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INCREASED NEAR-ULTRAVIOLET INDUCED DNA FRAGMENTATION IN XERODERMA PIGMENTOSUM VARIANTS

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SUMMARY

Immediate fragmentation of parental DNA by near-ultraviolet irradiation at 313 nm was measured in cultured skin fibroblasts from normal individuals, patients with Xeroderma pigmentosum of complementation group A (XPA) and Xeroderma pigmentosum variants (XPV) by the alkaline elution procedure. For a dose of 2.25 KJm^{-2} given at 0^{o} fragmentation was comparable in all cell strains. However, fragmentation was strongly increased relative to 0^{o} in XPV but not in normal fibroblasts and the XPA strains when irradiation was carried out at 37^{o}. From our results it appears that a step in the repair of parental DNA is abnormal in XPV.

INTRODUCTION

Cultured skin fibroblasts from most patients with the autosomal recessive disease Xeroderma pigmentosum (XP), which is characterized by increased skin sensitivity to solar radiation and increased incidence of skin cancer, are deficient in the excision of thymine containing photodimers induced by 254 nm ultraviolet light (1-3). Cells from a small group of patients with the typical clinical symptoms of XP possess normal capacities for the removal of photodimers from parental DNA, however (4,5) and are referred to as XP variants (XPV). XPV skin fibroblasts in culture are slightly more sensitive to 254 nm light

The abbreviations are : XPA, Xeroderma pigmentosum skin fibroblasts of complementation group A; XPV, skin fibroblasts from Xeroderma pigmentosum variants; PBS, phosphate buffered saline.

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(6) than normal controls and possess decreased capacities for the reactivation of irradiated adenovirus (7). Following 254 nm irradiation of XPV, daughter strand fragments are synthesized which are of lower molecular weights than for cells from normal individuals or patients with excision deficient forms of XP and it takes longer to reach control molecular weights (8,9). All these studies were carried out with far-ultraviolet light. However, the near- rather than far-ultraviolet portion of solar radiation is responsible for erythema formation and most probably for the induction of skin cancer in the human (10,11). It appears particularly interesting to study the near-ultraviolet photobiology of the human genetic diseases which are characterized by increased sensitivity to sunlight, therefore. The type and relative abundance of DNA lesions differ substantially for the different portions of the ultraviolet spectrum. While cyclobutyl dipyrimidines in general represent the predominant type of lesions other damage such as single-strand breaks (alkali-labile bonds) (12-14), products of the 5,6-dihydroxy-dihydrothymine type (15) and possibly "spore" products (16) gain in importance in the near- relative to the far-ultraviolet.

We have studied the formation of strand breaks (or alkali-labile bonds) in parental DNA in normal-, excision deficient XP- and XPV-skin fibroblasts following exposure to near-ultraviolet at 313 nm. It was found that 313 nm radiation at 37^{o} induced excessive fragmentation of parental DNA in XPV skin fibroblasts in comparison to normal control strains and XP-fibroblasts of complementation group A (XPA). Our results suggest that the primary defect in XPV may lie in a step of the repair of parental DNA.

METHODS

The skin fibroblast strains from normal individuals, CRL 1121 and CRL 1141, from XP patients of complementation Group A, XP 12 BE and XP 25 RO, from XP variants, XP 13 BE and XP 4 BE, were obtained from the American Type Culture Collection. All cells were grown in monolayer cultures in T 75 Falcon flasks in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The protocol for the labeling of parental DNA with ^{14}C-thymidine has been described previously (17, 18). The cultures were split into 60 mm Falcon dishes

and irradiated in phosphate buffered saline pH 7.4 (PBS) with monochromatic light at 313 nm (3 nm half-band width, 4.7 Jm^{-2} sec^{-1} dose rate) from a Schoeffel GM 250 high intensity quarter meter grating monochromator. The cultures, which had been irradiated at 37°, were harvested immediately by trypsinization at 37° (0.1% trypsin) or incubated in fresh complete media for different lengths of time. For experiments at 0° the culture dishes were placed on ice 15 min. before irradiation, 5 ml cold PBS was added and the irradiation carried out on a layer of ice. These cultures were washed with cold Versene and trypsinized at 0° (0.25% trypsin). The cell suspensions were applied to polyvinylchloride filters for cell lysis and alkaline elution as described previously (17,18). For unincubated samples the total handling time until application of the cells on the filters was 5 to 7 min.

RESULTS

Fragmentation of pre-existing "parental" DNA in human skin fibroblasts from normal individuals and XP patients was studied following exposure to monochromatic light at 313 nm by the alkaline elution method (17). In this method cells are applied and lysed on a polyvinylchloride filter. Single stranded DNA fragments of molecular weights below approximately 10^9 are eluted rapidly from the filter with a tetrapropylammonium buffer at pH 11.9 while DNA of higher molecular weight is eluted only very slowly. Individual elution curves for two normal strains, two XPA strains and two XPV strains are shown in Figure 1. Each panel contains curves for unirradiated controls and cultures which were irradiated and handled at 0° and 37°, respectively. The fraction of parental DNA retained on the filter is plotted on a logarithmic scale as a function of the volume of the elution buffer which has passed through. The curves are close to linear at low elution volumes and then slightly convex. The rates of elution of DNA of unirradiated controls from all normal- and XP-strains were comparable as were the increases in the rates of elution upon irradiation with 2.25 KJm^{-2} and handling at 0°. In contrast, when irradiation and handling were carried out at 37° the rate of elution increased by a small amount for the normal- and XPA-strains, but by a large factor for the XPV-strains.

A measure of the extent of immediate fragmentation of DNA in the different cell strains can be obtained from the fraction of DNA eluted by a constant amount of elution buffer (18). The table lists mean values of the fraction of

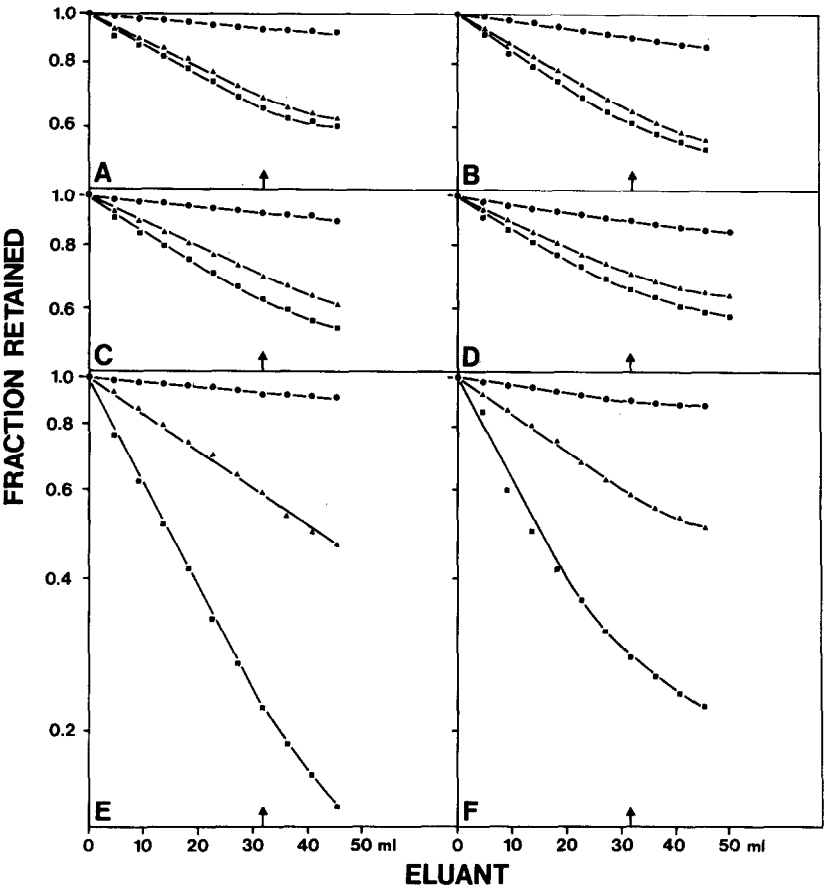

Figure 1 Alkaline elution analysis of parental DNA of normal-, XPA- and XPV-skin fibroblasts following irradiation with 2.25 KJm^{-2} of monochromatic light at 313 nm at 0^{o} or 37^{o}, respectively.

For all six panels: ● , unirradiated controls; ■ , irradiation and handling at 37^{o}; ▲ , irradiation and handling at 0^{o}.
A. Normal control CRL 1121; B. Normal control CRL 1141; C. XPA strain XP 12 BE; D. XPA strain XP 25 RO; E. XPV strain XP 13 BE; F. XPV strain XP 4 BE.

DNA eluted by 31.5 ml buffer of several experiments for each cell strain following irradiation with 2.25 KJm^{-2} at 0^{o} and 37^{o}, respectively. It is evident that the fraction eluted after irradiation and handling at 0^{o} was comparable for all cell strains and ranged from 0.20 to 0.31. Only small increments relative to 0^{o} were observed in the fraction eluted for the normal and XPA strains when irradiation and handling were at 37^{o}. In contrast, large increases from 0.31 to 0.63 for the variant strain XP 13 BE and from 0.29 to 0.48

TABLE 1 IMMEDIATE FRAGMENTATION OF PARENTAL DNA AT 313 nm

	Cell strain	0^{o} fraction eluted*	37^{o} fraction eluted*
Normals	CRL 1141	0.24	0.33
	CRL 1121	0.20	0.28
XP Group A	XP 12 BE	0.24	0.31
	XP 25 RO	0.27	0.36
XP Variants	XP 13 BE	0.31	0.63
	XP 4 BE	0.29	0.48

* The fraction of DNA eluted by 31.5 ml tetrapropylammonium buffer following irradiation with 2.25 KJm^{-2} and trypsinization at 0o and 37^{o}, respectively. The data was obtained from individual elution curves and is corrected for the fraction eluted in sham-irradiated samples (0.05-0.08). All experimental points were run in duplicates and the values listed are means of 5 independent experiments for the normal control strains and XP 13 BE and two experiments for all other strains. Reproducibility between duplicates was $\pm$ 3 % and between independent experiments $\pm$ 10 %.

for the variant strain XP 4 BE were observed upon changing the temperature from 0^{o} to 37^{o}.

The time course of the elongation-rejoining of the parental DNA following irradiation at 37^{o} is shown in Figure 2. Return to the elution properties of unirradiated control cultures was fastest for XPA and achieved within 30 min. and slowest for XPV for which the rates of elution were still significantly above the controls after 5 hours post-irradiation incubation.

DISCUSSION

For the interpretation of our results it should be appreciated that net fragmentation of parental DNA to molecular weights below approximately 10^{9} is measured by the alkaline elution method. Net DNA fragmentation at 0^{o}, where most cellular activities are strongly suppressed, is mostly due to the action of radiation per se, net fragmentation at 37^{o} is the result of multiple enzy-

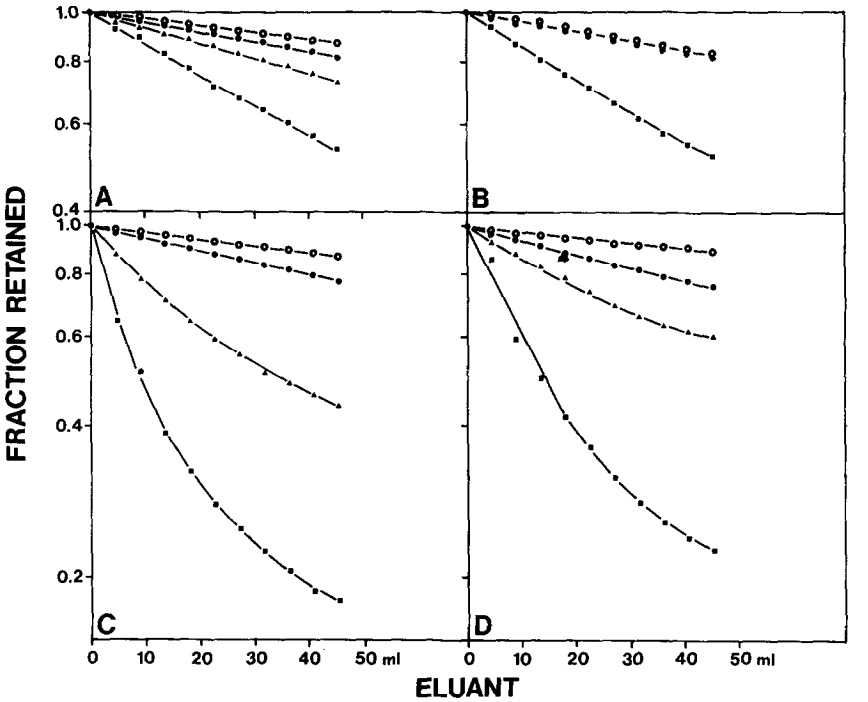

Figure 2 Alkaline elution analysis of parental DNA of normal-, XPA- and XPV-skin fibroblasts following irradiation with 2.25 KJm^{-2} of monochromatic light at 313 nm at 37^{0} as a function of post-irradiation incubation.

For all four panels : 0--0, unirradiated controls; ■, no incubation (handling time 5-7 min); ▲, 1 hour incubation; ●, 5 hours incubation. A, normal control CRL 1121; B. XPA strain XP 25 RO; elution curves after 30 min and 1 hour incubation (not shown) were not significantly different from that of the unirradiated control. C. XPV strain XP 13 BE; D. XPV strain XP 4 BE.

matic and non-enzymatic reactions, on the other hand. Since the level of immediate DNA fragmentation at 313 nm is only slightly higher at 37^{0} than at 0^{0} in the normal controls and XPA enzymatic strand fragmentation and elongation-rejoining during irradiation and handling of the cultures at 37^{0} are close to equilibrium in these cells under our experimental conditions. In contrast, a shift has occurred in the relative rates of these reactions strongly favoring strand fragmentation in XPV at 37^{0}. Correspondingly, the return of the elution properties of DNA to those of unirradiated controls was slower in XPV- than in normal- and XPA-strains. It is unlikely that our results are due to

increased radiation-chemical sensitivity of the DNA in XPV at 37^{0} relative to XPA and normal controls.

Since multiple reactions result in net fragmentation and net elongation-rejoining of parental DNA following UV-irradiation a number of alternative interpretations should be considered for our results. We would like to stress the following : (1) A late step in excision repair such as the ligation of parental DNA fragments may be deficient in XPV. This interpretation is supported by the fact that earlier steps in excision repair of cyclobutyl dipyrimidines are normal in XPV (4,5). The observation that the rejoining of X-ray induced strand breaks was normal in XPV argues against this interpretation, however (19). (2) A nuclease function involved in the removal of radiation lesions could be more active in XPV or (3) a glycosylase function could be more active. Glycosylases could be involved in the removal of monomeric lesions such as lesions of the 5,6-dihydroxy-dihydrothymine type. This would result in increased amounts of apyrimidinic-sites in XPV relative to normal controls and XPA and in increased DNA fragmentation by the action of apyrimidinic/apurinic-site endonucleases or hydrolysis during the alkaline elution procedure. The latter possibility appears unlikely since our elution curves were linear or slightly convex. It has been shown that the progressive hydrolysis of alkaline labile sites induced by methylating agents produced shouldered elution curves (20,21). Regardless of which interpretation turns out to be correct the abnormality in the metabolism of damaged DNA discovered in XPV strains affects parental DNA. Therefore, the possibility should be considered that the abnormality in de novo DNA synthesis in XPV described originally be Lehmann et al. (8) may be a reflection of a primary defect in the repair of the parental DNA template rather than a defect in "post-replication repair". Alternatively it is conceivable that the abnormal function in DNA metabolism in XPV affects in parallel the repair of parental DNA and de novo DNA synthesis.

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