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Cellular Effects of UVA: DNA Damages

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INTRODUCTION

Ultraviolet radiation between 320 nm and visible light (UVA) is a major component of both solar radiation and suntan lamps, which are being increasingly used in tanning booths. UVA has generally been considered innocuous, partially because DNA does not absorb appreciably in this region (Sutherland and Griffin, 1981), but UVB radiation (290-320 nm) has widely been considered to be the the major etiological factor in human skin carcinogenesis caused by solar UV radiation (Setlow, 1974; Parrish et al., 1978), largely because DNA absorbs photons of UVB, which is known to produce thymine photoproducts (cyclobutane dimers and adducts). It is true that patients with xeroderma pigmentosum are particularly prone to solar-UV-induced skin cancer, and cells derived from these people have been shown to lack ability to repair pyrimidine photoproducts by excision (Cleaver et al., 1984; Cleaver, 1987), evidence that pyrimidine photoproducts might play a role in carcinogenesis in certain specialized situations. Normal cells have the ability to repair these UVB-induced lesions (Mitchell, 1988a,b). However, UVA is considerably more penetrating and more abundant than UVB, and Tyrrell and Pidoux (1987) have performed a spectral analysis that claimed that 20-60% (depending upon the solar zenith angle) of the toxic biological effects of solar radiation can be attributed to UVA. The fact that UVA radiations are mutagenic (reviewed by Peak and Peak, in press) provides motivation for studying DNA changes that might be effected by this region of the electromagnetic spectrum. The

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following is a summary of the use of sensitive alkaline and neutral elution DNA filter assays to reveal and quantify various DNA damages resulting from exposures of human cells to isolated monochromatic UVB and UVA and visible light radiations.

METHODS

The use of P3 human teratocarcinoma cells in radiation studies was discussed by Hill et al., 1988. Culture and labelling of cells and use of monochromatic UV and visible light and x-rays were described by Hill et al. (1988), Peak et al., (1985), and Peak et al. (in press). Elution of DNA was as described by Kohn et al. (1981), and Peak et al. (in press). The modification of the assay for measurement of DNA-to-protein crosslinking in the special case where DNA breaks are also present was described by Peak et al. (1987). Calibration of the neutral elution assay for measurement of double-strand DNA breaks was described by Peak et al. (1988).

RESULTS AND DISCUSSION

Slowly developing alkali-labile sites: Figure 1 illustrates the different elution profiles that have been observed under various assay conditions after P3 cells were exposed to UVB and UVA as well as visible radiations. Standard alkaline elution for detection of total strand breaks [single-strand breaks (SSB) plus double-strand breaks (DSB) plus rapidly developing alkali-labile sites (RDALS)] usually gives profiles that are exponential (Figure 1, SSB). We observed this in the case of P3 cells exposed to 365-nm radiation and green light at 512 nm (Peak et al., in press). In

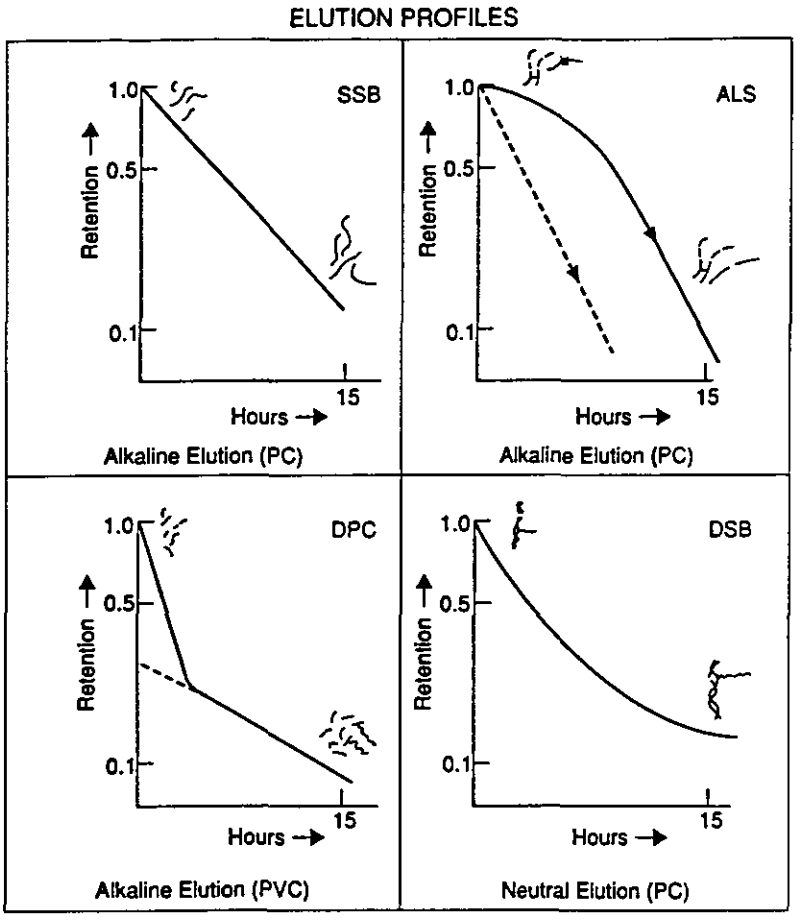

Figure 1

Schematic representation of different elution profiles observed after exposure of P3 cells to UVA radiations. Control, unirradiated profiles are omitted. The configuration of the DNA at the start and end of elution are shown by cartoons. SSB, single-strand break; PC, polycarbonate filters; ALS, alkali-labile site; DPC, DNA-to-protein crosslink; PVC, polyvinyl chloride filters; DSB, double-strand break.

in the same study we observed that alkaline elution profiles were down-turning (convex) after the cells were exposed to 405-nm near-blue and 434-nm blue light (Figure 1, ALS). After 6 h of elution at pH 12.1, the profiles became exponential. A logical explanation is that the DNA was broken during the first 6-h elution period, i.e, that these radiations induced a class of lesion that produced SSB slowly during the first 6-h elution. This theory was tested by holding the DNA on the filters at pH 12.1 for 6-h, to allow these putative

breaks to develop before commencing elution. With this treatment, profiles became entirely exponential, with exactly the same slopes as the post-6-h elution slopes, evidence that all slowly developing alkali-labile sites (SDAL) had developed by the start of elution. Table 1 shows calculated yields of SSB plus RDAL and SDAL, compared with those measured earlier (Peak et al., 1987) by alkaline sucrose sedimentation techniques. (Because sedimentation was for

Table 1. DNA Lesions Induced by 405-nm UVA

Method	Lesion	Number per 10^{10} per $J.m^{-2}$	%
ELUTION	FSB* + RDAL	1.2	35
	SDAL	1.7	65
		2.9	100
SEDIMENTATION		3.2	

*Frank strand breaks

20 h at pH 12.1, all SDAL were presumably developed in the initial part of the run, so that the DNA was fully broken during most of the sedimentation.) More than 60% of lesions induced by 405-nm UVA were in the form of SDAL. It is important to discover what DNA lesion this represents and what its biological significance may be. Since SSB are generally assumed to be completely sealed (Painter, 1980; Ward, 1985; Ward et al., 1985), it is of interest to investigate the extent of repair of SDAL induced by 405- and 434-nm radiations.

DNA-to-protein crosslinks: The assay for DNA-to-protein crosslinks (DPC) involves first exposing DNA to a large dose of x-rays to break the DNA into small pieces that elute rapidly unless they are covalently linked to protein. This gives the biphasic profiles shown by Figure 1 (DPC). Peak et al. (1987) developed an improved method for quantification of DPC caused by UV radiation, which compensates for the large number of SSB induced concomitantly. We demonstrated that UVA radiations are very effective at inducing these particular lesions (Peak and Peak, in press), even more so than ionizing radiation (x-rays) when computed on the basis of events per lethal fluence (dose) per cell. The biological role of the large numbers of DPC induced in cells by UVA remains completely enigmatic. In order to investigate possible damage to genetic activity of DNA, E. R. Blazek in our laboratory has recently crosslinked a variety of proteins and amino acids to single-stranded M13 viral template DNA by using crosslinking agents (UVA plus a porphyrin sensitizer or trans-dichlorodiammine Pt 11). Possible deleterious genetic damage was studied by using a sequencing technique to detect any DNA synthesis arrests, described by Ito et al. (1988). To date, few specific synthesis arrests are attributable to DPC caused by either treatment, and this is taken as evidence that DPC induced by these agents are linked to the DNA at sites that do not interfere with transcription (manuscript in preparation).

Neutral elution: An empirical method for the neutral elution assay (Figure 1, DSB) for measurement of DSB was described by Peak et al. (1988). The calibrated assay has been applied to analysis of DSB induced by UVB and UVA monochromatic radiations at 313, 365, and 405 nm (manuscript in preparation). As has been seen with ionizing radiation (Blazek et al., submitted), the dose response for DSB induction is not linear but quadratic,

indicating that DSB induction is entirely a two-hit event. In this case, the two hits must represent two SSB close enough to cause the DNA to part. There are two explanations for the data. Either the response is correct, implying that the DSB caused by UVB and UVA are due to SSB that must be non-random (clustered), or else that an unexplained artifact exists in the neutral elution assay. Resolution of these alternatives is under active investigation in our laboratory.

CONCLUSIONS

Elution techniques have been successfully applied to UVB and UVA photobiology, and their use has extended our understanding of the damage that these carcinogenic radiations cause to DNA.

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