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Repair of DNA-Protein Cross-Links in an Excision Repair-deficient Human Cell Line and Its Simian Virus 40-transformed Derivative

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ABSTRACT

DNA-protein cross-links are induced in mammalian cells by X-rays, ultraviolet light, fluorescent light, and numerous chemical carcinogens. Others have shown that these cross-links are repaired by normal cells but that excision repair-deficient xeroderma pigmentosum (XP) Group A cells, XP12BE, are deficient in repair of these bulky adducts. This paper compares the DNA-protein cross-link repair competency of another XP Group A strain, XP20S, with its more rapidly proliferating simian virus 40-transformed derivative line and with normal human skin fibroblasts. DNA-protein cross-links were induced with 20 μM *trans*-platinum(II)diamminedichloride and assayed by the membrane alkaline elution procedure of Kohn. Treated and untreated cells are lysed on a polycarbonate membrane filter, and the coelution rates of the DNA at pH 12.2 are compared; DNA-protein cross-links retard elution of DNA. The repair competency of XP20S cells for *trans*-platinum(II)diamminedichloride-induced DNA-protein cross-links was similar to that of XP12BE cells, but the competency of the simian virus 40-transformed XP20S cells was nearly equal to that of normal human skin fibroblasts. These results suggest that either cell cycling compensates for the genetic deficiency present in the nucleotide excision process of XP Group A cells or that a process other than nucleotide excision can repair these lesions; this process requires cell cycling or activation by the virus.

INTRODUCTION

DNA-protein cross-links are formed in mammalian cells by numerous agents including X-rays (24), UV light (1, 5), visible fluorescent light (9, 10), and a wide variety of chemicals (6, 22). These adducts are readily repaired by repair-proficient cells in culture (25). Although mutagenic activity is not detected when cross-links are formed with *trans*-platinum,[2] this bifunctional compound induces sister chromatid exchanges (2), and it accelerates transformation of 3T3 and 10T½ mouse fibroblasts (7). Since malignant transformation certainly requires more than a simple mutation, unrepaired DNA-protein cross-links may play an important genetic or epigenetic role in this transformation. XP cells, Complementation Group A, derived from individuals genetically predisposed to a high risk of cancer, are deficient in their ability to repair UV-induced thymidine dimers (3, 18) as well as DNA-protein cross-links (8). Since repair of the latter lesion seems to involve a cell cycle-related phenomenon (11) in addition to nucleotide excision, we compared this repair in SV40-transformed XP Group A cells which proliferate vigorously with the more slowly proliferating untransformed parent line and with normal skin fibroblasts. This paper presents evidence that SV40-transformed XP fibroblasts repair DNA-protein cross-links induced by *trans*-platinum virtually as competently as do normal human foreskin fibroblasts, whereas the untransformed cells are repair deficient.

MATERIALS AND METHODS

Cell Culture. Monolayer cultures of XP20S and SV40-transformed XP20S (21) (Institute for Medical Research, Camden, NJ, repository Numbers GM4419 and GM4312A, respectively) were carried in plastic T-25 flasks in Dulbecco's modified Eagle's medium (MA Bioproducts, Walkersville, MD) containing 100 μg of Gentamicin/ml (Schering-Plough Corp., Kenilworth, NJ) and supplemented, respectively, with 20% and 10% fetal bovine serum (Flow Laboratories, McLean, VA). At bimonthly intervals, stock cultures were grown in antibiotic-free medium for 3 weeks and, when tested for *Mycoplasma*, were found negative (Flow Laboratories) by direct and indirect tests. In logarithmic growth phase, the XP20S cells doubled their population in about 36 hr; the transformed cells doubled in about 26 hr. The XP20S cultures were subcultured 1:2, and the transformed cultures, 1:5 weekly and grown at 37°C with 10% CO_2:air gas phase. Normal human foreskin fibroblasts were initiated and cultured as described previously (19). In early passages, these cells double their population in about 22 hr, and this increases to as much as 40 hr in later passages. For comparison of DNA elution patterns, confluent T-25 flasks were harvested with 0.1% crystalline trypsin (Worthington Biochemical Corp., Freehold, NJ) in 0.02% EDTA and planted in T-75 flasks. The planting medium was replaced with fresh medium 48 hr later, and either [2-^{14}C]thymidine (0.4 μCi/ml) or [*methyl*-^{3}H]thymidine (0.8 μCi/ml) was added. After an additional 72 hr, the cells were harvested, and replicate cultures were prepared in thymidine-free medium at 3×10^5 cells/T-25 flask. After 24 hr, the cells are treated with 20 μM *trans*-platinum (Vega Biochemicals, Tucson, AZ) by adding to each culture 100 μl of a 1 mM solution prepared in complete medium 30 min earlier at 37°C. The *trans*-platinum remains in the medium unless the experiment continues beyond 48 hr, at which time the fluid is changed as usual. The final 20μM *trans*-platinum treatment was chosen, because it produces sufficient DNA-protein cross-links for measurement and has little or no toxic effect as reported for L1210 mouse cells by Zwelling *et al.* (26) and confirmed in our laboratory.

Assay of DNA-Protein Cross-Links. The membrane alkaline elution procedure of Kohn *et al.* (14, 15) was used to measure cross-links with minor modifications. Briefly, ^{14}C- and ^{3}H-prelabeled treated and untreated control cells were mixed together, placed on a polycarbonate filter (Unipore, 2-μm pore size; BioRad, Richmond, CA), and lysed with 5 ml of 20 mM sodium EDTA (pH 10.2) containing 0.3% Sarkosyl (w/v, Ciba-Geigy, Greensboro, NC), and 2 M NaCl was followed by a burst of air forced through the membrane with a syringe to fragment the DNA (9). The DNA was then eluted at a flow rate of 0.33 ml/min with 20 mM EDTA:tetrapropylammonium hydroxide (free acid form of EDTA titrated to pH 12.2 with tetrapropylammonium hydroxide). Fractions were collected every 15 min for 3.5 to 4 hr. Glacial acetic acid (0.1 ml) was added to each fraction, and the ^{14}C and ^{3}H were counted as a gel in 10 ml of Ready-Solv MP (Beckman Instruments, Palo Alto, CA) at 61 and 34% efficiency, respectively, in a Beckman Model LS250 scintillation counter. The radioactivity remaining on the filter was solubilized with dilute HCl (1

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[2] The trivial name and abbreviation used are: *trans*-platinum, *trans*-platinum(II)diamminedichloride; XP, xeroderma pigmentosum.

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M, 70°C for 30 min) and counted as described (16). The cells and culture medium were shielded from light of wavelength less than 500 nm, and the elution procedure was carried out in the dark.

Although polycarbonate filters and the inclusion of sodium dodecyl sulfate in the elution solution are recommended for minimizing protein adsorption to the filter (16), under the above elution conditions without sodium dodecyl sulfate, these filters were as sensitive as were polyvinyl chloride filters for measuring DNA-protein cross-links. Also, as with polyvinyl chloride filters, addition of proteinase K (0.5 mg/ml; E. Merck, Darmstadt, Germany) to the lysis buffer restores the elution rate of DNA from *trans*-platinum-treated cells to that of the untreated controls.

RESULTS

The formation of DNA-protein cross-links by *trans*-platinum and their subsequent repair in mammalian cells have been studied in detail by Zwelling *et al.* (25). However, the underlying mechanism(s) of this repair has not been as intensively studied. The finding that an XP Group A cell line, XP12BE, is deficient in repair of these lesions (8) provides evidence that nucleotide excision is one mechanism for repair. Exploratory experiments in our laboratory corroborate these results; XP12BE cells as well as a Group C and a Group E line (American Type Culture Collection repository Numbers CRL 1223, CRL 1158, and CRL 1259, respectively) are deficient in repair of DNA-protein cross-links induced with 20 μM *trans*-platinum, but these cells could completely repair the cross-links within 144 hr. In view of the above results, the repair competency of SV40-transformed XP20S cells, which also belong to complementation Group A but proliferate rapidly, was compared with that of normal human skin fibroblasts. The alkaline elution profiles of DNA from cells harvested immediately after 20 μM *trans*-platinum treatment and after 24- and 48-hr repair periods show that the virus-transformed XP cells repair the DNA-protein cross-links nearly as proficiently as do the normal cells (Chart 1). However, comparison of the repair competency of SV40-transformed XP20S cells with their untransformed counterparts revealed that the untransformed XP20S cells were repair deficient (Chart 2), similar to XP12BE cells (8). Alternatively, the more rapid DNA elution rate of *trans*-platinum-treated SV40-transformed XP20S cells could result from the accumulation of DNA strand breaks during the repair period rather than from proficient repair. To test this possibility, proteinase K digestion was included in the elution assay at 48 hr after *trans*-platinum treatment. The treated-cell DNA eluted at the same rate as that of the untreated controls. This result indicates that the increased elution rate of DNA from the *trans*-platinum-treated transformed cells was not due to accumulation of DNA strand breaks. Another point to note is that the transformed and untransformed cells are cultured in 10 and 20% serum supplement, respectively. Although there is no detectable difference in level of DNA-protein cross-links induced in 10 and 20% serum, the untransformed cells are even less proficient at repair in 10% serum.

One difference between virus-transformed cells and untransformed cells is the tendency of the transformed cells to continue cycling under conditions which restrain normal cells. Growth

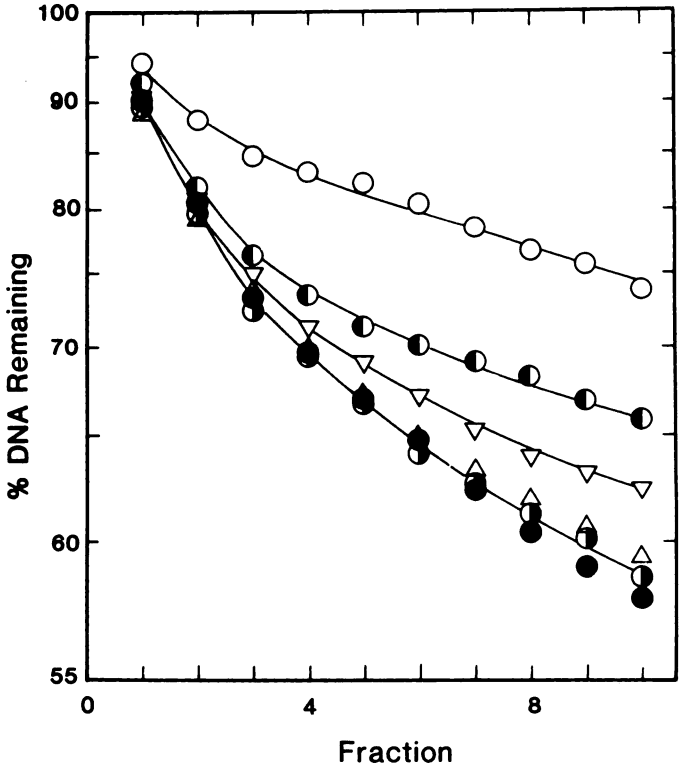

Chart 1. DNA alkaline elution profiles of SV40-transformed XP20S and normal human skin fibroblasts treated with 20 μM *trans*-platinum. Loss of DNA-protein cross-links is indicated by more rapid elution of DNA. Treated and untreated normal cell DNA elution profiles at zero time were within 3% of the corresponding SV40-transformed XP20S cell profiles and were omitted for clarity. ○, ◐, and ◑, *trans*-platinum-treated SV40-XP20S cells after zero, 24-, and 48-hr repair, respectively; and ●, untreated zero time control. ▽ and △, treated normal human fibroblasts after 24 and 48 hr, respectively.

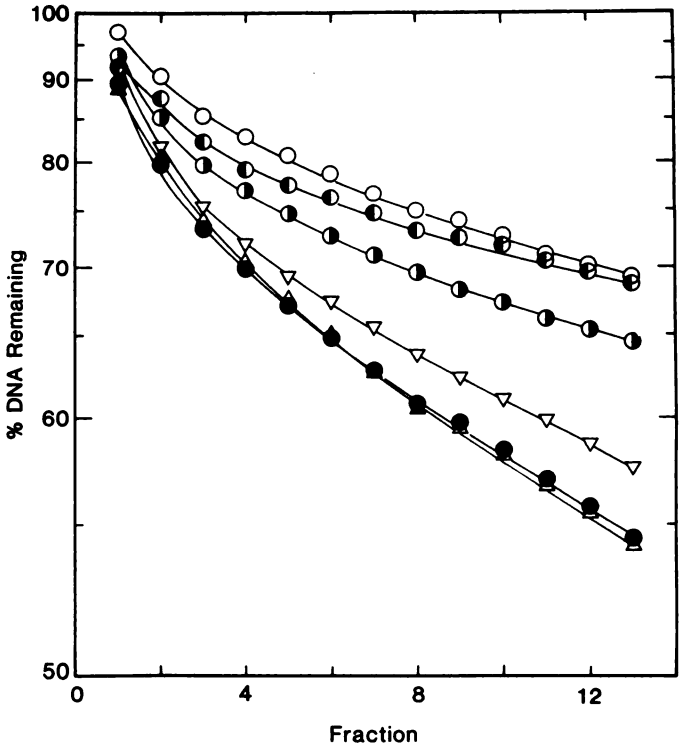

Chart 2. DNA alkaline elution profiles of SV40-transformed and untransformed XP20S fibroblasts treated with 20 μM *trans*-platinum. Loss of DNA-protein cross-links is indicated by more rapid DNA elution. Treated and untreated SV40-transformed XP20S cell DNA elution profiles at zero time were within 3% of the corresponding untransformed XP20S cell profiles and were omitted for clarity. ○, ◐, and ◑, *trans*-platinum-treated XP20S cells after zero, 24-, and 48-hr repair, respectively; and ●, untreated zero time control. ▽ and △, treated SV40-transformed fibroblasts after 24 and 48 hr, respectively.

Table 1

Dose response of SV40-XP20S and XP20S fibroblasts to trans-platinum

Replicate cultures were prepared at $2 \times 10^{+5}$ cells/T-25 flask, and 24 hr later, *trans*-platinum was introduced. Two hr later, the platinum-containing medium was replaced with fresh growth medium. After 72 hr, the cells were dispersed with 0.1% trypsin, suspended in 0.1% trypan blue, and counted. More than 98% of the cells excluded trypan blue.

Cells	μM *trans*-platinum	10^{-5} × cell no., 72 hr posttreatment
SV40-XP20S fibroblasts	0	5.37 ± 0.78[a]
	50	4.85 ± 0.68
	100	3.99 ± 0.15
	150	2.98 ± 0.62
XP20S fibroblasts	0	4.53 ± 0.91
	50	2.72 ± 0.21
	100	2.87 ± 0.67
	150	2.63 ± 0.21

[a] Mean ± S.D. of 3 replicate cultures.

Table 2

UV sensitivity of normal human foreskin and SV40-XP20S Group A fibroblasts

An inoculum of 10^{+5} cells/60-mm dish was used; 24 hr later, the dishes were exposed to 8 J of UV irradiation (determined with an IL 700 radiometer) from a 15-watt germicidal lamp. After 5 days, the remaining cells were dispersed with 0.1% trypsin, suspended in 0.1% trypan blue, and counted. More than 96% of the cells observed excluded the dye.

Cells	10^{-5} × cell no. 5 days postexposure	% of control
Foreskin fibroblasts		
Control	4.51 ± 0.14[a]	100
UV exposed	1.11 ± 0.20	25
SV40-XP20S fibroblasts		
Control	7.17 ± 0.94	100
UV exposed	<0.01	<0.2

[a] Mean ± S.D. of 3 replicate cultures.

comparison of untransformed XP20S with SV40-transformed XP20S cells after treatment with several concentrations of *trans*-platinum shows that the virus-transformed cells treated with 50 μM *trans*-platinum doubled in number, and those cells treated with 100 μM *trans*-platinum nearly doubled in number as well (Table 1). However, the untransformed XP20S cells essentially stopped growing after 50 μM *trans*-platinum treatment. At least 98% of the cells counted were judged metabolically competent by the trypan blue exclusion test. These results are consistent with the idea that cell cycling of the transformed cells may be important for excision-deficient cells to repair *trans*-platinum-induced DNA-protein cross-links.

The similarity of these transformed XP cells to normal cells in survival following *trans*-platinum treatment raised a question as to the identity of the XP cells. Since a hallmark of XP cells is their sensitivity to UV light, we exposed both the normal fibroblasts and SV40-transformed XP cells to UV light, 8 J/sq m. The results show (Table 2) that 25% of the normal cells survived, 96% of which excluded trypan blue, while no surviving transformed XP cells were found (<0.2%). This clear difference in UV light sensitivity confirms the identity of the SV40-transformed cells.

DISCUSSION

Repair of damaged DNA in mammalian cells is complex. Numerous investigators have suggested that DNA synthesis or cell cycling in some way enhances repair of various kinds of damage (for a review, see Ref. 4). In a preliminary paper (11), we presented evidence suggesting that cell cycling also enhances repair of DNA-protein cross-links in mouse L1210 cells and normal human skin fibroblasts. We now report that an excision-deficient, SV40-transformed XP Group A cell line (XP20S) repairs DNA-protein cross-links nearly as rapidly as normal human skin fibroblasts, whereas the untransformed XP parent line is repair deficient. It has been suggested that XP Group A cells may be deficient in an endonuclease activity required for nucleotide excision repair (20, 23). Since the continued sensitivity to UV light demonstrates that infection and integration of the SV40 viral genome do not introduce new information directly affecting DNA repair capacity of UV light damage, the present results suggest that another pathway in addition to the nucleotide excision pathway reported by Fornace and Seres (8) exists for the repair of DNA-protein cross-links. Presumably, some direct or indirect consequence of cell transformation, such as cell cycling (12) and/or the activation and expression of cell information present before transformation, leads to this proficiency in DNA-protein cross-link repair. This second pathway is not post-replication repair, because these experiments measure changes in the parental strand only, rather than daughter strand repair.

Various investigators have found that nucleosome structure inhibits repair of DNA damage (for a review, see Ref. 17). It is probable that even higher orders of chromatin structure complicate further the problem of repair. An explanation of the deficiency in XP Group A cells, alternative to the loss of endonuclease activity, was suggested by Mortelmans *et al.* (18) and Kantor and Setlow (13). These investigators propose that Group A cells are not deficient in the actual repair process but are deficient in enzymes, which render the damage in chromatin accessible to repair. In accordance with this concept, the proficient repair of DNA-protein cross-links by the transformed cells may result from cell cycling rendering the lesions more accessible to nucleotide excision repair enzymes. However, the results also suggest that the repair of DNA-protein cross-links may proceed by a pathway other than nucleotide excision.

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