damage excised are needed since all the genotoxic agents induce a large pattern of various lesions which could have different individual excision potential by cell repair machinery. In the case of the antitumor agent *cis*-diamminedichloroplatinum(II) (*cis*-DDP), its effectiveness is believed to result from covalent binding to DNA (for review, (4)). The reaction of *cis*-DDP with DNA appears to be a two step process (5–7). This compound initially binds monofunctionally, primarily to guanine residues at the N7 position. It then reacts to produce difunctional lesions including rare DNA-protein cross-links (8). The predominant adducts formed by *cis*-DDP on DNA both *in vitro* and *in vivo* are 1,2-intrastrand cross-links d(GpG) (65% of total adducts) and d(ApG) (20–25%) while 1,3-intrastrand adducts and interstrand cross-links are minor products (~5%) (5, 9, 10). Although the adducts formed in the reaction of *cis*-DDP with DNA have been extensively studied, it is still uncertain which are responsible for cytotoxicity. In addition, the mechanism of cell lethality is also unknown, but a role of apoptosis has been recently demonstrated (11). Nevertheless, DNA repair has been emphasized as a mechanism of resistance to *cis*-DDP toxicity in various cell types (for reviews, (12–14)). Moreover, it is well established that nucleotide excision-repair greatly contributes to cell survival after *cis*-DDP treatment since it is very toxic to cell lines from individuals with DNA repair defect like xeroderma pigmentosum (XP) or Fanconi's anemia (FA) (15, 16). However, platinum adducts are not efficiently repaired within the cell ; for example, studies on murine L1210 cells have shown only a partial removal of *cis*-DDP or related drug adducts from the chromosome when either total platinum adducts (17) or d(GpG) intrastrand lesions (18) were examined. Some difference exists also between intra and interstrand cross-link repair since the former have been shown to be preferentially removed from transcribed regions as opposed to the latter (10). However, some *cis*-DDP resistant cells display increased repair of interstrand but not intrastrand cross-links (19).

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The relative repair of individual adducts formed on DNA by *cis*-DDP could then account for their respective cytotoxicity in normal as well as in resistant cells.

In order to investigate this question, we have used a cell-free system that can mediate DNA excision-repair by extracts from mammalian cells (20). These extracts can carry out repair synthesis in plasmid DNA damaged by UV light (20), psoralen (20–22), acetylaminofluorene (21, 23) and *cis*-DDP (24). This *in vitro* repair assay sufficiently resembles genomic repair in cells with regard to several features : defective repair occurred with extracts from repair deficient XP cells (20, 25, 26), DNA polδ/ε appears to be involved both *in vivo* and *in vitro* in the repair synthesis step ((27) and references therein) and the patch size measured *in vitro* is closed to *in vivo* estimates (28, 29). Differences in excision repair *in vitro* between the two major UV photoproducts (30, 31) or psoralen mono or diadducts (22) have already been established. In the present study, we have assessed the relative excision repair extent of platinum monoadducts and *cis*-DDP 1,2-intrastrand and interstrand cross-links by human cell extracts.

MATERIALS AND METHODS

Cell lines and extracts

Epstein-Barr virus-immortalized human lymphoblastoid cell lines used were : GM1953 (healthy donor) and GM2345 (XP group A), both from the National Institute of General Medical Sciences Human Genetic Mutant Cell repository (Coriell Institute, Camden, NJ), and AHH1 (healthy donor) from Dr W.Thilly via Dr E. Moustacchi (Institut Curie, Paris, France). The HeLa S3 cell line was obtained from the stock of European Molecular Biology Laboratories (Heidelberg, Germany). All the cells were cultured in suspension in RPMI 1640 medium (Gibco BRL) supplemented with glutamine (2 mM), 10% fetal calf serum (Gibco BRL), penicillin (2×10^5 U/l) and streptomycin (50 mg/l). Cells were regularly tested and found to be free of contamination by Mycoplasma (Mycoplasma detector kit, Boehringer).

Whole cell extracts were performed according to the method of Manley (32) with minor modifications as described (20). After preparation, extracts were immediately frozen and stored at −80°C.

Chemicals

cis-DDP was a gift from R. Bellon Cie. [Pt(dien)Cl]Cl, a monofunctionally DNA-binding platinum derivative compound, was a gift from Dr. N.P.Johnson (CNRS, Toulouse, France). Stock solutions of platinum(II) compounds were prepared at 1 mg/ml in water and stored at −80°C.

Preparation of plasmids and treatments with platinum(II) compounds

pBS (pBluescript KS+ from Stratagene) is a 2959 bp plasmid carrying ampicillin resistance and pHM is a 3738 bp derivative plasmid (gift from Dr R.D. Wood, ICRF, UK). Plasmids were prepared by the alkaline lysis method from *E.coli* JM109 (relevant genotype: *recA1*, *endA1*, *gyrA96*, *hsdR17*). Both plasmids were carefully purified by one cesium chloride and two neutral sucrose gradient centrifugations as described (33) pBS plasmid was treated with platinum(II) compounds according to Hansson and Wood (24). Briefly, plasmid DNA (0.1 mg/ml) was incubated with different concentrations of [Pt(dien)Cl]Cl or *cis*-DDP in TE-buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) for 12–14 h at 37°C in the dark (drug/nucleotide ratio between 0.001 and 0.01 depending on the desired overall platination level). The reaction was stopped by NaCl (added to 0.5 M). Plasmid DNA was ethanol precipitated, washed in 70% ethanol, dried and redissolved in TE-buffer. Total platinum content was determined by atomic absorption spectroscopy. pBS plasmid was incubated in parallel without drug and used further as control in repair reactions.

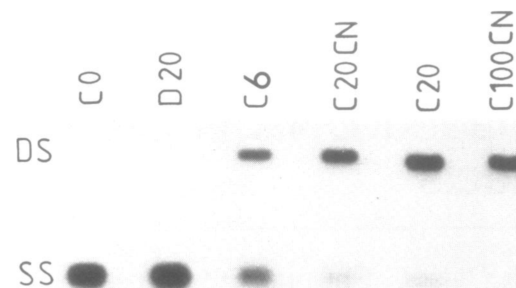

Figure 1. Platinated plasmids analysis for interstrand cross-links. Unplatinated C0 and platinated pBS plasmids (see Table 1) were linearized, end-labelled, heat-denatured and analyzed by electrophoresis on agarose gel. Migration was from the top. The picture represents the autoradiograph of the dried gel. Bands correspond to double-stranded cross-linked DNA (DS) and single-stranded non-cross-linked DNA (SS). The% of SS DNA for each plasmid was determined by scintillation counting of excised bands.

NaCN treatment and measurement of DNA interstrand cross-links

Preferential removal of platinum intrastrand cross-links was performed by incubating platinated DNA in the presence of cyanide ions as described (34, 35) with the following modifications : platinated or untreated pBS DNA (20 μg/ml) was incubated for various times in 0.2 M NaCN, 20 mM Tris-HCl, (pH 8.3) at 37°C. Plasmid DNA was then ethanol precipitated, washed in 70% ethanol, dried and redissolved in TE-buffer. For measurement of interstrand cross-links, ~150 ng HindIII linearized DNA samples were end-labelled for 15 min at 30°C in a 20 μl reaction mixture containing 200 μM each of dTTP, dGTP, dCTP, 1 μCi [α-^{32}P]dATP (800 Ci/mmol, Amersham) and 2 units of *E.coli* DNA PolI large fragment (Gibco BRL). After ethanol precipitation, washing and drying, DNA samples were treated according to Hartley et al.(36). DNA was redissolved in 10 μl denaturation buffer (30% dimethyl sulfoxide, 1 mM EDTA, 0.04% bromophenol blue), heated at 90°C for 3 min, chilled on ice, loaded on a 0,8% agarose gel and run for 12 h in 40 mM Tris-HCl, 20 mM acetic acid, 2 mM EDTA, pH 8. After drying, gel was autoradiographed (Fig 1). The bands corresponding to single and double-stranded DNA were excised and radioactivity was quantified by scintillation counting. The average number of interstrand cross-links per plasmid was calculated according to the Poisson distribution from the fraction of non-cross-linked single-stranded DNA.

In vitro repair reactions

Standard 50 μl reaction mixtures contained 300 ng each of (platinum ± NaCN) treated or mocked treated pBS and untreated pHM plasmids, 2 μCi of [α-^{32}P]dATP (800 Ci/mmol,

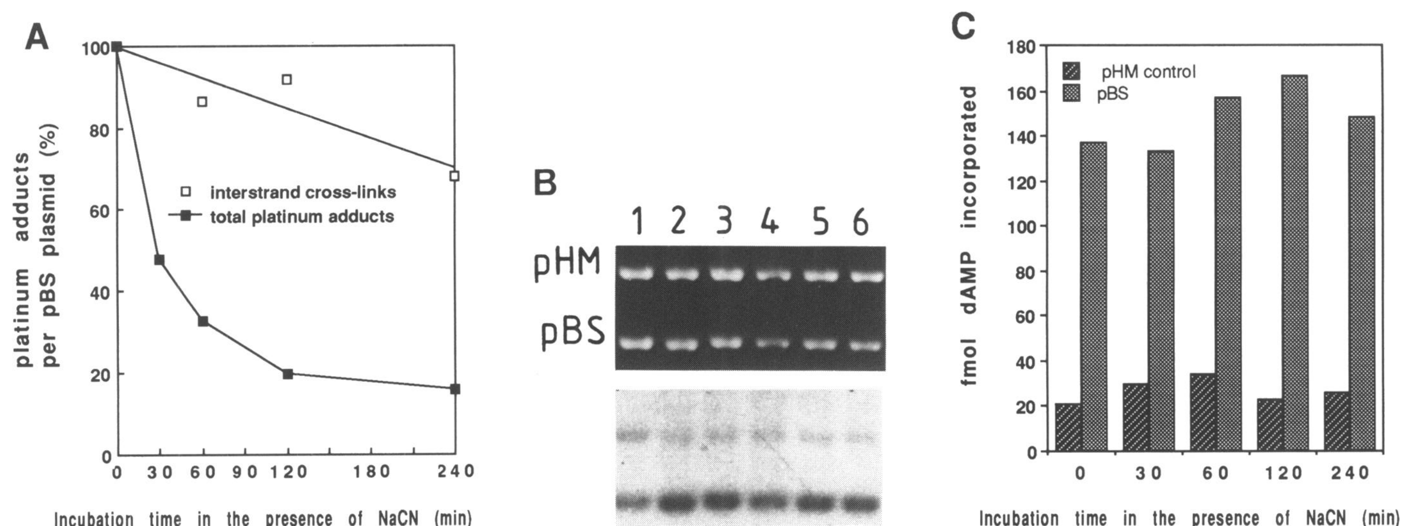

Figure 2. Kinetics of plasmid treatment with cyanide ions. **A.** Time course of platinum adducts removal in the presence of NaCN. C20 pBS plasmid was treated with cyanide ions for the indicated time. Total platinum bound per plasmid (■) was measured by atomic absorption spectroscopy. Interstrand cross-links per plasmid (□) were quantified as in Fig. 1. **B.** Repair synthesis by AHH1 cell extracts. NaCN treated pBS plasmids and pHM control plasmids were incubated with AHH1 repair proficient whole cell extracts. *Upper panel* : photograph of the ethidium bromide-stained agarose gel. *Lower panel* : autoradiograph of the dried gel. Incubation was for 3 h at 30°C in 50 μl standard reaction mixture containing 300 ng each of pBS and pHM plasmids and 100 μg whole cell extract protein. Plasmids were pHM control and pBS as follows: lane 1 : C0CN mocked-platinated plasmid, 4 h NaCN treatment ; lanes 2–6 : C20 plasmid, 0, 30, 60, 120, 240 min NaCN treatment respectively. **C.** Quantification of data from B. Incorporation is in femtomoles dAMP per plasmid. Marginal damage-independent incorporation in COCN pBS plasmid was subtracted from repair synthesis in C20 plasmids for each time point.

Amersham), 100 μg extract protein and 60 mM KCl in reaction buffer as described Incubation was 3 h at 30°C. Plasmid DNA was purified from reaction mixtures as described (20) linearized with HindIII and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. Data were quantifed by autoradiography, scintillation counting of excised DNA bands and densitometry of the photographic negative of the gel to normalize for plasmid DNA recovery in each reaction sample (Scanning Laser Densitometer, Biocom, France). Incorporation in pHM plasmid yielded background synthesis. Incorporation in mocked-platinated (C0) and mocked-platinated NaCN treated (C0CN) pBS plasmids yielded damage-independent synthesis. Since treatment of undamaged plasmid with cyanide ions led to a slight increase in background DNA synthesis possibly resulting from a few random nicks introduced in plasmid DNA during incubation, it was subtrated from repair synthesis in damaged plasmid to calculate damage-specific synthesis.

RESULTS

Consequences of removing cyanide-reversible platinum DNA adducts on DNA repair synthesis

Preferential removal of d(GpG) and d(ApG) *cis*-DDP intrastrand diadducts occurred in the presence of cyanide ions (CN^-) (34, 35, 37). In order to determine the contribution of these adducts to DNA repair synthesis *in vitro*, we measured simultaneously (i) the kinetics of adduct removal from platinated plasmid DNA during treatment with cyanide ions, (ii) the damage-dependent incorporation in the resulting substrates during excison-repair by human cell extracts. A plasmid containing ~20 total platinum adducts (C20, Table 1) was treated with NaCN for various time intervals up to 4 h. For each time point, total platinum content was determined by atomic absorption spectroscopy and interstrand

Table 1. Platinated plasmid substrates.

pBS plasmid	damage	NaCN treatment	total platinum adducts per plasmid	interstrand cross-links per plasmid[(a)]
D20	[Pt(dien)Cl]Cl	–	19.5	0
C6	*cis*-DDP	–	6.4	0.5
C20CN	*cis*-DDP	+	5.5	1.5
C20	*cis*-DDP	–	19.3	2.2
C100CN	*cis*-DDP	+	22.9	>4.6

(a) the average number of interstrand cross-links per pBS plasmid was calculated according to the Poisson distribution from the fraction of single-stranded non-cross-linked DNA determined by gel electrophoresis after heat-denaturation (see Figure 1).

cross-links were quantified by plasmid denaturation experiments (see Material and Methods and Fig. 1). After 4 hours, 85% of total platinum adducts were removed but about 70% of interstrand cross-links still remained in plasmid DNA (Fig. 2A). When the resulting DNA samples were tested in standard *in vitro* DNA repair conditions with human cell extracts (AHH1 cell line), no significant variation in damage-dependent dAMP incorporation occurred (Fig. 2B and 2C), implying that the remaining lesions after NaCN treatment accounted almost exclusively for the DNA repair synthesis yield.

Construction and characterization of plasmid substrates containing various proportions of mono-, intra- and interstrand platinum DNA-adducts

In order to check more precisely for the relative repair of the various platinum-DNA adducts by human cell extracts *in vitro*, we constructed three types of plasmid DNA substrates containing mainly either monoadducts, 1,2-intrastrand cross-links or interstrand cross-links lesions.

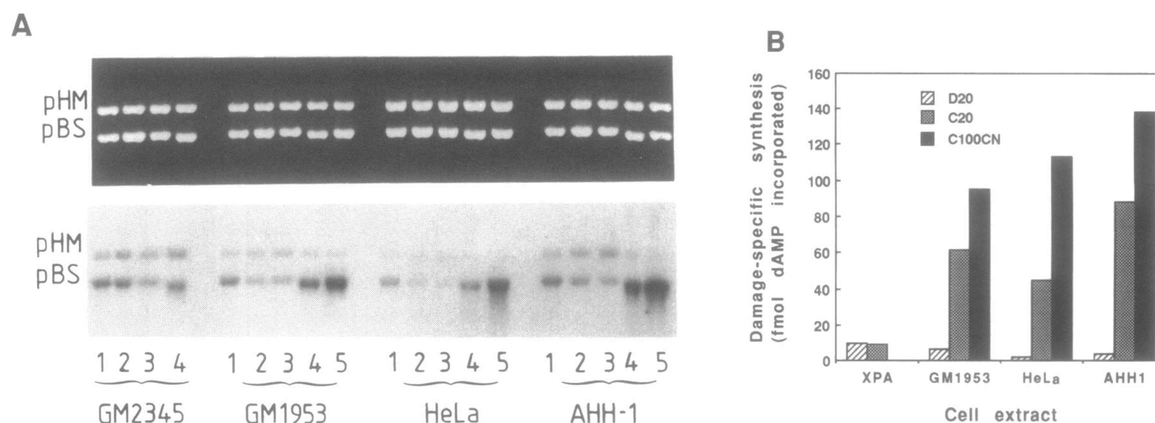

Figure 3. DNA repair synthesis by extracts from XPA (GM2345) and repair proficient cells (GM1953, HeLa, AHH1). **A.** Upper panel : photograph of the ethidium bromide-stained agarose gel. *Lower panel* : autoradiograph of the dried gel. Incubation conditions were as in Fig. 2. Plasmids were pHM control and pBS as follows: lanes 1, C0CN mocked-platinated, NaCN treated; lanes 2, C0 mocked-platinated, NaCN untreated; lanes 3, D20; lanes 4, C20; lanes 5, C100CN. **B.** Quantification of data from A. The histogram shows femtomoles of dAMP incorporated into D20, C20 and C100CN plasmids by cell extracts as indicated. For each sample, incorporation was normalized for the amount of DNA recovered and the small amount of background incorporation in pHM control plasmid was subtracted from incorporation in pBS plasmid. Damage-independent incorporation in unplatinated C0CN and C0 pBS plasmids (lanes 1 and 2, panel A) was subtracted from repair synthesis in C100CN and C20 pBS plasmids, respectively (lanes 4 and 5, panel A) in order to calculate damage-specific synthesis.

Monoadducted DNA was obtained after treatment with the monofunctionally binding compound [Pt(dien)Cl]Cl (D20 plasmid, Table 1). It has been established that 1,2-intrastrand cross-links between adjacent bases in d(GpG) and d(ApG) sequences account for about 90% of total platinum adducts after *cis*-DDP binding to cellular or plasmid DNA (9, 38). Therefore, we incubated pBS plasmid under conditions that produced mainly intrastrand adducted plasmid DNA (C6 and C20 plasmids, Table 1) ; 8 to 11% of *cis*-DDP adducts were interstrand cross-links in these plasmids (Fig. 1 and Table 1), according to published results (24).

To get higher proportions of interstrand cross-links, plasmid DNA was first treated with *cis*-DDP in order to obtain 20 or 100 adducts per circle ; purified plasmids were then incubated for 4 h in the presence of cyanide ions which removed preferentially intrastrand diadducts, leading to plasmid DNA enrichment for interstrand cross-links (Fig. 1, Table 1). Control C0 plasmids devoid of platinum or D20 plasmids contained no interstrand cross-links as determined on agarose gel after heat-denaturation (Fig. 1).

Repair synthesis by human cell extracts in platinated plasmid DNA substrates

Whole cell extracts were prepared from human cell lines either from lymphoid (GM1953, GM2345, AHH1) or epithelial origin (HeLa S3). Fig. 3A shows the result of a representative experiment comparing DNA repair synthesis in control and platinated DNA mediated by 100 μg of extracts in standard *in vitro* DNA repair conditions. An extract from GM2345 cell line was included for comparison as representative of DNA repair deficiency (xeroderma pigmentosum group A). As already reported (20, 25, 26) XPA cell extracts showed only residual damage-dependent repair synthesis (Fig. 3B), which provides strong evidence that this assay measures nucleotide excision repair. Surprisingly, D20 plasmid modified with up to 20 platinum monoadducts did not induce significant DNA repair synthesis by all three human repair proficient cell extracts tested.

The background synthesis caused by platination conditions or cyanide treatment was taken into account in order to calculate damaged-dependent repair synthesis *cis*-DDP damaged plasmid treated or not with cyanide ions (Fig. 3B). All three independent extracts from unrelated human repair proficient cells exhibited an increase in DNA repair synthesis on C100CN plasmid bearing a more than two fold higher proportion of interstrand cross-links as compared to C20 plasmid, whereas both plasmids were modified with about 20 total platinum adducts per plasmid. The increase in DNA repair synthesis ranged from 1.6-fold for GM1953 and AHH1 cell extracts to 2.5-fold for HeLa cell extracts. When the experiment was repeated with C6 and C20CN plasmids modified with about 6 platinum adducts, a similar 2-fold increase was found, comparing repair synthesis on plasmid C20CN enriched for interstrand cross-links to C6 ($\sim$27 and $\sim$8% interstrand cross-links, respectively) (not shown).

DISCUSSION

Cis-DDP modified plasmid DNA has been reported to be repaired *in vitro* by nucleotide excision activity in extracts from human cells (21, 24−26, 39). The platinum(II) coordination complexes initially bind to DNA as monoadducts that rearrange afterwards to form either intra- or interstrand diadducts (4).

It is generally believed that platinum monoadducts are biologically inactive, based on the ineffectiveness of the monofunctionally DNA binding compound [Pt(dien)Cl]Cl as a toxic agent or block to replication *in vitro* and *in vivo* (39−43). By using [Pt(dien)Cl]Cl-adducted plasmid DNA, we report a defect in platinum monoadduct repair by mammalian cell extracts. Plasmids modified with up to 20 platinum monoadducts per circle were marginally repaired while significant DNA repair synthesis occurred in plasmid bearing four fold less total *cis*-DDP adducts. These results imply that monoadducts are poor substrates for eukaryotic nucleotide excision-repair. It has been reported that UvrABC bacterial excision-nuclease *in vitro* removes *cis*-DDP monofunctional adducts, even more efficiently than intrastrand d(GpG) or d(ApG) diadducts (44). However, the experiments were performed on synthetic oligonucleotides, that could conceivably alter lesion recognition and modify UvrABC affinity for the various platinum adducts. For example, DNA negative

supercoiling greatly stimulates incision of psoralen cross-links whereas it has no effect on monoadducts incision (45). Moreover, the human repair system may prefer different substrates than UvrABC excinuclease. Our results on the lack of platinum monoadducts excision *in vitro* suggest also that the absence of a toxic effect of the monofunctionally binding platinum compound *in vivo* is not related to repair, but rather to a tolerance mechanism.

Results from the *in vitro* repair synthesis experiments performed on cyanide-treated platinated plasmids clearly demonstrated that (i) DNA repair proteins in cell extracts discriminated between platinum adducts on target DNA, (ii) DNA repair synthesis occurred preferentially in the presence of cyanide-resistant platinum adducts.

With 20 total platinum adducts per plasmid, there is a plateau in repair synthesis in the absence of NaCN treatment ((24) and data not shown). The two fold increase in repair synthesis that was observed above the plateau could rely partly on an alleviation of DNA synthesis inhibition by intrastrand diadducts that were removed during NaCN treatment and that could block recognition and/or DNA polymerization steps in the nucleotide excision process. Hanson and Wood (24) already noticed that *cis*-DDP adducts can suppress extension of patches associated with non specific background DNA synthesis. However, we also observed a two fold increase in repair synthesis in interstrand cross-link enriched plasmids modified with about 6 total *cis*-DDP adducts, which is far below the number of total adducts leading to a plateau in repair synthesis. Moreover, after 30 min incubation in the presence of NaCN where less than 10% of interstrand cross-links were removed from DNA, no increase in repair synthesis was observed, although more than 50% of total platinum adducts were already removed. Then, an alleviation of repair synthesis inhibition by intrastrand cross-links removal during cyanide treatment cannot solely account for these results.

As demonstrated for psoralen cross-links (22), the *in vitro* assay we used is dealing with true repair, leading to lesion removal. We then took advantage of the easy visualization of interstrand DNA cross-linking on agarose gel after heat-denaturation to quantify the global disappearance of interstrand diadducts after incubation with cell extracts in excision repair conditions. By using C20 plasmids initially containing ~2 interstrand cross-links (11% of total adducts), we estimated that excision repair eliminates ~20% of these adducts, that is at least 2% of total adducts (unpublished results). Since the in vitro repair efficiency concerns about 0.5 to 3% of total lesions (24), this suggests that interstrand cross-links represent a major lesion contributing to DNA repair synthesis in the *in vitro* assay. Recent data corroborate our findings since it has been shown that a site-specifically placed unique 1,2-d(GpG) *cis*-DDP adduct in duplex M13 DNA induced very poor repair synthesis by cell extracts *in vitro* (46).

We cannot exclude that the repair of other minor lesion(s) resistant to removal by cyanide ions could participate to the repair synthesis signal. To our knowledge, the cyanide reversibility of 1,3-intrastrand cross-links has not been precisely characterized. Knowing that *trans*-DDP preferentially forms 1,3-intrastrand diadducts *in vitro* in addition to interstrand cross-links (47), it is of interest that *trans*-DDP treated plasmids stimulated about twice as much repair synthesis by cell extracts than plasmids modified with *cis*-DDP (24). In addition, preincubation in cell extracts reactivated more readily *trans*-DDP modified plasmids for replication *in vitro* than plasmids treated with *cis*-DDP, in conditions were interstrand cross-links were almost absent (39). These results and ours taken together suggest that platinum adducts other than the predominant 1,2-intrastrand diadduct are responsible for DNA repair synthesis in DNA damaged with *cis*-DDP. Similar *in vitro* experiments using repair proficient cell extracts have also established that psoralen cross-links induced a higher level of repair synthesis compared to monoadducts (22).

A model for interstrand cross-link repair has been established *in vitro* (48–50) and requires first incision on one strand, gap filling by recombination, removal of the cross-linked oligomer and second gap filling by DNA polymerization. During repair of interstrand diadducts, a gap size larger than for intrastrand lesions could conceivably contribute to the increase in repair synthesis that we observed in interstrand cross-link enriched plasmids. Therefore, the precise repair patch size remain to be established and we have undertaken experiments with a site-specifically positioned *cis*-DDP interstrand diadduct.

What could be the relation of these results obtained *in vitro* with the *in vivo* situation? It has been reported that in Chinese hamster ovary cells, *cis*-DDP interstrand cross-links were removed more efficiently from DNA than intrastrand adducts (10). Moreover, the most abundant 1,2-intrastrand adducts are only partially repaired in murine L1210 (18). The role of DNA repair in modulating *cis*-DDP cytotoxicity has also been clearly emphasized by studies on the mechanisms of acquired cellular resistance to *cis*-DDP (for reviews, (12–14). Two *cis*-DDP DNA structure-specific recognition protein SSRP1 and HMG1 have been characterized and shown to recognize specifically d(GpG) and d(ApG) 1,2-intrastrand cross-links (51, 52). It has been hypothesized that *in vivo* binding of these proteins to platinated DNA may prevent recognition of these adducts by cell repair process (51). The results we have obtained *in vitro* where 1,2-intrastrand *cis*-DDP diadducts seemed to contribute poorly to DNA repair synthesis by human cell extracts might mimic this *in vivo* situation. In that view, the *in vitro* repair assay represents a powerful system to elucidate interaction of cellular repair or structure-specific recognition proteins with platinum adducts on DNA.

From a clinical point of view, a repair defect of the predominant *cis*-DDP adducts in DNA could reasonably account for drug efficiency. *Cis*-DDP cytotoxicity has already been suggested to rely on the repair defect of the major drug adducts (4, 53). Moreover, the alleviation of this repair defect might conceivably lead to cell resistance. In one study, cells resistant to *cis*-DDP have been shown to remove a higher proportion of d(GpG) intrastrand cross-links from DNA than their sensitive counterpart (47). However, a recent report emphasized an increased DNA repair efficiency of interstrand cross-links as the basis for acquired cellular resistance to *cis*-DDP (19). Non exclusive participation of both mechanisms in cell resistance can be hypothesized. We are currently investigating DNA repair involvement in cell resistance to *cis*-DDP by means of the *in vitro* DNA repair assay (54) in order to determine wether changes in cellular discrimination of platinum adducts on DNA are a basis for this resistance mechanism.

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